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Synthesis of *N*-[4-(alkyl)cyclohexyl]-substituted benzamides with anti-inflammatory and analgesic activities

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Abstract

Two series of *N*-[4-(alkyl)cyclohexyl]-substituted benzamides, i.e. a series of *N*-[4-(*tert*-butyl)cyclohexyl]-substituted benzamides and a series of *N*-[4-(ethyl)cyclohexyl]-substituted benzamides, were synthesised and evaluated for their anti-inflammatory and analgesic potencies, and gastrointestinal irritation liability. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: *N*-[4-(Alkyl)cyclohexyl]-substituted benzamides; Anti-inflammatory agents; Analgesic agents

1. Introduction

Interesting anti-inflammatory and analgesic activities of some *N*-[3-(methyl)cyclohexen-2-yl]-, *N*-[3-(methyl)-cyclohexyl]- and *N*-[3-(methyl)cyclohexyl]-substituted benzamides (**1–3**; see Fig. 1) have recently been reported by us [1].

The best pharmacological profiles were obtained by compounds bearing a substituted benzoyl moiety, such as **1b**, **2b** and **3a**,**b**, or an unsubstituted cinnamoyl moiety, such as **1c** and **3c**.

In particular, compound **3a** showed anti-inflammatory and analgesic activities comparable to those of the indomethacin, even if at a higher dose.

In order to acquire new insights on the structure–activity relationships of this new class of compounds, we synthesised and pharmacologically evaluated the new derivatives **4a–h** and **5a–h** (Fig. 2) in which, with respect to compounds of general structure **3**, the methylic moiety in the 4-position of the cyclohexane was substituted by a *tert*-butylic (**4a–h**) or an ethylic (**5a–h**) group, while the residue R was a (un)substituted benzoyl moiety bearing electron-drawing or electron-donor substituents. The aim of this study is to investigate the influence on the pharmacological activity of the bulkiness of the substituent in the 4-position of the cyclohexane and the importance of the electronic density on the benzoyl moiety.

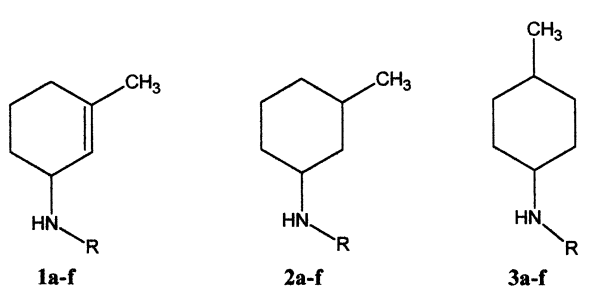

Fig. 1. *N*-[3-(Methyl)cyclohexen-2-yl]-, *N*-[3-(methyl)cyclohexyl]- and *N*-[4-(methyl)cyclohexyl]-amides (**1–3**, respectively) previously synthesised. **a**: R = 4-Cl–C_6H_4–CO; **b**: R = 3,4,5-$(CH_3O)_3$–C_6H_2–CO; **c**: R = C_6H_5–CH=CH–CO; **d**: R = 4-Cl–C_6H_4–CH=CH–CO; **e**: R = 3,4,5-$(CH_3O)_3$–C_6H_2–CH=CH–CO; **f**: R = 3,4-(CH_2O_2)–C_6H_3—CH=CH–CO.

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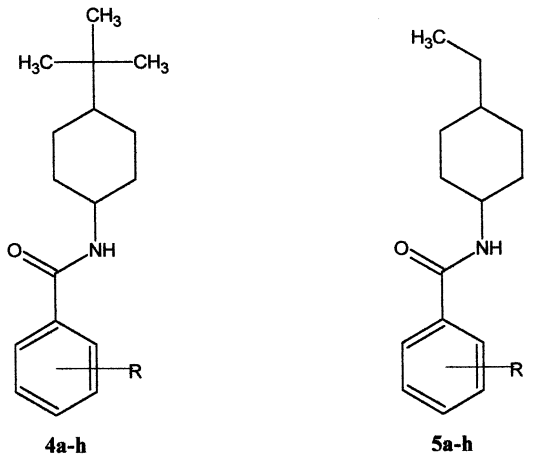
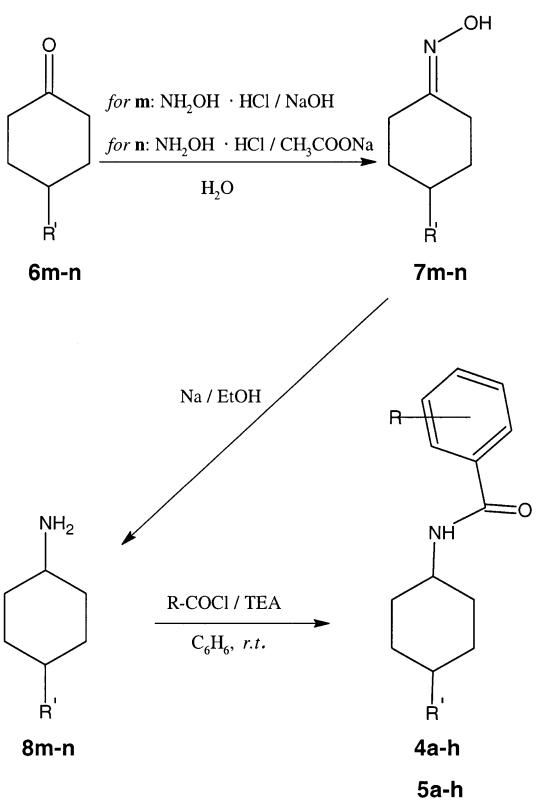


Fig. 2. *N*-[4-(Alkyl)cyclohexyl]-substituted benzamides **4a–h** and **5a–h**. **a**: R = 3,4,5-$(CH_3O)_3$; **b**: R = 4-Cl; **c**: R = 4-F; **d**: R = 4-Br; **e**: R = 2,4-diCl; **f**: R = H; **g**: R = 4-CH_3; **h**: R = 4-NO_2.

2. Chemistry

The synthesis of *N*-[4-(alkyl)cyclohexyl]-substituted benzamides **4a–h** and **5a–h** is depicted in Scheme 1. 4-(*tert*-Butyl)- and 4-(ethyl)cyclohexanone (**6m,n**), which are commercially available, were readily converted into their oximes (**7m,n**) according to Lachman [2] and Adams et al. [3], respectively. Compound **7n** was not obtained by the Lachman method; thus, the procedure was replaced by a more convenient method i.e. we obtained the 4-ethyl-cyclohexanone oxime **7n** (in good yields) directly from **6n** according to the procedure of Adams et al. [3], which was modified by us. The reaction time for the appearance of **7n** and the exhaustion of 4-(ethyl)cyclohexanone **6n** was monitored by TLC. The reduction of **7m,n** with sodium in boiling anhydrous ethanol [4,5] afforded the 4-(*tert*-butyl)- and 4-(ethyl)cyclohexylamine (**8m,n**).



Scheme 1. Synthesis of compounds **4a–h**, and **5a–h**. Key: **m**: R′ = *tert*-C_4H_9; **n**: R′ = C_2H_5; R = see Fig. 2.

The synthesis of (un)substituted benzoyl chlorides obtained from the appropriate acids in the presence of thionyl chloride is described in Section 5.

Finally, condensation of **8m,n** with the appropriate (un)substituted benzoyl chlorides in triethylamine/anhydrous benzene at room temperature (r.t.) (see Section 5) gave the required compounds **4a–h** and **5a–h** in good yields (57–97%).

The physical characteristics and yields of cyclohexylamides **4a–h** and **5a–h** are reported in Section 5. The assigned structures were generally supported by IR and GC/MS data (Table 1), and ^1H and ^{13}C NMR spectra (see Section 5).

3. Pharmacology

Compounds **4a–h** and **5a–h** (racemic mixtures) 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 molar 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 a reference drug.

4. Results and discussion

Results of the anti-inflammatory assay are shown in Table 2. Results of the analgesic assay are shown in Table 3. The ulcerogenic activity is shown in Table 4.

As regards the anti-inflammatory activity, the best results were shown by **4f**, followed by **4h**, **5h** and **4g**.

Anti-inflammatory and analgesic activities were evident in most of the tested compounds, although they were tested at a dosage more than ten times higher than indomethacin, which was used as a comparison.

As regards the analgesic activity, the best results were shown by **4f**, followed by **4d**, but many other compounds showed pharmacological potency.

As regards the ulcerogenic action, the most potent compound was **5d**, followed by **4c** and **4f**, but in general all compounds showed a fairly high irritative capacity.

Table 1
IR and GC/MS spectra of compounds **4a–h** and **5a–h**

Comp.	t_r (min)	GC/MS (most important fragments (m/z))	IR (cm^{-1})	
			ν(NH)	ν(C=O)
4a	19.0	349: (M^+); 292: [3,4,5-$(CH_3O)_3$–C_6H_2–CO–NH–C_6H_{10}]; 211: [3,4,5-$(CH_3O)_3$–C_6H_2–CO–NH_2]; 195: [3,4,5-$(CH_3O)_3$–C_6H_2–CO]; 167: [3,4,5-$(CH_3O)_3$–C_6H_2].	3230	1620
4b	14.5	293: (M^+); 236: [4-Cl–C_6H_4–CO–NH–C_6H_{10}]; 155: [4-Cl–C_6H_4–CO–NH_2]; 139: [4-Cl–C_6H_4–CO]; 111: [4-Cl–C_6H_4].	3230	1620
4c	13.8	277: (M^+); 220: [4-F–C_6H_4–CO–NH–C_6H_{10}]; 139: [4-F–C_6H_4–CO–NH_2]; 123: [4-F–C_6H_4–CO]; 95: [4-F–C_6H_4].	3300	1670
4d	23.1	338: (M^+); 281: [4-Br–C_6H_4–CO–NH–C_6H_{10}]; 200: [4-Br–C_6H_4–CO–NH_2]; 184: [4-Br–C_6H_4–CO]; 156: [4-Br–C_6H_4].	3220	1620
4e	15.2	328: (M^+); 271: [2,4-Cl_2–C_6H_4–CO–NH–C_6H_{10}]; 190: [2,4-Cl_2–C_6H_4–CO–NH_2]; 174 [2,4-Cl_2–C_6H_4–CO]; 146: [2,4-Cl_2–C_6H_4].	3215	1630
4f	15.9	259: (M^+); 202: [C_6H_4–CO–NH–C_6H_{10}]; 121: [C_6H_4–CO–NH_2]; 105: [C_6H_4–CO]; 77: [C_6H_4].	3320	1630
4g	14.9	273: (M^+); 216: [4-CH_3–C_6H_4–CO–NH–C_6H_{10}]; 135: [4-CH_3–C_6H_4–CO–NH_2]; 119: [4-CH_3–C_6H_4–CO]; 91: [4-CH_3–C_6H_4].	3230	1620
4h	19.6	304: (M^+); 247: [4-NO_2–C_6H_4–CO–NH–C_6H_{10}]; 166: [4-NO_2–C_6H_4–CO–NH_2]; 150: [4-NO_2–C_6H_4–CO]; 122: [4-NO_2–C_6H_4].	3230	1640
5a	19.5	321: (M^+); 292: [3,4,5-$(CH_3O)_3$–C_6H_2–CO–NH–C_6H_{10}]; 211: [3,4,5-$(CH_3O)_3$–C_6H_2–CO–NH_2]; 195: [3,4,5-$(CH_3O)_3$–C_6H_2–CO]; 167: [3,4,5-$(CH_3O)_3$–C_6H_2].	3300	1620
5b	15.9	265: (M^+); 236: [4-Cl–C_6H_4–CO–NH–C_6H_{10}]; 155: [4-Cl–C_6H_4–CO–NH_2]; 139: [4-Cl–C_6H_4–CO]; 111: [4-Cl–C_6H_4].	3290	1625
5c	13.8	249: (M^+); 220: [4-F–C_6H_4–CO–NH–C_6H_{10}]; 139: [4-F–C_6H_4–CO–NH_2]; 123: [4-F–C_6H_4–CO]; 95: [4-F–C_6H_4].	3300	1630
5d	16.9	310: (M^+); 281: [4-Br–C_6H_4–CO–NH–C_6H_{10}]; 200: [4-Br–C_6H_4–CO–NH_2]; 184: [4-Br–C_6H_4–CO]; 156: [4-Br–C_6H_4].	3290	1630
5e	16.8	300: (M^+); 271: [2,4-Cl_2–C_6H_4–CO–NH–C_6H_{10}]; 190: [2,4-Cl_2–C_6H_4–CO–NH_2]; 174: [2,4-Cl_2–C_6H_4–CO]; 146: [2,4-Cl_2–C_6H_4].	3280	1635
5f	14.1	231: (M^+); 202: [C_6H_5–CO–NH–C_6H_{10}]; 121: [C_6H_5–CO–NH_2]; 105: [C_6H_5–CO]; 77: [C_6H_5].	3300	1630
5g	15.3	245: (M^+); 216: [4-CH_3–C_6H_4–CO–NH–C_6H_{10}]; 135: [4-CH_3–C_6H_4–CO–NH_2]; 119: [4-CH_3–C_6H_4–CO]; 91: [4-CH_3–C_6H_4].	3300	1630
5h	18.0	276: (M^+); 247: [4-O_2N–C_6H_4–CO–NH–C_6H_{10}]; 166: [4-O_2N–C_6H_4–CO–NH_2]; 150: [4-O_2N–C_6H_4–CO]; 122: [4-O_2N–C_6H_4].	3300	1630

These pharmacological results allow us to draw some preliminary conclusions on the structure–activity relationship:

- In general, derivatives bearing a *tert*-butyl moiety (**4a–h**) showed a better pharmacological profile; for example, compound **4f** was three-fold more potent as anti-inflammatory than **5h**, the most potent in the series of the ethyl derivatives (**5a–h**).
- The presence of substituents on the aromatic ring was not necessary: in fact, the best pharmacological result was shown by the unsubstituted **4f**. Anyway, among the substituted compounds the best results were shown by compounds bearing an NO_2 (**4h**, **5h**) or a bromine (**4d**) group.
- In many cases, especially in the analgesic test, a fairly good activity appeared at low doses, but without enhancement from increased doses.
- Concerning the ulcerogenic potency, all compounds were less ulcerogenic than indomethacin (at 14 μmol/kg) (especially if the severe doses are considered i.e. 375 μmol/kg). Anyway, often it does not correspond to the anti-inflammatory and/or analgesic effect. In fact, the ulcerogenic potency of the most active compounds was not significantly different from the one of the compounds lacking in anti-inflammatory and analgesic activities: on the other hand, compounds having weak anti-inflammatory and/or analgesic activity (**4a–c**, **5d**) proved to be gastric irritants.

5. Experimental

5.1. Chemistry

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

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

IR spectra were recorded on a Perkin–Elmer model 298 spectrophotometer, including solid samples in KBr pellets and analysing liquid samples as films.

GC–MS spectra were obtained from a HP5970A (Hewlett–Packard) apparatus, equipped with a capillary column HP-5 (25 m × 0.2 mm × 0.11 μm). Programmed temperatures ranged from 100 to 300°C (10°C/min), the detector temperature was set to 300°C and carrier gas was helium at 10 psi of pressure.

The ^{1}H and ^{13}C NMR measurements were performed on a Bruker AMX 500 MHz spectrometer equipped with a Bruker X-32 computer, using $CDCl_3$ as solvent and TMS as internal standard. Chemical shifts are expressed in ppm downfield from TMS and coupling constants (J) are expressed in Hertz.

Commercially available solvents and chemicals were usually used for syntheses.

*5.1.1. Synthesis of 4-(tert-butyl)cyclohexanone oxime (**7m**)*

It was prepared by using the procedure already described by Lachman [2]: 82% yield; m.p. 138–139°C; IR (KBr, cm^{-1}) 1665 (νC=N–OH); MS m/z 169 (M^+).

*5.1.2. Synthesis of 4-(ethyl)cyclohexanone oxime (**7n**) [3]*

A total of 6.0 g (86 mmol) of hydroxylamine hydrochloride and 9.0 g (66 mmol) of sodium acetate hydrate crystals ($NaC_2H_3O_2 \cdot 3H_2O$) were dissolved in 20 ml of water in a two-necked round-bottomed flask fitted with a thermometer and a mechanical stirrer.

The solution was then warmed up to about 40°C and 5 g (5.5 ml, 40 mmol) of 4-(ethyl)cyclohexanone (**6n**) (previously distilled in vacuo using a Kugelrohr apparatus) were added. The mixture was vigorously stirred for

Table 2
Carrageenan rat paw edema: anti-inflammatory activity

Comp.	Dose (μmol/kg p.o.)	% Edema inhibition relative to control at:			
		3 h	ED_{50} (μmol/kg p.o.)	4 h	ED_{50} (μmol/kg p.o.)
Indomethacin	14	−63		−68	
4a	150	−28		−25	
4b	150	−27		−39	
4c	150	−24		−19	
	300	−46		−54	
4d	150	−42		−37	*252.7 (203.4–314.0)*
	75	−5		−18	
	300	−19		−18	
4e	150	−16		−12	
	75	−22		−18	
	150	−84		−72	
4f	75	−43	81.6 (72.3–92.2)	−55	75.8 (64.1–89.6)
	37.5	−11		−23	
	300	−62		−52	
4g	150	−42	*193.8 (152.4–246.5)*	−50	*206.9 (105.8–404.4)*
	75	−27		−39	
	300	−62		−54	
4h	150	−46	*145.9 (212.5–267.1)*	−52	*190.0 (123.5–292.1)*
	75	−43		−34	
5a	150	−3		−18	
	300	−62		−52	
5b	150	−27	*238.2 (212.5–267.1)*	−23	*273.9 (241.0–311.5)*
	75	−2		−2	
5c	150	−16		−30	
5d	150	−27		−9	
5e	300	−46		−43	
	150	−32		−39	
	75	−22		−18	
5f	300	−49		−43	
	150	−46		−39	
	75	−32		−30	
5g	300	−49		−57	*282.7 (236.6–337.7)*
	150	−22		−18	
	75	−10		−9	
5h	300	−64	*233.4 (208.7–261.1)*	−70	*228.9 (178.5–228.9)*
	150	−27		−39	
	75	−2		−5	

Table 3
Acetic acid writhing test: analgesic activity

Compound	Dose (μmol/kg po)	Mean number of writhes in 25 min period after treatment ± S.E.	% Decrease relative to controls	ED_{50} (μmol/kg po)
Controls	–	45.6 ± 5.3	–	
Indomethacin	14	22.7 ± 4.1	−50	
4a	150	29.5 ± 3.7	−35	
4b	150	33.2 ± 2.8	−27	
4c	150	30.6 ± 2.5	−33	
4d	300	21.9 ± 3.9	−52	
	150	24.0 ± 3.1	−47	229.2
	75	27.3 ± 4.1	−40	(110.7–474.6)
4e	300	27.1 ± 4.2	−41	
	150	28.1 ± 4.2	−38	
	75	31.6 ± 5.1	−41	
4f	150	20.9 ± 3.5	−54	
	75	25.6 ± 3.1	−44	118.2
	37.5	26.9 ± 4.3	−41	(60.8–233.9)
4g	300	23.7 ± 5.1	−48	
	150	26.2 ± 7.1	−42	
	75	28.5 ± 2.8	−37	
4h	300	23.2 ± 3.8	−49	
	150	24.3 ± 4.2	−47	
	75	27.7 ± 4.1	−39	
5a	150	33.2 ± 2.9	−27	
5b	300	24.1 ± 5.2	−47	
	150	24.8 ± 2.6	−46	
	75	27.7 ± 6.1	−39	
5c	150	31.0 ± 2.5	−32	
5d	300	26.5 ± 5.2	−42	
	150	28.0 ± 4.1	−39	
	75	31.7 ± 2.8	−30	
5e	150	28.4 ± 3.1	−38	
5f	300	25.3 ± 4.3	−44	
	150	25.9 ± 1.9	−43	
	75	29.2 ± 4.3	−36	
5g	300	24.2 ± 5.1	−47	
	150	24.4 ± 3.3	−46	
	75	27.5 ± 5.1	−40	
5h	150	32.5 ± 2.7	−29	

30 min, then warmed to 80°C for 3 h and after cooling at r.t., the resulting oil was extracted with ethyl ether. The organic extracts were dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure. The crude oil was distilled on a Kugelrohr apparatus under vacuum (b.p. 130–135°C, 7 mmHg) to yield 4.7 g (86%) of the 4-ethyl-cyclohexanone oxime (**7n**) as a clear liquid. TLC (benzene/acetone 8:2 v/v); IR (film, cm^{-1}) 1660 (νC=N–OH); MS m/z 141 (M^+).

5.1.3. 4-(tert-Butyl)cyclohexylamine (8m) [4,5]

To a solution of 8 g (47 mmol) of 4-(*tert*-butyl)cyclohexanone oxime (**7m**) in 136 ml of boiling anhydrous ethanol were added portionwise 20 g (870 mmol) of clean sodium (previously cut into small pieces), keeping the vigorous reaction under control. When the sodium had reacted completely, the reaction mixture was cooled and diluted with aqueous ethanol solution (1/1) and then it was treated with conc. HCl with stirring until the solution was acidic 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 in the process. The aqueous layer was basified with 5 N NaOH solution and extracted with diethyl ether which was dried with anhydrous sodium sulfate, filtered and evaporated under vacuum to leave an oily residue. The crude oil was distilled on a Kugelrohr apparatus under vacuum (b.p. 60–70°C, 7 mmHg) to yield 5 g (68%) of the 4-*tert*-butyl-cyclohexylamine (**8m**) as a clear liquid. TLC (benzene/acetone 8:2 v/v); IR (film, cm^{-1}) 3330 (ν_{asym} N–H), 3270 (ν_{sym} N–H); MS m/z 155 (M^+).

5.1.4. 4-(Ethyl)cyclohexylamine (8n)

This compound was prepared in a similar manner as for **8m** using 4.7 g (33 mmol) of **7n**. The crude clear

yellow oil obtained was distilled on a Kugelrohr apparatus under vacuum (b.p. 40–60°C, 7 mmHg) to yield 3.8 g (90%) of the 4-ethyl-cyclohexylamine (**8n**) as a clear liquid. TLC (benzene/acetone 8:2 v/v); IR (film, cm^{-1}) 3340 (ν_{asym} N–H), 3280 (ν_{sym} N–H); MS m/z 127 (M^+).

5.1.5. General procedure for the synthesis of acyl chlorides

A total of 15 mmol of (un)substituted benzoic acid and 35 mmol of thionyl chloride was heated to reflux under stirring for 2h and the excess thionyl chloride was distilled off. The remaining acyl chloride, prepared as crude material, was used for the next step without further purification; yields 58–87%.

*5.1.6. General procedure for the preparation of N-[4-(tert-butyl)cyclohexyl]-substituted benzamides (**4a–h**) and N-[4-(ethyl)cyclohexyl]-substituted benzamides (**5a–h**)*

A solution of substituted cyclohexylamine (5 mmol) in anhydrous benzene (ca. 30 ml) and triethyl amine was added dropwise, via an addition funnel, to an ice-cold solution of the appropriate acyl chloride (6 mmol) in the same solvent, under magnetic stirring. The mixture was allowed to warm to r.t. and was vigorously stirred for a time varying from 30 min to 2 h, until the TLC analysis (eluant benzene/acetone 8:2 v/v) indicated the disappearance of the starting materials. At the end of the reaction, the precipitate was collected by filtration and sequentially washed with 5% $NaHCO_3$, water and dried to give the title products **4a–h** and **5a–h** as white crystals, which were crystallised from alcohol or a hydro-alcoholic mixture.

Table 4
Induction of gastric lesions in rats

Comp.	Dose (μmol/kg p.o.)	6 h after treatment, animals with:	
		Hyperaemia (%)	Ulcers (%)
Indomethacin	14	80	60
4a	375	60	40
4b	375	30	20
4c	375	70	50
4d	375	60	40
4e	375	50	30
4f	375	70	50
4g	375	60	40
4h	375	30	30
5a	375	30	30
5b	375	30	20
5c	375	20	20
5d	375	70	60
5e	375	30	20
5f	375	30	20
5g	375	40	20
5h	375	50	40

The initial filtrate (benzene solution) was shaken with a solution of 5% $NaHCO_3$ and water to enhance the reaction yield. The organic phase was dried (anhydrous magnesium sulfate) and evaporated under vacuum to afford the crude amide compound, which was purified by silica-gel chromatography or flash-chromatography using a misture of benzene/acetone (8:2 v/v) as eluant. Finally, the appropriate fractions were collected and evaporated to afford the desired products **4a–h** and **5a–h**, which were stored together with the amounts obtained as described above.

*5.1.6.1. N-[4-(tert-Butyl)cyclohexyl)]-3,4,5-trimetoxybenzamide (**4a**).* Formula ($C_{20}H_{31}NO_4$), M_w 349.48, m.p. 196–197°C, yield 86%. ^1H ($CDCl_3$): 0.90 (s, 9H, 4-C(CH_3)$_3$), 1.05 (m, 1H, H-4), 1.22 (m, 4H, H-2 and H-6), 1.83 (bd, 2H, $J = 8.0$, H_{eq}-3 and H_{eq}-5), 2.08 (bd, 2H, $J = 8.0$, H_{ax}-3 and H_{ax}-5), 3.86 (m, 4H, H-1 and 4′-OCH_3), 3.92 (s, 6H, 3′-OCH_3 and 5′-OCH_3), 5.94 (d, 1H, $J = 6.8$, NH), 6.98 (s, 2H, H-2′ and H-6′). ^{13}C ($CDCl_3$): 26.16 (2C, C-3 and C-5), 27.58 (3C, 4-C(CH_3)$_3$), 32.38 (1C, 4-C(CH_3)$_3$), 33.67 (2C, C-2 and C-6), 47.39 (1C, C-4), 49.40 (1C, C-1), 56.35 (2C, 3′-OCH_3 and 5′-OCH_3), 60.90 (1C, 4′-OCH_3), 104.31 (2C, C-2′ and C-6′), 130.62 (1C, C-1′), 153.14 (3C, C-3′, C-4′ and C-5′), 166.49 (CO).

*5.1.6.2. N-[4-(tert-Butyl)cyclohexyl]-4-chlorobenzamide (**4b**).* Formula ($C_{17}H_{24}NOCl$), M_w 293.84, m.p. 199°C, yield 88%. ^1H ($CDCl_3$): 0.88 (s, 9H, 4-C(CH_3)$_3$), 1.02 (m, 1H, H-4), 1.20 (m, 4H, H-2 and H-6), 1.82 (bd, 2H, $J =$ 8.0, H_{eq}-3 and H_{eq}-5), 2.06 (bd, 2H, $J = 8.0$, H_{ax}-3 and H_{ax}-5), 3.88 (m, 1H, H-1), 6.02 (d, 1H, $J = 6.8$, NH), 7.42 (d, 2H, $J = 8.5$, H-3′ and H-5′), 7.70 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 26.13 (2C, C-3 and C-5), 27.54 (3C, 4-C(CH_3)$_3$), 32.35 (1C, 4-C(CH_3)$_3$), 33.64 (2C, C-2 and C-6), 47.35 (1C, C-4), 49.39 (C-1), 128.29 (2C, C-3′ and C-5′), 128.68 (2C, C-2′ and C-6′), 133.14 (1C, C-1′), 137.39 (C-4′), 165.64 (1C, CO).

*5.1.6.3. N-[4-(tert-Butyl)cyclohexyl]-4-fluorobenzamide (**4c**).* Formula ($C_{17}H_{24}NOF$), M_w 277.38, m.p. 192°C, yield 73%. ^1H ($CDCl_3$): 0.86 (s, 9H, 4-C(CH_3)$_3$), 0.98 (m, 1H, H-4), 1.17 (m, 4H, H-2 and H-6), 1.82 (bd, 2H, $J = 7.7$, 2H, H_{eq}-3 and H_{eq}-5), 2.11 (bd, 2H, $J = 7.7$, 2H, H_{ax}-3 and H_{ax}-5), 3.88 (m, 1H, H-1), 6.01 (d, 1H, $J = 6.8$, NH), 7.05 (t, 2H, $J = 8.5$, H-3′ and H-5′), 7.74 (dd, 2H, $J = 8.5$, 5.1, H-2′ and H-6′). ^{13}C ($CDCl_3$): 26.14 (2C, C-3 and C-5), 27.57 (3C, 4-C(CH_3)$_3$), 32.38 (1C, 4-C(CH_3)$_3$), 33.66 (2C, C-2 and C-6), 47.33 (1C, C-4), 49.35 (1C, C-1), 115.45 (2C, d, $J_{C-F} = 22.9$, C-3′ and C-5′), 129.17 (2C, d, $J_{C-F} = 9.5$, C-2′ and C-6′), 131.02 (1C, C-1′), 164.63 (1C, d, $J_{C-F} = 251.8$, C-4′), 165.71 (1C, CO).

*5.1.6.4. N-[4-(tert-Butyl)cyclohexyl]-4-bromobenzamide (**4d**).* Formula ($C_{17}H_{24}NOBr$), M_w 338.29, m.p. 200–201°C, yield 56%. ^1H ($CDCl_3$): 0.88 (s, 9H, 4-C(CH_3)$_3$), 1.00 (m, 1H, H-4), 1.18 (m, 4H, H-2 and H-6), 1.84 (bd, 2H, $J = 7.4$, H_{eq}-3 and H_{eq}-5), 2.14 (bd, 2H, $J = 7.4$, 2H, H_{ax}-3 and H_{ax}-5), 3.84 (m, 1H, H-1), 6.02 (d, 1H, $J = 6.8$, NH), 7.58 (d, 2H, $J = 8.5$ H-3′ and H-5′), 7.64 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 26.13 (2C, C-3 and C-5), 27.56 (3C, 4-C(CH_3)$_3$), 32.37 (1C, 4-C(CH$_3$)$_3$), 33.62 (2C, C-2 and C-6), 47.33 (1C, C-4), 49.40 (1C, C-1), 125.82 (1C, C-4′), 128.52 (2C, C-3′ and C-5′), 131.67 (2C, C-2′ and C-6′), 133.88 (1C, C-1′), 165.76 (1C, CO).

*5.1.6.5. N-[4-(tert-Butyl)cyclohexyl]-2,4-dichlorobenzamide (**4e**).* Formula ($C_{17}H_{23}NOCl_2$), M_w 328.28, m.p. 179–180°C, yield 73%. ^1H ($CDCl_3$): 0.89 (s, 9H, 4-C(CH_3)$_3$), 1.02 (m, 1H, H-4), 1.19 (m, 4H, H-2 and H-6), 1.85 (bd, 2H, $J = 8.5$, 2H, H_{eq}-3 and H_{eq}-5), 2.17 (bd, 2H, $J = 8.5$, 2H, H_{ax}-3 and H_{ax}-5), 3.86 (m, 1H, H-1), 6.00 (d, 1H, $J = 6.8$, NH), 7.32 (d, 1H, $J = 8.5$ H-5′), 7.42 (s, 1H, H-3′), 7.61 (d, 1H, $J = 8.5$, H-6′). ^{13}C ($CDCl_3$): 26.07 (2C, C-3 and C-5), 27.50 (3C, 4-C(CH_3)$_3$), 32.32 (4-C(CH$_3$)$_3$), 33.44 (2C, C-2 and C-6), 47.33 (1C, C-4), 49.71 (1C, C-1), 127.41, 129.88 and 131.14 (1C, C-3′, C-5′ and C-6′), 131.24 (1C, C-1′), 133.93 and 136.43 (2C, C-2′ and C-4′), 164.79 (1C, CO).

*5.1.6.6. N-[4-(tert-Butyl)cyclohexyl]benzamide (**4f**).* Formula ($C_{17}H_{25}NO$), M_w 259.39, m.p. 172–173°C, yield 94%. ^1H ($CDCl_3$): 0.88 (s, 9H, 4-C(CH_3)$_3$), 0.98 (m, 1H, H-4), 1.18 (m, 4H, H-2 and H-6), 1.82 (bd, 2H, $J = 7.5$, 2H, H_{eq}-3 and H_{eq}-5), 2.12 (bd, 2H, $J = 7.5$, 2H, H_{ax}-3 and H_{ax}-5), 3.86 (m, 1H, H-1), 6.06 (d, 1H, $J = 6.8$, NH), 7.42 (t, 2H, $J = 8.5$, H-3′ and H-5′), 7.48 (t, 1H, $J = 8.5$, H-4′), 7.78 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 26.16 (2C, C-3 and C-5), 27.56 (3C, 4-C(CH_3)$_3$), 32.35 (1C, 4-C(CH$_3$)$_3$), 33.65 (2C, C-2 and C-6), 47.35 (1C, C-4), 49.23 (1C, C-1), 126.86 (2C, C-3′ and C-5′), 128.43 (2C, C-2′ and C-6′), 131.16 (1C, C-4′), 135.09 (1C, C-1′), 166.89 (1C, CO).

*5.1.6.7. N-[4-(tert-Butyl)cyclohexyl]-4-methylbenzamide (**4g**).* Formula ($C_{18}H_{27}NO$), M_w 273.42, m.p. 178–179°C, yield 94%. ^1H ($CDCl_3$): 0.85 (s, 9H, 4-C(CH_3)$_3$), 0.96 (m, 1H, H-4), 1.16 (m, 4H, H-2 and H-6), 1.82 (bd, 2H, $J = 7.5$, 2H, H_{eq}-3 and H_{eq}-5), 2.12 (bd, 2H, $J =$ 7.5, H_{ax}-3 and H_{ax}-5), 2.38 (s, 3H, 4′-CH_3), 3.86 (m, 1H, H-1), 6.03 (d, 1H, $J = 6.8$, NH), 7.20 (d, 2H, $J = 8.5$, H-3′ and H-5′), 7.66 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 21.33 (1C, 4′-CH_3), 26.13 (2C, C-3 and C-5), 27.52 (3C, 4-C(CH_3)$_3$), 32.31 (1C, 4-C(CH$_3$)$_3$), 33.65 (2C, C-2 and C-6), 47.34 (1C, C-4), 49.09 (1C, C-1), 126.81 (2C, C-3′ and C-5′), 129.04 (2C, C-2′ and C-6′), 131.95 (1C, C-1′), 141.43 (1C, C-4′), 166.61 (1C, CO).

*5.1.6.8. N-[4-(tert-Butyl)cyclohexyl]-4-nitrobenzamide (**4h**).* Formula ($C_{17}H_{24}N_2O_3$), M_w 304.39, m.p. 180°C, yield 97%. ^1H ($CDCl_3$): 0.82 (s, 9H, 4-C(CH_3)$_3$), 0.98 (m, 1H, H-4), 1.12 (m, 4H, H-2 and H-6), 1.84 (bd, 2H, $J = 8.0$, 2H, H_{eq}-3 and H_{eq}-5), 2.16 (bd, 2H, $J = 8.0$, 2H, H_{ax}-3 and H_{ax}-5), 3.86 (m, 1H, H-1), 6.14 (d, 1H, $J = 6.8$, NH), 7.90 (d, 2H, $J = 8.5$ H-3′ and H-5′), 8.24 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 26.10 (2C, C-3 and C-5), 27.55 (3C, 4-C(CH_3)$_3$), 32.38 (1C, 4-C(CH$_3$)$_3$), 33.53 (2C, C-2 and C-6), 47.28 (1C, C-4), 49.79 (1C, C-1), 123.74 (2C, C-3′ and C-5′), 128.10 (2C, C-2′ and C-6′), 140.63 (1C, C-1′), 149.42 (1C, C-4′), 164.74 (1C, CO).

*5.1.6.9. N-[4-(Ethyl)cyclohexyl]-3,4,5-trimetoxybenzamide (**5a**).* Formula ($C_{18}H_{27}NO_4$), M_w 321.42, m.p. 207–208°C, yield 94%. ^1H ($CDCl_3$): 0.88 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.18 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.82 (bd, 2H, $J = 8.0$, H_{eq}-3 and H_{eq}-5), 2.08 (bd, 2H, $J = 8.0$, H_{ax}-3 and H_{ax}-5), 3.86 (m, 4H, H-1 and 4′-OCH_3), 3.92 (s, 6H, 3′-OCH_3 and 5′-OCH_3), 5.94 (d, 1H, $J = 6.8$, NH), 6.98 (s, 2H, H-2′ and H-6′). ^{13}C ($CDCl_3$): 11.61 (1C, 4-CH_2-CH_3), 29.48 (1C, 4-CH_2-CH_3), 31.44 (2C, C-3 and C-5), 33.26 (2C, C-2 and C-6), 38.69 (1C, C-4), 49.45 (1C, C-1), 56.35 (2C, 3′-OCH_3 and 5′-OCH_3), 60.90 (1C, 4′-OCH_3), 104.25 (2C, C-2′ and C-6′), 130.65 (1C, C-1′), 153.16 (3C, C-3′, C-4′ and C-5′), 166.46 (1C, CO).

*5.1.6.10. N - [4 - (Ethyl)cyclohexyl] -4-chlorobenzamide (**5b**).* Formula ($C_{15}H_{20}NOCl$), M_w 265.79, m.p. 168–170°C, yield 75%. ^1H ($CDCl_3$): 0.85 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.16 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.82 (bd, 2H, $J = 8.0$, 2H, H_{eq}-3 and H_{eq}-5), 2.10 (bd, 2H, $J = 8.0$, 2H, H_{ax}-3 and H_{ax}-5), 3.86 (m, 1H, H-1), 5.90 (d, 1H, $J = 6.8$, NH), 7.40 (d, 2H, $J = 8.5$, H-3′ and H-5′), 7.70 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 11.60 (1C, 4-CH_2-CH_3), 29.45 (1C, 4-CH_2-CH_3), 31.40 (2C, C-3 and C-5), 33.23 (2C, C-2 and C-6), 38.64 (1C, C-4), 49.43 (1C, C-1), 128.28 (2C, C-3′ and C-5′), 128.74 (2C, C-2′ and C-6′), 133.40 (1C, C-1′), 137.43 (1C, C-4′), 165.63 (1C, CO).

*5.1.6.11. N-[4-(Ethyl)cyclohexyl]-4-fluorobenzamide (**5c**).* Formula ($C_{15}H_{20}NOF$), M_w 249.33, m.p. 190–191°C, yield 78%. ^1H ($CDCl_3$): 0.87 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.19 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.82 (bd, 2H, $J = 7.7$, 2H, H_{eq}-3 and H_{eq}-5), 2.11 (bd, 2H, $J = 7.7$, 2H, H_{ax}-3 and H_{ax}-5), 3.88 (m, 1H, H-1), 6.01 (d, 1H, $J = 6.8$, NH), 7.05 (t, 2H, $J = 8.8$, H-3′ and H-5′), 7.74 (dd, 2H, $J = 8.8$, 5.1, H-2′ and H-6′). ^{13}C ($CDCl_3$): 11.61 (1C, 4-CH_2-CH_3), 29.45 (1C, 4-CH_2-CH_3), 31.47 (2C, C-3 and C-5), 33.28 (2C, C-2 and C-6), 38.70 (1C, C-4), 49.45 (1C, C-1), 115.45 (2C, d, $J_{C-F} = 22.9$, C-3′ and C-5′), 129.17 (2C, d, $J_{C-F} = 9.5$, C-2′ and C-6′), 131.00 (1C, C-1′), 164.63 (1C, d, $J_{C-F} =$ 251.8, C-4′), 165.71 (1C, CO).

*5.1.6.12. N - [4 - (Ethyl)cyclohexyl] -4-bromobenzamide (**5d**).* Formula ($C_{15}H_{20}NOBr$), M_w 310.24, m.p. 200–201°C, yield 67%. ^1H ($CDCl_3$): 0.85 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.18 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.84 (bd, 2H, $J = 7.4$, H_{eq}-3 and H_{eq}-5), 2.16 (bd, 2H, $J = 7.4$, H_{ax}-3 and H_{ax}-5), 3.88 (m, 1H, H-1), 5.92 (d, 1H, $J = 6.8$, NH), 7.58 (d, 2H, $J = 8.5$ H-3′ and H-5′), 7.64 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 11.60 (1C, 4-CH_2-CH_3), 29.46 (1C, 4-CH_2-CH_3), 31.40 (2C, C-3 and C-5), 33.22 (2C, C-2 and C-6), 38.65 (1C, C-4), 49.43 (1C, C-1), 125.85 (C-4′), 128.48 (2C, C-3′ and C-5′), 131.72 (2C, C-2′ and C-6′), 133.87 (1C, C-1′), 165.70 (1C, CO).

*5.1.6.13. N-[4-(Ethyl)cyclohexyl]-2,4-dichlorobenzamide (**5e**).* Formula ($C_{15}H_{19}NOCl_2$), M_w 300.23, m.p. 161–162°C, yield 83%. ^1H ($CDCl_3$): 0.88 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.16 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.82 (bd, 2H, $J = 8.5$, H_{eq}-3 and H_{eq}-5), 2.17 (bd, 2H, $J = 8.5$, 2H, H_{ax}-3 and H_{ax}-5), 3.92 (m, 1H, H-1), 6.00 (d, 1H, $J = 6.8$, NH), 7.32 (d, 1H, $J = 8.5$ H-5′), 7.40 (s, 1H, H-3′), 7.61 (d, 1H, $J = 8.5$, H-6′). ^{13}C ($CDCl_3$): 11.59 (1C, 4-CH_2-CH_3), 29.43 (1C, 4-CH_2-CH_3), 31.34 (2C, C-3 and C-5), 32.98 (2C, C-2 and C-6), 38.59 (1C, C-4), 49.72 (1C, C-1), 127.47, 128.48 and 131.15 (1C, C-3′, C-5′ and C-6′), 131.24 (1C, C-1′), 133.92 and 136.47 (2C, C-2′ and C-4′), 164.67 (1C, CO).

*5.1.6.14. N-[4-(Ethyl)cyclohexyl]benzamide (**5f**).* Formula ($C_{15}H_{21}NO$), M_w 231.34, m.p. 217°C, yield 57%. ^1H ($CDCl_3$): 0.86 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.22 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.82 (bd, 2H, $J = 7.5$, 2H, H_{eq}-3 and H_{eq}-5), 2.08 (bd, 2H, $J =$ 7.5, 2H, H_{ax}-3 and H_{ax}-5), 3.92 (m, 1H, H-1), 5.96 (d, 1H, $J = 6.8$, NH), 7.42 (t, 2H, $J = 8.5$, H-3′ and H-5′), 7.48 (t, 1H, $J = 8.5$, H-4′), 7.76 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 11.61 (1C, 4-CH_2-CH_3), 29.48 (1C, 4-CH_2-CH_3), 31.43 (2C, C-3 and C-5), 33.26 (2C, C-2 and C-6), 38.66 (1C, C-4), 49.27 (1C, C-1), 126.82 (2C, C-3′ and C-5′), 128.50 (2C, C-2′ and C-6′), 131.23 (1C, C-4′), 135.09 (1C, C-1′), 166.71 (1C, CO).

*5.1.6.15. N - [4 - (Ethyl)cyclohexyl] - 4 - methylbenzamide (**5g**).* Formula ($C_{16}H_{23}NO$), M_w 245.37, m.p. 175–176°C, yield 60%. ^1H ($CDCl_3$): 0.87 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.19 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.82 (bd, 2H, $J = 7.5$, H_{eq}-3 and H_{eq}-5), 2.08 (bd, 2H, $J = 7.5$, H_{ax}-3 and H_{ax}-5), 2.38 (s, 3H, 4′-CH_3), 3.84 (m, 1H, H-1), 5.96 (d, 1H, $J = 6.8$, NH), 7.21 (d, 2H, $J = 8.5$, H-3′ and H-5′), 7.63 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 11.61 (1C, 4-CH_2-CH_3), 21.43 (1C, 4′-CH_3), 29.49 (1C, 4-CH_2-CH_3), 31.45 (2C, C-3 and C-5), 33.27 (2C, C-2 and C-6), 38.67 (1C, C-4), 49.17 (1C, C-1), 126.82 (2C, C-3′ and C-5′), 129.14 (2C, C-2′ and C-6′), 132.19 (1C, C-1′), 141.56 (1C, C-4′), 166.63 (1C, CO).

*5.1.6.16. N-[4-(Ethyl)cyclohexyl]-4-nitrobenzamide (**5h**).* Formula ($C_{15}H_{20}N_2O_3$), M_w 276.35, m.p. 220°C, yield 80%. ^1H ($CDCl_3$): 0.86 (t, 3H, $J = 7.0$, 4-CH_2-CH_3), 1.18 (m, 7H, H-4, H-2, H-6 and 4-CH_2-CH_3), 1.82 (bd, 2H, $J = 8.0$, 2H, H_{eq}-3 and H_{eq}-5), 2.10 (bd, 2H, $J = 8.0$, 2H, H_{ax}-3 and H_{ax}-5), 3.92 (m, 1H, H-1), 6.06 (d, 1H, $J = 6.8$, NH), 7.92 (d, 2H, $J = 8.5$ H-3′ and H-5′), 8.28 (d, 2H, $J = 8.5$, H-2′ and H-6′). ^{13}C ($CDCl_3$): 11.59 (1C, 4-CH_2-CH_3), 29.42 (1C, 4-CH_2-CH_3), 31.34 (2C, C-3 and C-5), 33.11 (2C, C-2 and C-6), 38.59 (1C, C-4), 49.82 (1C, C-1), 123.76 (2C, C-3′ and C-5′), 128.07 (2C, C-2′ and C-6′), 140.64 (1C, C-1′), 149.43 (1C, C-4′), 164.68 (1C, CO).

5.2. Pharmacology

Tested compounds were administered orally by gavage in 1% methylcellulose suspension, using a dose of 150 μmol/kg (≅ 40 mg/kg) and then, if significant pharmacological activity was detected, higher (300 μmol/kg) and lower (75, 37.5 μmol/kg) doses in order to determine the ED_{50} values.

Gastric ulcerogenic action was studied in rats which were treated orally with higher doses (375 μmol/kg, ≅ 100 mg/kg).

Indomethacin was included in all tests for comparison purposes at the dose level of 14 μmol/kg (5 mg/kg).

The following experimental procedures were employed.

5.2.1. Anti-inflammatory activity

Paw edema inhibition test was used on rats [6]. Groups of five rats of both sexes (body weight 180–250 g), pregnant females excluded, were given a dose of a test compound and 30 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, and 4 h later. The mean increase of paw volume at each time interval was compared with that of the control group (five rats treated with carrageenan, but not treated with test compounds) at the same time intervals and percent inhibition values were calculated. Experimental results at 3 and 4 h are listed in Table 2.

5.2.2. Analgesic activity

Acetic acid writhing test was used on mice [7]. Groups of five mice (body weight 20–30 g) of both sexes, pregnant females excluded, were given a dose of a test compound and 30 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 (five mice not treated with test compounds) were calculated. Experimental results are listed in Table 3.

5.2.3. Ulcerogenic action

Groups of ten 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 [8]. 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 4.

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