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Hypotensive and antinociceptive effects of ether-linked and relatively non-pungent analogues of *N*-nonanoyl vanillylamide

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Summary — *N*-nonanoyl vanillylamide (**1**) is a synthetic substitute of capsaicin (**2**), a pungent principle in red pepper. **1** was reacted with succinic anhydride, 3-chloro-1,2-propanediol, sodium chloroacetate, and 2-chloroethanol to furnish *N*-nonanoyl vanillylamide-4-succinyl ester (**3**), *N*-nonanoyl vanillylamide-4-glyceryl ether (**4**), sodium *N*-nonanoyl vanillylamide-4-*O*-acetate (**5**) and *N*-nonanoyl vanillylamide-4-glycol ether (**6**), respectively. A 3,4-methylenedioxy derivative of **1**, *N*-nonanoyl piperonylamide (**7**), was synthesized from piperonylamine HCl and nonanoyl chloride. The ether analogues **4**–**6** all demonstrated marked antinociceptive and hypotensive effects without producing any overt irritation. In addition, these compounds revealed no untoward vagus reflex or transient hypertensive effect as previously found in **1** or **2**.

capsaicin / hypotensive activity / antinociceptive activity / non-pungent analogues

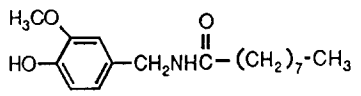
Introduction

Capsaicin, *trans*-8-methyl-*N*-vanillyl-6-nonenamide (**2**, fig 1), is a pungent constituent of red pepper. Early studies of its pharmacological effects revealed a wide spectrum of activities [1–3], including analgesic effects. However, its therapeutic value is limited by the concomitant hypothermia and irritation accompanying its use [4].

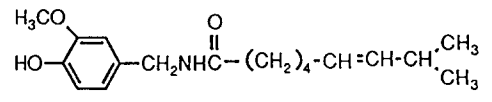
A number of derivatives of **2** have been synthesized and evaluated for their pungent potencies, desensitizing effects, nociceptive activities, and hypothermic effects [4–7]. However, none have been found to surpass the effectiveness of **2**. The most potent of the analogues synthesized thus far is *N*-nonanoyl vanillylamide (**1**, fig 1). In view of the pharmacological profile similar to that of **2**, **1** is used in substitution of **2** in experimental studies and pharmacological tests [8], and is an active ingredient in skin irritant preparations used for rheumatic disorders. It is available today both commercially and in reagent-grade purity.

Most of the analogues of **2** have been derived from modifications of either the acylamide linkage or the alkyl chain, and a few have been derived from alterations of the aromatic ring [7]. Some observations have pointed to the central role of the phenolic hydroxyl group in the bioactivity of these analogues. However, to date very few derivatives have been synthesized by replacing the phenolic hydroxyl group with different hydrophilic substituents.

The aim of the present study is to synthesize an intravenously activated, non-pungent antinociceptive, hypotensive analogue of **2** that shows less acute and cardiac toxicity than that of capsaicin or of its analogue, **1**. To this end a series of ether analogues were prepared by introducing side chains to the

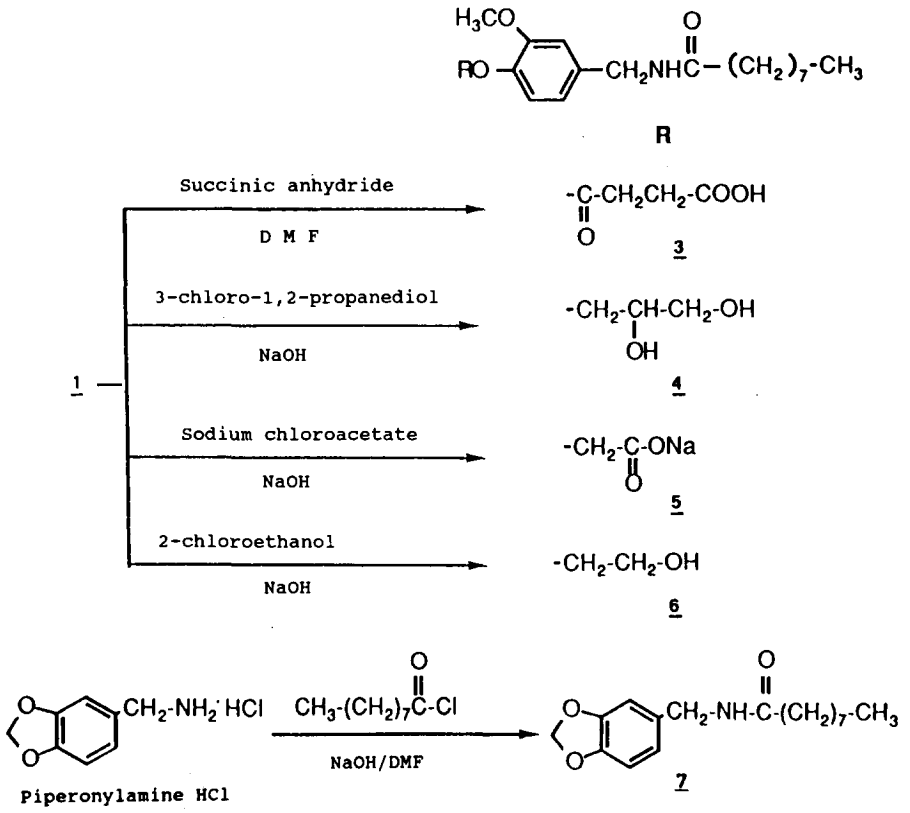


Nonivamide ; nonanoyl vanillylamide (NVA;1)



Capsaicin ; 8-methyl-N-vanillyl-nonenamide (CAP;2)

Fig 1. Chemical structures of nonivamide and capsaicin.



Scheme 1. Synthesis of capsaicin analogues.

Table I. Blood pressure response to intravenous injection of capsaicin and its analogues in anesthetized rats.

Compd[a]	*Blood pressure (mm Hg)*[b]		*Duration*	*Phase*	*Vagus*
	Before	*After*[c]	*(min)*		*reflex*[d]
CAP	118.2 ± 3.5	63.8 ± 4.4[e]	65.3 ± 13.3	3	45.0 ± 17.6
1	111.2 ± 6.0	61.8 ± 10.0[e]	53.2 ± 10.5	3	52.1 ± 16.6
3	121.8 ± 6.9	68.2 ± 11.4[e]	51.5 ± 6.5	3	48.5 ± 20.1
4	138.0 ± 13.0	91.7 ± 17.8[e,f]	7.0 ± 0.8[g,h]	1	nr
5	129.4 ± 7.4	108.2 ± 9.3[e,f]	11.2 ± 3.7[g,h]	1	nr
6	110.3 ± 6.4	68.3 ± 16.6[e]	43.5 ± 4.6[g]	1	nr
7	125.8 ± 16.4	115.1 ± 17.3[e,f]	0.9 ± 0.5[g,h]	1	nr

[a]Single dose of 0.1 mg kg^{-1} was administered for each compound. [b]Data are means ± SD (n = 6). [c]Data are values of the delayed fall in blood pressure. [d]Data are values of the initial fall in blood pressure. nr = no response. [e]Significant difference between 'Before' and 'After', $P < 0.05$ (Student's paired t-test). [f]Significant difference between 'Before' and 'After' as compared relative to capsaicin, $P < 0.05$ (Student's t-test). [g]Significant difference as compared to CAP, $P < 0.05$ (Student's t-test). [h]Significant difference as compared to compound **1**, $P < 0.05$ (Student's t-test).

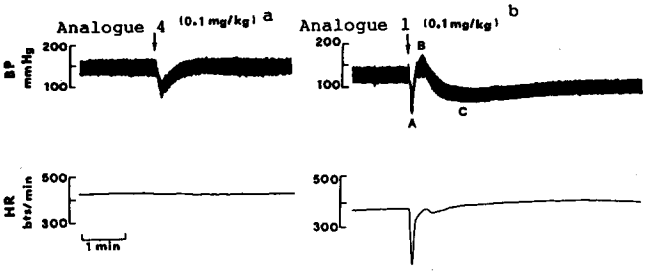


Fig 2. Typical records of systemic blood pressure (BP) and heart rate (HR) following iv injection of analogue **4** and **1** in anesthetized rats. [a]Similar patterns also shown for BP changes induced by analogue **5**, **6** and **7**, all of which were shown to be uninhibited by the pretreatment of atropine (1.0 mg kg^{-1}, ip) and bilateral vagotomy. [b]Similar patterns also shown for BP changes induced by analogue **3**. The initial fall (phase A) of triphasic BP changes was inhibited by the pretreatment of atropine (1.0 mg kg^{-1}, ip) and bilateral vagotomy.

phenolic hydroxyl group in position 4 on the aromatic ring (scheme 1). Each compound was tested for irritant and antinociceptive activities in mice, and for blood pressure changes and untoward vagus reflex in the cardiovascular system of the rat.

Chemistry

Ether analogues **4**, **5** and **6** were obtained by reacting **1** with 3-chloro-1,2-propanediol, sodium chloroacetate, and 2-chloroethanol respectively in basic medium. The ester **3** was synthesized by treating **1** with succinic anhydride in DMF, while compound **7** was prepared from piperonylamine HCl and nonanoyl chloride.

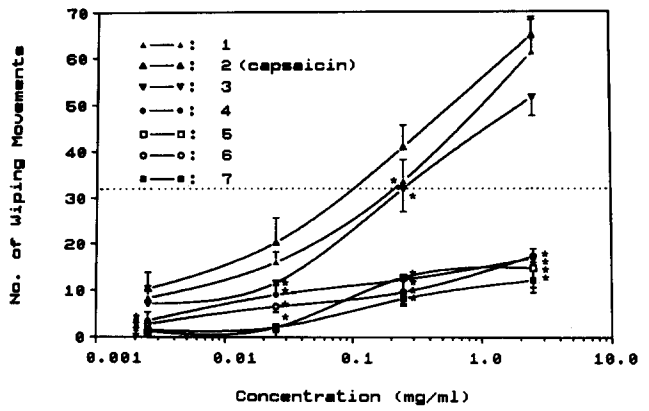


Fig 3. Dose–response curves plotted on the basis of the number of protective movements in response to instillation of solutions of capsaicin and its analogues into the eye of rats. Each value is the means ± SD of 6 experiments. The dotted line indicates the median response induced by capsaicin. *Significantly different from capsaicin, $P < 0.05$.

Table II. The relative pungent potencies of capsaicin and its analogues as measured by the wiping test. Methods are described by Szolcsányi and Jancsó-Gábor [4]. MPP = moderate pain-producing potency. RPP = relative pain-producing potency. –: Relatively non-pungent as compared to capsaicin (no of wiping movements are far less than the median scratchings induced by capsaicin throughout the concentrations tested; see also figure 3).

Compound	*MPP (mg/ml)*	*RPP*
Capsaicin	0.09	1000
1	0.22[a]	409
3	0.27[a, b]	333
4	–	–
5	–	–
6	–	–
7	–	–

[a]Significantly different as compared to capsaicin, $P < 0.05$, $n = 8$ (Student's t-test). [b]Non-significantly different as compared to compound **1**, $P > 0.05$, $n = 8$ (Student's t-test).

Pharmacological results

The data in table I indicate that all compounds significantly decreased blood pressure in the rat. Of these compounds, **7** was the least effective. Bradycardia and apnea, resulting from vagus reflex, occurred only with **1–3**. Vagus reflex was not observed with **4–7**. Thus, while **1**, **2** and **3** exhibited triphasic blood pressure changes, ether analogues of **1** (**4–6**) showed only monophasic blood pressure change. Pretreatment of rats with atropine (1.0 mg kg^{-1}, ip) inhibited the hypotensive vagus reflex of **1–3**, but did not inhibit the hypotensive effect of **4–7** (fig 2).

The pungent potency of the reference compounds **1**, **2** and of derivatives **3–7** was assessed in rats by the wiping test, according to the method of Szolcsányi and Jancsó-Gábor [5]. The results indicate that all but one of the analogues are non-pungent in comparison to **1** and **2** (**3** showed almost the same pungency as **1**) (fig 3 and table II).

All mice treated with these compounds showed a dose-related decrease in writhing counts induced by acetic acid. Indomethacin was used as reference drug. Writhing was most pronounced for 30 min following the administration of acetic acid, gradually subsiding after having reached a peak in the first 10–15 min. The ED_{50} values (table III) clearly show analogue **5** to be the most potent in its antinociceptive effect.

Discussion

The irritant and hypothermic effects of **2** were first noted by Hayes *et al* [4]. The irritant effect of **2** may result from the release of substance P from the central terminals of primary afferent fibres in the dorsal horn of the spinal cord [6, 9, 10]. Hypothermia is probably due to peripheral vasodilation initiated by the release of substance P from peripheral terminals of primary afferent fibres *via* the axon reflex collaterals [11].

It has been shown that even a minor chemical modification of the substituents on the aromatic ring of **2** can result in a marked reduction of pain-producing potency, and may also lead to a complete loss of antinociceptive and hypothermic activities [4, 5].

It has been proposed by Szolcsányi and Janscó-Gábor that the 3-methoxy,4-hydroxy phenyl ring is essential to the pungency of the capsaicin molecule. These authors also observed that replacement of the free phenolic OH group may lead to marked decrease, or loss of pungency [5, 6]. This finding is supported by the results of the present study, which demonstrated that the pungency of **3** is nearly equivalent to that of **1** and **2**, while analogues **4–7** are relatively non-pungent. In the case of **3**, its pungent and hypotensive activities, very similar to those of **1**, suggested a metabolic cleavage of the ester to the parent compound. This hypothesis is consistent with the observed cleavage of **3** into **1** and succinic acid in water.

Table III. Antinociceptive effects of capsaicin analogues on acetic acid-induced writhing in mice. Writhes were counted for 30 min after injection of acetic acid (ip), see details in text. ED_{50} and 95% confidence limits were calculated by the Litchfield and Wilcoxon method (run using Basic program in IBM PC-AT).

Compound	*ED_{50} (95% CL) (mg/kg)*	*Potency ratio*
Indomethacin	1.10 (2.57–0.47)	1.00
1	0.08 (0.42–0.02)	13.75
2	0.07 (0.33–0.02)	15.71
3	0.06 (0.30–0.01)	18.33
4	0.09 (0.38–0.02)	12.22
5	0.04 (0.13–0.01)	27.50
6	0.14 (0.43–0.04)	7.86
7	0.15 (0.48–0.04)	7.33

Intravenous administration of the analogues **4–7** showed only monophasic hypotension, indicating that modification of the free phenolic OH of **1** or **2** with ether linkage not only reduced the pungent activity but also abolished the untoward vagus reflex hypotensive effect as previously found in **1** or **2**. Dose–response of blood pressure and thoracic aorta relaxation will be described in a later report, in which the mechanisms of action of these compounds will also be discussed.

Literature data on the analgesic effects of **2** and analogues have been found to vary, depending upon test animals and methods used [4–6, 13, 14]. In the present study, the acetic acid-induced writhing syndrome of mice was used to evaluate the antinociceptive effects of **1–7**. The ED_{50}s ranged from 0.04 to 0.15 mg kg^{-1}, and were markedly lower than that of indomethacin, a positive control in this test.

The antinociceptive mechanism of the analogues synthesized in this study is still unknown. However, since a positive correlation has been found between pain-producing potencies and immunoreactive substance P (I-SP) releasing potencies of capsaicin-type compounds [15], the acute antinociceptive effect of **2** could be attributable to a depletion of terminally stored substance P following the blockage of axoplasmic transport [12, 16]. This would suggest that the antinociceptive effects of these analogues may act on mechanisms other than those affected by **1** and **2**.

In conclusion, 4-phenolic ether-substituted analogues of **1**, *ie*, **4–7**, still retain marked antinociceptive and hypotensive effects without demonstrating overt irritant effects.

Experimental protocols

Chemistry

All melting points were determined in a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The putative structures of all compounds were supported by data derived from infrared (IR) and nuclear magnetic resonance (NMR) spectra. IR spectra were determined with the Hitachi Model 260–30 recording spectrometer, and NMR spectra were recorded on a Varian T–200 spectrometer. Mass spectra were determined on a Jeol JMS–HX 110 mass spectrometer. Compound **1**, sodium chloroacetate, 3-chloro-1,2-propanediol, 2-chloroethanol and pipronylamine HCl were all obtained from

Tokyo Chemical industry Co (TCI). Succinic anhydride and sodium hydroxide were products of E Merck, capsaicin (98% pure) and indomethacin were products of Sigma Co. All other reagents used in this study were EP grade products of E Merck.

N-(4-O-succinic acid-3-methoxybenzyl)-nonamide (N-nonanoyl vanillylamide-4-succinyl ester 3)
A solution in DMF (10 ml) of **1** (2.00 g, 6.82 mmol) and succinic anhydride (0.8 gm, 8.0 mmol) in a 3-necked flask, was heated at 100°C for 1 h, then diluted with ethyl acetate (20 ml) and extracted with 20 ml of sodium hydroxide solution (0.5%) in a separatory funnel. The lower layer was acidified with conc HCl to pH 4.0 and extracted with ethyl acetate. The organic layer was then separated and evaporated to dryness under reduced pressure. The residue crystallized from benzene to give **3** (1.3 g, 48%) as colorless needles : mp 104–106°C. UV λ_{Max}^{MeOH} nm (log ε): 275 (3.49), 279 (3.48). NMR ($CDCl_3$) δ: 0.87 (t, 3H, CH_3), 1.25–2.25 (m, 14H, CH_2 x 7), 2.77–2.90 (m, 4H, CH_2 x 2), 3.75 (s, 3H, OCH_3), 4.34 (d, 2H, Ar-CH_2), 6.32 (s, lH, NH), 6.76–6.97 (m, 3H, Ar), 9.92 (br s, lH, COOH); IR (KBr): 1698, 1700 cm^{-1}; MS *m/z* 394 $(M+H)^+$. Anal $C_{21}H_{31}NO_6$ (C, H, N).

N-(4-O-glycerol-3-methoxybenzyl)-nonamide (N-nonanoyl vanillylamide-4-glyceryl ether 4)
A mixture of **1** (2.64 g, 9.0 mmol), 3-chloro-1,2-propanediol (1 ml) and 30% NaOH (1.2 ml), was heated at reflux for 1 h. After cooling, conc HCl was added until pH 4.0, the inorganic salt filtered off and the filtrate diluted with methanol. The solid separated was recrystallized from benzene to afford **4** (2.1 g, 64%) as colorless needles: mp 133°C. UV λ_{Max}^{MeOH} nm (log ε): 227.3 (4.57), 275.6 (3.90). NMR ($CDCl_3$) δ: 0.86 (m, 3H, CH_3), 1.25–2.22 (m, 14H, CH_2 x 7), 2.37 (t, lH, CHOH), 3.05 (s, lH, CH_2OH), 3.79 (d, 2H, CH_2OH), 3.84 (s, 3H, OCH_3), 4.05 (d, 2H, OCH_2CH), 4.15 (t, lH, OCH_2CH), 4.37 (d, 2H, Ar-CH_2), 5.8 (s, lH, NH), 6.80-6.87 (m, 3H, Ar); IR (KBr): 3280, 1640, 1020, 1150 cm^{-1}; MS *m/z* 367 $(M)^+$. Anal $C_{20}H_{33}NO_5$ (C, H, N).

N-(4-O-acetic acid sodium-3-methoxybenzyl)-nonamide (sodium N-nonanoyl vanillylamide-4-O-acetate 5)
Sodium chloroacetate (0.80 g, 9.76 mmol) was added to a solution of **1** (2.00 g, 6.82 mmol) in 30 ml 0.4 M NaOH. The mixture was heated at reflux for 2 h, concentrated under reduced pressure and the residue was crystallized from absolute ethanol to afford **5** (1.2 g, 44%) as colorless needles : mp 159–160°C. UV λ_{Max}^{MeOH} nm (log ε): 231 (4.25), 280 (3.92). NMR (DMSO–d_6) δ: 0.79–0.96 (t, 3H, CH_3), 1.24–2.17 (m, 14H, CH_2 x 7), 3.74 (s, 3H, OCH_3), 4.07 (s, 2H, OCH_2O), 4.13–4.19 (d, H, Ar-CH_2), 6.72–6.82 (d, 3H, Ar), 8.21 (s, lH, NH); IR (KBr): 1520, 1580, 1600, 1640, 3300 cm^{-1}; MS *m/z* 396 $(M+Na)^+$. Anal $C_{19}H_{28}NO_5Na{\cdot}2H_2O$ (C, H, N, O).

N-(4-O-glycol-3-methoxybenzyl)-nonamide (N-nonanoyl vanillylamide-4-glycol ether 6)

In a 3-necked flask a mixture of **1** (2.00 g, 6.82 mmol), 0.46 ml 2-chloroethanol and 1 ml 30% sodium hydroxide was heated at 100°C for 1 h. Following the procedure reported for **4** a product was isolated which crystallized from benzene to give 1.4 g (61%) of **6** as colorless needles: mp 116–117°C. UV λ_{Max}^{MeOH} nm (log ε): 230 (4.05), 279 (1.83). NMR ($CDCl_3$) δ: 0.84 (m, 3H, CH_3), 1.23–2.18 (m, 14H, CH_2 x 7), 2.27 (s, lH, OH), 3.82 (s, 3H, OCH_3), 3.89 (t, 2H, CH_2OH), 4.08 (t, 2H, OCH_2), 4.33 (d, 2H, Ar-CH_2), 5.77 (s, lH, NH), 6.79 (m, 3H, Ar); IR (KBr): 3280 cm^{-1}; MS *m/z* 337 $(M)^+$. Anal $C_{19}H_{31}NO_4$ (C, H, N)

N-(3,4-methylenedioxy benzyl)-nonylamide (N-nonanoyl piperonylamide 7)
Ten grams (0.053 mol) of piperonylamine HCl was dissolved by heating in 50 ml DMF, then 10 ml of nonanoyl chloride sodium hydroxide were added under stirring. After heating at 100°C for 2 h, the mixture was filtered and evaporated to dryness under reduced pressure. The residue was then chromatographed on a silica gel column using ethylacetate as the eluent. After evaporation of the solvent, the eluted product was crystallized from ethanol to give 6.2 g (40%) of **7** as colorless needles : mp 85.5–87.0°C. UV λ_{Max}^{MeOH} nm (log ε): 280.4 (4.05), 234.6 (4.67); 209.5 (4.69). NMR (DMSO–d_6) δ: 0.85 (t, 3H, CH_3), 1.23–1.78 (m, 12H, CH_2 x 6), 2.1 (m, 2H, -CH_2-), 4.11–4.17 (d, 2H, CH_2-NH), 5.96 (s, 2H, -O-CH_2-O-), 6.73–6.79 (m, 3H, Ar), 8.20 (t, lH, -CH_2-NH-C-); IR (KBr): 1258, 1650, 3320 cm^{-1}; MS *m/z* 291 $(M)^+$. Anal $C_{17}H_{25}NO_3$ (C, H, N).

Pharmacology

Measurement of blood pressure
Male Wistar rats, weighing 250–300 g were anesthetized with sodium pentobarbital (50 mg kg^{-1}, ip). Following tracheal cannulation, systemic arterial blood pressure and heart rate were recorded from the femoral artery with a pressure transducer (GOULD, Model P50). Body temperature was maintained at 37°C. The stock solutions (10 mg ml^{-1}) of compound **1**, **2**, **4**, **6** and **7** were prepared with the aid of 10% ethanol and 10% Tween 80, and appropriate dilutions were made up with saline. Compounds **3** and **5** were dissolved in Tween 80 (1%) and propyleneglycol (5%) and diluted with normal saline. A femoral vein was cannulated for iv injections.

Evaluation of antinociceptive effects
Following the method described by Koster [17], antinociceptive tests were carried out in male mice after intraperitoneal administration of **1** and its analogues. Briefly, 4 groups of 8 male mice (ddk strain) weighing 18–22 g were brought to the laboratory on the day prior to study, and housed overnight with free access to food and water. Solutions of **1** and its analogues, as well as indomethacin were made up in 10% ethanol, 10% Tween 80 and 80% saline, and then diluted with saline to the required concentrations. The test solution was administered by intraperitoneal injection with single dose of 0.2 ml (vehicle administered as control). Twenty min after injection, 0.2 ml of 0.7% acetic acid was injected intraperitoneally to induce writhing. Following injection, the mice were placed in separate clear glass cages and the number of writhes was counted for 18 consecutive 5-min periods beginning 5 min after acetic acid injection, a writhe being defined as a sequence of arching of the back, followed by pelvic rotation and hind limb extension.

Evaluation of the potency of pungency
The wiping test was performed as described by Szolcsányi and Jancsó–Gábor [5]. Briefly, solutions of **2** and its analogues were prepared as described above in successive 10-fold dilutions. Each dilution was dropped into the right eye (vehicle being administered to the left eye as negative control) of male Wistar rats weighing 180–250 g, and the total number of protective movements (scratching, wiping of the eye with the foreleg) was counted for 30 min. Each concentration was

applied to a total of 6 rats, and a dose–response curve were obtained from the mean value of each group. MPPs (the concentrations having a moderate pain-producing potency) were calculated from the dose-response curve and those concentrations inducing equal reactions of 32 scratchings (the median response induced by **2**; fig 3) were recorded. On the basis of the MPP values thus obtained, RPP (relative pain-producing potency) values were determined with respect to the pain-producing potency of **1**, which was taken as 1 000.

Statistical methods

Student's *t*-test and paired *t*-test were used to establish significant differences in the experiments. ED_{50}s were estimated by the Litchfield and Wilcoxon method [18].

Acknowledgments

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