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Antinociceptive properties of *N*-aryl-glutaramic acids and *N*-aryl-glutarimides

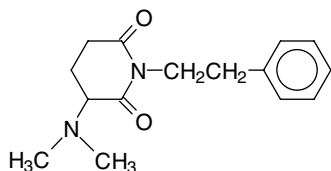
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This study describes the antinociceptive activity of some *N*-aryl-glutaramic acids and *N*-aryl-glutarimides in writhing and formalin tests, two classical models of pain in mice. These compounds show high activity, being more active than acetyl salicylic acid, acetaminophen and indomethacin, used as standard drugs for comparison. The introduction of different substituent groups in the aromatic ring caused a significant change in activity. The results obtained here are promising from a pharmacological point of view, since these simple compounds might be used as models to obtain new and potent analgesic drugs.

1. Introduction

Phyllanthimide is a naturally occurring alkaloid, which was isolated from the aerial parts of *Phyllanthus sellowianus* (Euphorbiaceae) [1] being one of the active compounds responsible for the antispasmodic effects of this plant [2]. We used this compound as a model and synthesized several analogues, especially maleimide and succinimide derivatives and determined their different kinds of activities, such as antispasmodic [3, 4], analgesic [5–8], antibacterial [9–13] and antifungal [14, 15] effects.

As part of our research programme to obtain new analgesic compounds structurally related to cyclic imides, we have now synthesized and evaluated the antinociceptive activity of *N*-aryl-glutaramic acids and *N*-aryl-glutarimides using the abdominal constriction test and formalin induced-pain test in mice.



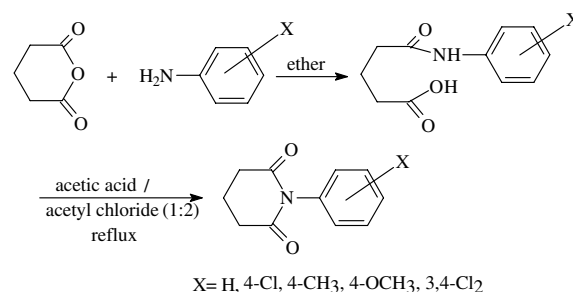
Molecular structure of phyllanthimide isolated from *P. sellowianus*

2. Investigations, results and discussion

We have recently demonstrated that some cyclic imides, including succinimides, maleimides, naphthalimides and related compounds, exhibited antinociceptive properties when tested against acetic acid-induced writhing in mice [5–8]. Since current analgesia inducing drugs (opiates, NSAIDS) are not useful in all cases, especially because of their side effects and potency [16], this field remains open to the discovery of new and better analgesic drugs. Thus, in order to discover substances with potential analgesic, some glutaramic acid and glutarimides derivatives were obtained by simple reactions, as shown in the Scheme.

Several methodologies were employed for the dehydration of amic acids into the respective glutarimide derivatives, such as treatment with hot acetic anhydride/sodium acetate, acetic acid under reflux or acetic acid with increasing amounts of acetyl chloride under reflux. The best experimental condition proved to be the use of acetic acid and acetyl chloride (1:2). Maleamic acid derivatives, which possess a double bond in the imido ring of five members and consequently take a planar configuration, may be eas-

Scheme

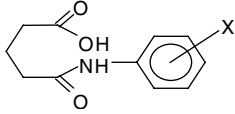


ily cyclized to maleimides using the reagents mentioned above and other condensation agents, such as phosphorus pentoxide, thionyl chloride, etc. [17, 18]. In contrast, glutaramic acids, which possess an imido ring of six members are not easily closed, due some physico-chemical factors [19]. According to Eliel [20], the ease of formation of a six-membered ring is less than for a five-membered one because the slight improvement in the strain factor is outweighed by a deterioration in the distance factor.

The substituent groups introduced in the aromatic ring (4-CH₃, 4-OCH₃, 4-Cl, 3,4-Cl₂) were chosen based on the Topliss method [21] which enables the prediction of new synthetic routes to obtaining more potent compounds. However, this method will be used in a further work.

Tables 1 and 2 show the antinociceptive activity of *N*-aryl-glutaramic acids in writhing and formalin tests at 10 mg/kg, given intraperitoneally. For comparison, we have included the data of some well-known non-steroidal antiinflammatory and analgesic drugs, which were evaluated in the same experimental procedures. As can be seen, all the compounds significantly inhibited the abdominal contractions, causing considerable inhibition (>45%), these being more active than standard drugs, which inhibited the abdominal contractions by 35 (ASA) and 38% (acetaminophen).

When evaluated against formalin-induced pain in mice at 10 mg/kg, i.p., all acids, except **3**, showed marked antinociceptive effects in relation to both phases of formalin induced-pain (neurogenic and inflammatory). They were considerably more efficacious than indomethacin, which produced inhibition of 33%. In contrast, compound **3** inhibited the first but not the second phase of the formalin test. This suggests that it may be acting by a mechanism of action different from non-steroidal antiinflammatory

Table 1: Antinociceptive effect of *N*-aryl-glutaramic acids, acetyl salicylic acid and acetaminophen against acetic acid-induced abdominal constriction in mice


Compd.	X	Number of constrictions	Inhibition (%)
Control	—	36.0 ± 3.7	—
1	H	12.8 ± 3.7	64.4
2	4-CH ₃	10.2 ± 3.9	71.6
3	4-OCH ₃	21.5 ± 3.9	40.3
4	3,4-Cl ₂	18.8 ± 2.8	47.8
5	4-Cl	19.6 ± 4.9	45.5
ASA (6)	—	23.4 ± 2.0	35.0
ACE (7)	—	22.3 ± 1.0	38.0

Each group represents the mean ± s.e.m. of 6–8 experiments; compounds were given intraperitoneally in a dose of 10 mg/kg. ASA = acetyl salicylic acid; ACE = acetaminophen

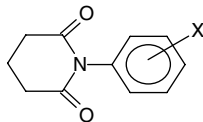
Table 2: Antinociceptive effect of *N*-phenyl-glutaramic acids and indomethacin against formalin induced-pain in mice

Compd.	First phase ^a	Inhibition (%)	Second phase ²	Inhibition (%)
Control	72.0 ± 8.2	—	133.0 ± 17.3	—
1	42.8 ± 7.6	40.8	38.5 ± 6.4	71.0
2	47.3 ± 10.8	34.3	54.8 ± 6.7	58.8
3	27.5 ± 3.9	61.8	123.7 ± 11.2	7.0
4	16.2 ± 2.4	77.5	22.8 ± 8.2	82.9
5	24.0 ± 3.9	66.7	56.0 ± 8.3	58.0
Indo-methacin	67.2 ± 1.0	6.6	89.1 ± 5.0	33.0

Each group represents the mean ± s.e.m. of 6–8 experiments; ^a 0–5 min licking (s); ² 15–30 min licking (s); * P < 0.05 compared with respective control values. Compounds were given intraperitoneally in a dose of 10 mg/kg

agents [22]. The effect produced in the first phase may be due to immediate and direct effects on sensory receptors, bradykinin receptors or glutamatergic way whereas for the last phase the antinociceptive effect is related to the inflammatory responses induced by the arachidonic acid cascade [23, 24].

Table 3 shows the antinociceptive activity of *N*-aryl-glutarimides against the writhing test. All compounds, except **11** (inactive), exhibited excellent effects being more active than their acids and standard drugs shown in Table 1. In the formalin model, these glutarimides inhibited both

Table 3: Antinociceptive effect of *N*-aryl-glutarimides against acetic acid-induced abdominal constriction in mice


Compd.	X	Number of constrictions	Inhibition (%)
Control	—	39.4 ± 1.9	—
8	H	13.0 ± 6.3	67.0
9	4-CH ₃	8.3 ± 2.7	78.9
10	4-OCH ₃	1.3 ± 0.5	96.7
11	3,4-Cl ₂	28.5 ± 5.9	27.6
12	4-Cl	5.0 ± 1.5	87.3

Each group represents the mean ± s.e.m. of 6–8 experiments; compounds were given intraperitoneally in a dose of 10 mg/kg

Table 4: Antinociceptive effect of *N*-phenyl-glutarimides against formalin induced-pain in mice

Compd.	First phase ^a	Inhibition (%)	Second phase ^b	Inhibition (%)
Control	75.0 ± 4.4	—	144.3 ± 8	—
8	33.5 ± 4.3	55.3	61.2 ± 12.0	57.6
9	36.0 ± 4.1	52.0	65.4 ± 3.5	54.7
10	51.0 ± 9.8	31.7	16.7 ± 9.9	88.5
12	33.8 ± 9.3	54.9	33.0 ± 8.8	77.1

Each group represents the mean ± s.e.m. of 6–8 experiments; ^a 0–5 min licking (s); ^b 15–30 min licking (s); * P < 0.05 compared with respective control values. Compounds were given intraperitoneally in a dose of 10 mg/kg

neurogenic and inflammatory phases of pain, with results (Table 4) similar to those of their respective acids (Table 2). Surprisingly, compound **10** caused a marked inhibition in relation to the second phase of the formalin test (88.5% of inhibition), in contrast to its acid **3**, which was active only against the first phase of this experimental model.

Although several studies have demonstrated different kinds of biological properties for glutarimide and related compounds [25], only a few papers reported the analgesic effects of these compounds [26, 27].

The results obtained in our study are promising from a pharmacological point of view, since these simple compounds might be used as leads to obtain new and potent analgesic drugs. Presently pharmacological studies are in progress to confirm the antinociceptive properties of the imides reported here in other models of pain as well as to elucidate the mechanism of action of the more active compounds.

3. Experimental

3.1. Synthesis: general procedures

Compounds **1–5** were obtained by reaction of glutaric anhydride with an appropriate aniline in ether. The dehydration of the corresponding glutaramic acid by treatment with acetic acid/acetyl chloride 1:2 under reflux (2 h) gave compounds **6–10**. All the compounds were synthesized in moderate to good yields (40–90%) and characterized by ¹H NMR, IR and microanalysis. The purity of the tested substances was determined by TLC using several solvent systems of different polarity. Spots were visualized by short-wave UV light and iodine vapor.

3.2. Pharmacological analysis: evaluation of antinociceptive activity

3.2.1. Abdominal constriction response caused by intraperitoneal injection of diluted acetic acid

Abdominal constriction was induced by intraperitoneal injection of acetic acid (0.6%), according to the procedures described previously [28, 29] with minor modifications. Animals (male Swiss mice, 25–30 g) were pretreated with the compounds or standard drugs intraperitoneally (10 mg · kg⁻¹) 30 min before the acid acetic injection. Control animals received a similar volume of 0.9% NaCl (10 ml · kg⁻¹, i.p.). All experiments were carried out at 23 ± 2 °C. After challenge, pairs of mice were placed in separate boxes and the number of constrictions of the abdominal muscles, together with stretching, were cumulatively counted over a period of 20 min. Antinociceptive activity was expressed as the reduction of the number of abdominal contractions comparing control animals and mice pretreated with compounds or standard drugs.

3.2.2. Formalin-induced pain

The procedure used was essentially similar to that previously described [22, 30]. Animals (male Swiss mice, 25–30 g) from the same strain were slightly anaesthetized with ether, except when used to analyse the first phase of formalin-induced pain, and 20 µl of 2.5% (0.92% formaldehyde) made up PBS (phosphate buffered solution, containing: NaCl 137 mM; KCl 2.7 mM and phosphate buffer 10 mM) was injected s.c. under the plantar surface of the left hindpaw with a Hamilton syringe. Animals were acclimatized to the laboratory for at least 24 h before experiments. Two mice (control and treated) were simultaneously observed from 0 up to 30 min following formalin injection. The initial nociceptive scores nor-

mally peaked after 5 min (first phase, representing the neurogenic pain), and 15–30 min after formalin injection (second phase, representing the inflammatory pain) [22]. Animals were treated with saline 0.9% (10 ml/kg, i.p.), with the compounds or with indomethacin (10 mg/kg, i.p.) 60 min before formalin injection. After intraplantar irritant application, the animals were immediately placed into a glass cylinder (20 cm diameter). The time spent by animals licking or biting the injected paw was timed with a chronometer and was considered indicative of pain.

3.2.3. Statistical analysis

The pharmacological results are presented as mean \pm s.e.m., and the statistical significance between groups was analysed by means of the t test or analysis of variance followed by Dunnett's multiple comparison test, when appropriate. P values less than 0.05 were considered as indicative of significance.

Acknowledgements: This work was supported by grants from CNPq and ProPPEX/UNIVALI.

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Received February 21, 2000

Accepted April 5, 2000

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