Antinociceptive Effects of Tetrahydrophthalimides and Related Compounds

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This paper describes the antinociceptive effects of tetrahydrophthalimides and related compounds in mice. Twenty compounds were obtained by the reaction of *cis*-1,2,3,6-tetrahydrophthalic anhydride with appropriate amines, dehydration, and addition to the imidic double bond. They were analyzed in the writhing test at 10 mg/kg given intraperitoneally. The most active compound 2-benzyl-5-morpholin-4-yl-hexahydroisoindole-1,3-dione (**19**) was studied on formalin, capsaicin, glutamate and hot plate models. The antinociceptive activity demonstrated by some studied compounds is promising, and some of them were more active than acetylsalicylic acid and paracetamol used as reference drugs in writhing tests in mice. Compound **19** was about 5-fold more potent than the reference drugs, being also effective by oral route and against the inflammatory response in the formalin test. The results suggest that compound **19** could be used as a model to obtain new and more potent antinociceptive agents. It exhibits an interesting antinociceptive profile, and does not interact with opioid systems.

Key words: Antinociceptive Effects, Tetrahydrophthalimides, Mice

Introduction

Cyclic imides are an important class of compounds due to their variety of biological properties and reactional versatility (Hargreaves *et al*., 1970; Cechinel-Filho *et al*., 2003). Our research group has focused the attention to these compounds since the discovery of phyllanthimide, an alkaloid present in low concentration in the active aerial parts of *Phyllanthus sellowianus* (Tempesta *et al*., 1988). Using this compound as a model, we have synthesized a great number of compounds belonging to the different sub-classes of cyclic imides, including maleimides, 3,4-dichloromaleimides, succinimides, glutarimides, naphthalimides, among others, and determined their different kinds of biological activities, such as antispasmodic, antibacterial, antifungal, and analgesic or antinociceptive effects (Cechinel-Filho *et al*., 2003; Campos-Buzzi *et al*., 2003; Lopez *et al*., 2003; Prado *et al*., 2004).

Extending our research program related to the biological properties of cyclic imides, we have now synthesized twenty new tetrahydrophthalimides and related compounds and evaluated initially their antinociceptive action using the writhing test in mice at 10 mg/kg by the intraperitoneal route. The most active compound was analyzed using other classical and specific models of pain in order to confirm its antinociceptive effect as well as to investigate the possible mechanism of action. The effects of acetylsalicylic acid and paracetamol were included in this study with the purpose of comparison.

Materials and Methods

Chemistry: general procedures for the synthesis of studied compounds

Compounds **1**–**6** were obtained by the reaction of *cis*-1,2,3,6-tetrahydrophthalic anhydride with appropriate amines in ether, which were dehydrated by the treatment with acetic acid/reflux to give the respective cyclic compounds **7**–**18** according to a methodology previously described (Scheme 1) (Cechinel Filho *et al*., 1994; Costa, 2004). Compounds **19** and **20** were obtained directly from compound **14** by the reaction with morpholine and piperidine in benzene, respectively (Costa, 2004). The purity of the synthesized substances was monitored by thin-layer chromatography (tlc) using silica precoated plastic plates,

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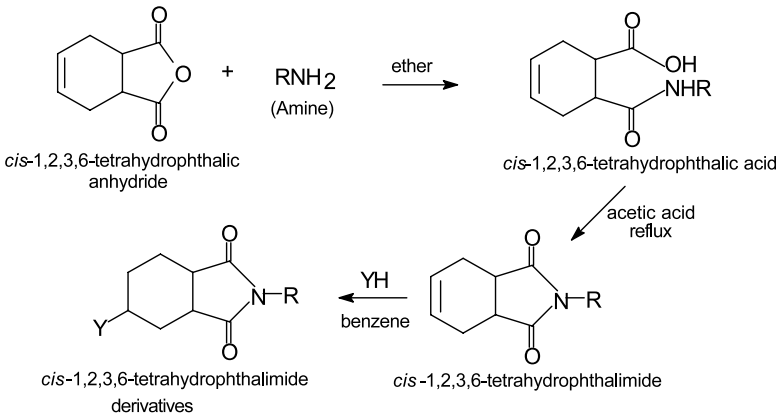

Scheme 1. Synthesis of compounds studied.

200 μm in thickness (Sigma Chemicals, St. Louis, USA), with several solvent systems of different polarities. Spots were visualized by short-wave UV light and iodine vapor. Spectral data (IR, ^{1}H and ^{13}C NMR) and elemental analyses were in good agreement (Costa, 2004) with the structures shown in Fig. 1.

Animals

Swiss male mice (25–30 g) were obtained from Central Bioterio of the University of Vale do Itajaí (Itajaí, Brazil). They were kept in a temperature-controlled environment [(23 ± 2) °C] with a 12 h light-dark cycle. Food and water were freely available. The allocation of animals in the different groups was randomized and the experiments were carried out in blind conditions. Since some suffering might result from experiments, the IASP's Committee for Research and Ethical Issues Guidelines (Zimmermann, 1983) were followed.

Drugs and reagents

The following drugs and reagents were used: ASA (acetylsalicylic acid), acetaminophen, indomethacin and PBS (phosphate buffered saline) (all from Sigma Chemical), formalin and acetic acid (Merck, Darmstadt, Germany). The compounds studied as well as the reference drugs were dissolved in Tween 80 (Merck) plus 0.9% of NaCl solution and 0.5% carboxymethylcellulose plus 0.9% of NaCl solution, respectively. The final content of Tween and ethanol did not exceed 5% and did not cause any effect *"per se"*.

Abdominal constriction response caused by injection of acetic acid

The abdominal constriction induced by intraperitoneal (i.p.) injection of acetic acid (0.6%), which consisted of a contraction of the abdominal muscle together with a stretching of hind limbs, was carried out according to previously described procedures (Collier *et al.*, 1968). Animals were pretreated with the compounds or standard drugs intraperitoneally (10 mg/kg) 30 min before the acetic acid injection. The dose was chosen considering previous results with this class of compounds. Compound **19**, the most active compound tested, was also analyzed intraperitoneally at 6, 8, 10 mg/kg or orally at 100 mg/kg. We have chosen here an oral dose about 10-times higher because of pharmacokinetics parameters. Control animals received a similar volume of 0.9% NaCl solution (10 ml/kg). All the experiments were carried out at 20–22 °C. After the challenge, pairs of mice were placed in separate boxes, and the number of abdominal constrictions was cumulatively counted over a period of 20 min. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with compounds or standard drugs.

Formalin-induced pain

The procedure was similar to that described previously (Hunskaar and Hole, 1987). Animals from the same strain were slightly anaesthetized with ether, except when used to analyze the first phase of formalin-induced pain, and 20 μl of 2.5% formalin

(0.92% formaldehyde) made up in phosphate buffer solution were injected under the paw surface of the right hindpaw. Two mice (control and treated) were observed simultaneously from 0 to 30 min following formalin injection. The time spent licking the injected paw was considered as indicative of pain. The initial nociceptive scores normally peaked 5 min (early phase) and 15–30 min after formalin injection (late phase), representing the tonic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). Animals were treated with compound **19** intraperitoneally at 10 mg/kg, 30 min before formalin injection. Following intraplantar injection of formalin, animals were immediately placed into a glass cylinder of 20 cm in diameter, and the time spent licking the injected paw (second phase of formalin test) was determined.

Capsaicin-induced pain

The procedure used was similar to that described previously (Sakurada *et al.,* 1993). Animals were placed individually in transparent glass cylinders. Following the adaptation period, 20 μl of capsaicin (1.6 μg/paw) were injected under the skin of the plantar surface of the right hindpaw, using a microsyringe. Animals were observed individually for 5 min following capsaicin injection. The time spent licking the injected paw was taken with a chronometer and considered as indicative of nociception. Animals were intraperitoneally treated with compound **19** at 10 mg/kg or saline (10 ml/kg, i.p.) 1 h before administration of capsaicin. Control animals received a similar volume of 0.9% NaCl (10 ml/kg, i.p.).

Glutamate-induced nociception

Animals were treated with compound **19** intraperitoneally (10 mg/kg) 30 min before glutamate injection. A volume of 20 μl of glutamate solution (30 μmol/paw), made up in phosphate buffered saline (PBS), was injected intraplantarly under the surface of the right hindpaw as described previously (Beirith *et al.*, 1998). After injection of glutamate, the animals were individually placed into glass cylinders of 20 cm in diameter and observed from 0 to 15 min. The time spent licking and biting the injected paw was taken with a chronometer and considered as indicative of pain.

Hot-plate test

The hot-plate test was used to estimate the latency of responses according to the method described by Eddy and Leimback (1953) with minor modifications. The temperature of the hot-plate was maintained at (56 ± 3) °C. Animals (n = 8) were placed on glass funnels on the heated surface and the time between placing the animals and the beginning of licking paws or jumping was recorded as latency of response in non-treated (saline 10 ml/kg, i.p.) or with compound **19** treated (10 mg/kg, i.p.) animals.

Statistical analysis

Results are presented as mean ± s.e.m., except the mean ID_{50} values (*i.e.* the dose of drugs or compounds reducing the algesic responses by 50% relative to the control value) which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance between groups was analyzed by variance followed by Dunnett's multiple comparison test. p-Values less than 0.05 were considered as indicative of significance. ID_{50} values were determined by graphical interpolation of individual experiments.

Results and Discussion

We initially synthesized the *cis*-1,2,3,6-tetrahydrophthalic acids from the reaction of *cis*-1,2,3,6-tetrahydrophthalic anhydride and appropriate amines in ether, and, after purification procedures, the amic acids were dehydrated to give the respective cyclic compounds (Costa, 2004) according to Scheme 1. The addition of piperidine and morpholine to the double bond of the imidic ring was carried out using different solvents, however, benzene furnished the most suitable profile. All the compounds were generally obtained in good yields (40–70%).

Thus, tetrahydrophthalic acids and tetrahydrophthalimide derivatives shown in Fig. 1 were tested by the writhing test at 10 mg/kg by the intraperitoneal route, whose results are indicated in Table I. As can be seen, some of them caused interesting antinociceptive effects, being more effective the reference drugs acetylsalicylic acid and paracetamol. All the synthetic process was conducted in order to obtain active compounds, according to Fig. 1. In some cases (compounds **1**–**4** and **7**–**11**) the substitution pattern suggested by Topliss (1977) was used for rational selection of phenyl substituents. However, significant differences between the parameters involved were not ob-

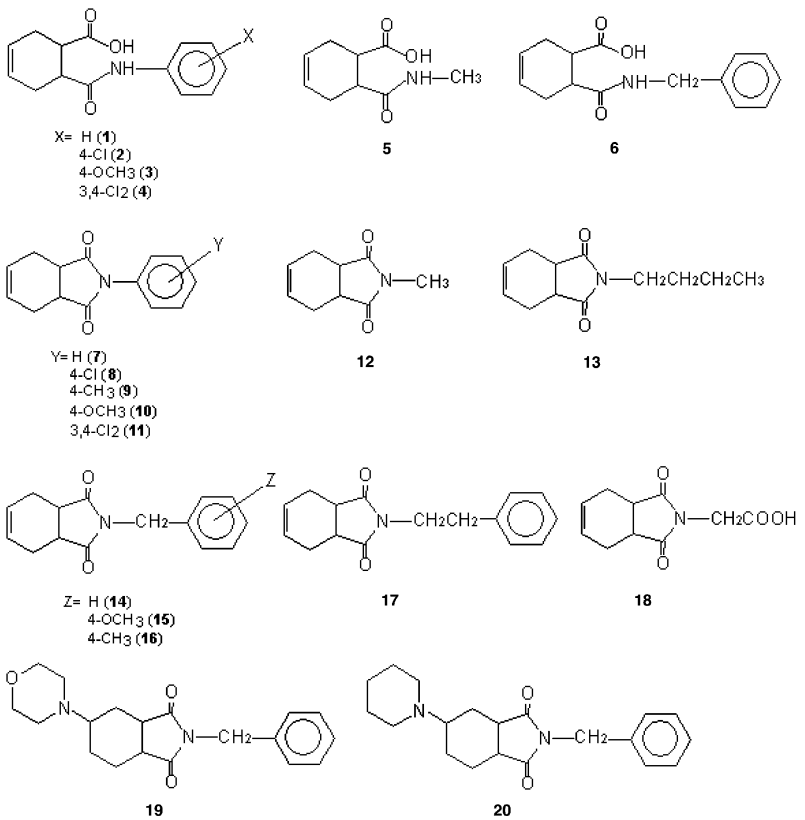

Fig. 1. Molecular structures of tetrahydrophthalic acids and tetrahydrophthalimide derivatives studied.

served. Compound **19**, which exhibited the most efficacy and reduced about 65% of the abdominal constrictions, was studied in more detail. Fig. 2 indicates that it dose-dependently inhibited the abdominal constrictions in the writhing test with an ID_{50} value of 8.1 (7.6–8.6) mg/kg [27.9 (26.2–30.0) μmol/kg], being about 5-fold more potent than the mentioned reference drugs.

Given orally, compound **19** was also effective in this model (writhing test), reducing by 64% the

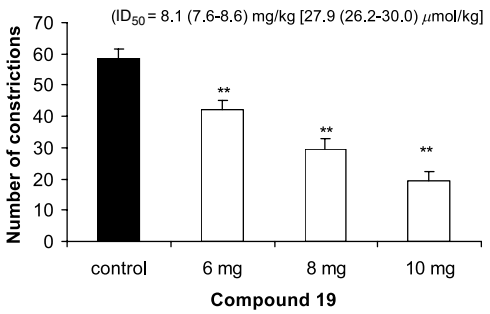

Fig. 2. Effect on acetic acid-induced pain in mice test of compound **19**, administered intraperitoneally at 6, 8 and 10 mg/kg. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$ compared with corresponding control value.

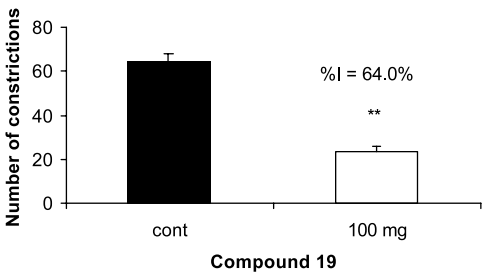

Fig. 3. Effect on acetic acid-induced pain in mice test of compound **19**, administered orally at 100 mg/kg. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$ compared with corresponding control value.

Table I. Antinociceptive activity of tetrahydrophthalamic acids, tetrahydrophthalimide derivatives and reference drugs against acetic acid-induced abdominal constriction (writhing test) in mice at 10 mg/kg, given intraperitoneally.

Compound	Inhibition (%)
1	10.8 ± 6.2
2	23.7 ± 7.2
3	48.7 ± 2.8**
4	26.7 ± 1.4
5	37.3 ± 4.5*
6	28.3 ± 10.5
7	15.8 ± 6.0
8	Inactive
9	36.4 ± 3.4*
10	40.4 ± 2.5**
11	50.9 ± 5.4**
12	17.7 ± 8.6
13	34.3 ± 8.8*
14	51.0 ± 5.4**
15	40.4* ± 3.5
16	33.3* ± 3.0
17	10.0 ± 4.8
18	32.9 ± 3.1*
19	65.4 ± 3.0**
20	30.0 ± 3.0
ASA	35.0 ± 2.0*
Paracetamol	38.0 ± 1.0**

Each group represents the mean ± s.e.m. of 5 to 7 experiments.
* $p < 0.05$; ** $p < 0.01$ compared with respective control values.
ASA, acetylsalicylic acid; Paracetamol, *N*-acetyl-*p*-aminophenol.

number of abdominal constrictions induced by acetic acid (Fig. 3), whereas acetylsalicylic acid and paracetamol were less active by this administration route (Vaz *et al.*, 1996). Such a result is important from a medical point of view, because it strongly suggests that compound **19** is absorbed from the gastrointestinal tract. Compound **20**, which presents the piperidino group instead of a morpholino group attached to the imidic ring, was less active in the writhing test at 10 mg/kg (i.p.) with a reduction of 30% of the abdominal constrictions. This indicates that the increase of hydrophilicity improves the antinociceptive effects. The oxygen atom present in the morpholino moiety contributes to the increased activity because of hydrogen bonding with different receptors.

When evaluated by the formalin-induced pain, compound **19** at 10 mg/kg (i.p.) was inactive against the first phase (neurogenic pain), but significantly inhibited (36.3%) the inflammatory response (second phase) (Fig. 4) with a very similar profile to those of acetylsalicylic acid and indomethacin reported in previous studies (Bresciani *et al.*, 2003).

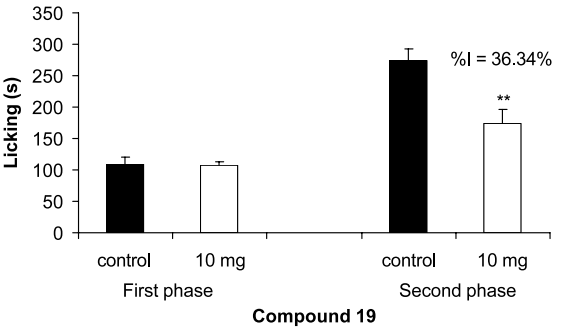

Fig. 4. Effect of compound **19**, administered intraperitoneally against formalin-induced pain in mice. Each column represents the mean ± s.e.m. of six experimental values. ** $p < 0.01$ compared with corresponding control value.

In the capsaicin test, **19** caused only a weak antinociceptive effect with inhibition of 21.5% (results not shown). This result is in agreement with that observed in the formalin test, since it provides more direct evidence of the antinociceptive action on neurogenic pain (Sakurada *et al.*, 1993). Another important finding, which confirms these results, was obtained in the hot-plate test and shows the lack of an antinociceptive effect of compound **19** at 10 mg/kg (i.p.) (results not shown). It is a technique that presents a selectivity for opioid-derived analgesics (Abbott and Franklin, 1986).

The results also demonstrated that compound **19** does not interact with excitatory amino acids, since it caused a weak inhibition (15%, 10 mg/kg, results not shown) of the hyperalgesia induced by intraplantar injection of glutamate.

Although additional studies are required to elucidate the exact mechanism of the antinociceptive action of compound **19**, the results verified in formalin, capsaicin and hot plate tests strongly suggest that it acts in a non-opioid pathway. Finally, the results show that **19** could be used as a model to obtain new and more potent antinociceptive agents.

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