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Further studies on analgesic activity of cyclic imides

Valdir Cechinel Filho [a,*], Rogério Corrêa [a], Zulma Vaz [b], João Batista Calixto [b], Ricardo José Nunes [c], Tânia Rosely Pinheiro [c], Adriano Defini Andricopulo [c], Rosendo Augusto Yunes [c]

[a] *Núcleo de Investigações Químico-Farmacêuticas (NIQFAR)/FAQFAR, Universidade do Vale do Itajaí (UNIVALI), 88302-202 Itajaí, SC, Brazil*

[b] *Department of Pharmacology, Universidade Federal de Santa Catarina (UFSC), 88040-900 Florianópolis, SC, Brazil*

[c] *Department of Chemistry, Universidade Federal de Santa Catarina (UFSC), 88040-900 Florianópolis, SC, Brazil*

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Abstract

As part of our research programme to obtain pharmacologically active compounds structurally related to cyclic imides, we have synthesized different compounds and examined their analgesic activities using the abdominal constriction test in mice. The results showed that some of the compounds studied, given intraperitoneally, exhibited graded and significant analgesia against acetic acid-induced abdominal constriction, being several times more potent than aspirin and paracetamol, two standard drugs used for comparison.

Keywords: Cyclic imides; Analgesia; Writhing test; Mice

1. Introduction

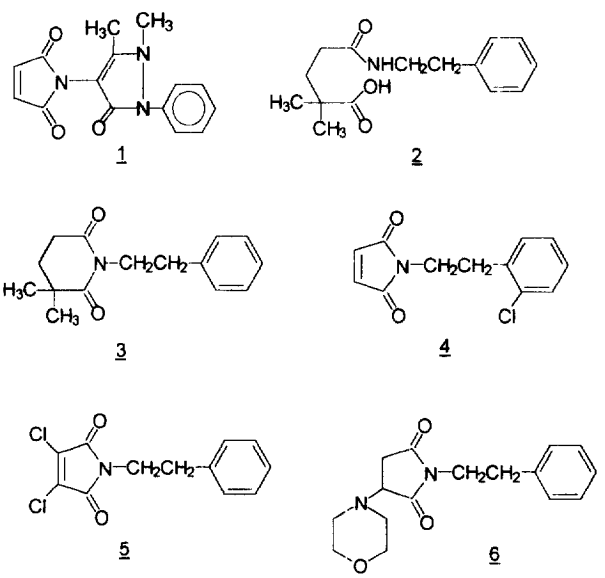
In some earlier studies, we reported the synthesis and biological activities of several succinimides and maleimides derivatives [1–8] which are analogues to phyllanthimide, an alkaloid isolated from *Phyllanthus sellowianus* [9].

In order to advance our extensive investigations about the chemical and biological aspects of cyclic imides, we have now synthesized different compounds of related structures and evaluated them as analgesic by using the writhing test in mice. In addition, we have also included the analgesic effects of aspirin and paracetamol in order to compare their potencies.

2. Results and discussion

In our program for obtaining compounds with analgesic properties, we have recently selected some succinimide and maleimide derivatives [4–8] and shown that some of them exerted a significant analgesic profile when analyzed against acetic acid-induced writhing in mice. Such findings led us to synthesize other substances structurally related to those previously described [5] and compare, in some cases, their analgesic effects using the same experimental model, in order to clarify some aspects about their structure–activity relationships. The compounds studied here are shown in Scheme 1.

All compounds were obtained using previously described methodologies [1–8], with minor modifications, which produced good yields (40–95%). Table 1 shows the analgesic effects of these compounds and of aspirin and paracetamol. As can be verified, some of them exhibited potent and dose-dependent analgesic effects. The most active compound



Scheme 1.

* Corresponding author.

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tested was from those containing antypirine directly attached to the imido ring (**1**). It was approximately 50-fold more active than the standard drugs, aspirin and paracetamol. However, all animals died after treatment with this compound, suggesting a high toxic effect, which therefore requires further investigation.

Another interesting result was given by compound **2**, which presented an ID_{50} value of 11 μmol kg^{-1}, whereas its derivative **3** was practically ineffective, causing only a discrete analgesic activity, inhibiting only 31% of the abdominal constrictions (30 mg kg^{-1}) [5]. Thus, compound **2** could be useful for obtaining other more active derivatives.

On the other hand, maleimide **4**, which possesses a chlorine atom introduced in position 2 of the aromatic ring, was more active than those in which such an atom is attached in position 4 [5], suggesting that steric parameters are involved in the analgesic activity.

Based on structure **4**, certainly other more potent derivatives could be obtained exploring the distance between the aromatic and imido rings or other structural factors, such as the introduction of two chlorine atoms in the double bond of the imido ring. This latter hypothesis is supported by the high analgesic action of compound **5**, which was about 30-fold more potent than aspirin and paracetamol.

In contrast to the above described results, compound **6** exhibited a moderate analgesic activity (ID_{50} = 35 μmol kg^{-1}). However, these data confirm our previous studies where we have suggested the biological importance of the double bond of maleimides [1–3,5].

Finally, the promising analgesic effects demonstrated here for some new cyclic imides, indicate the viability of using such a class of organic compounds to achieve more active substances, which might present new therapeutic possibilities. The studies are currently in progress to evaluate these compounds in other pharmacological models of pain as well to characterize their mechanisms of analgesic action.

3. Experimental

Melting points were determined with a Microquímica AP-300 apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer 720 spectrometer. ^{1}H NMR were recorded on a Varian XL 60 or on a Bruker 200 MHz. Elementary analyses were obtained on a Perkin-Elmer 2400. Percentages of elements determined (C, H, N) were in agreement with the product formula (within ±0.4% of theoretical values). The solvents and reagents were purified in the usual manner when necessary.

3.1. Chemical procedures

Compound **1** was obtained by the reaction of maleic anhydride with 4-aminoantypirine and dehydration of the corresponding maleamic acid by treatment with hot acetic anhydride/sodium acetate or acetic acid with reflux, as previously described [1–7]. Compounds **2–5** were obtained in the same way, using the appropriate anhydride and amine. Compound **6** was obtained from *N*-phenetylmaleimide by the addition of morpholin in benzene [5]. All the compounds were synthesized in good yields (45–90%) and characterized by ^{1}H NMR, IR and microanalysis. The purity of the tested substances was determined by thin layer chromatography (TLC) using several solvent systems of different polarity. Spots were visualized by short-wave UV light and iodine vapor. The physical characteristics of the prepared compounds are shown in Table 1.

Table 1
Physical characteristics of the prepared compounds

Compounds	M.p. (°C)	IR (cm^{-1}) a, Film NaCl; b, KBr	^{1}H NMR (δ, ppm) c, $CDCl_3$; d, CD_3OD	Analyses (C, H, N)
1 (a,c)	oil	1700, 1650 (CO), 1595 (C=C, Ar)	7.60 (s, 5H, Ar), 7.00 (s, 2H, CH=CH), 3.23 (s, 3H, CH_3), 2.20 (s, 3H, CH_3)	$C_{15}H_{13}N_3O_3$
2 (b,d)	115	3368 (OH), 1726, 1684 (CO), 1616 (C=C, Ar)	7.40–7.20 (m, 5H, Ar), 5.03 (s, 1H, NH), 3.35 (q, 2H, CH_2), 3.15 (t, 2H, CH_2COOH), 2.95 (t, 2H, CH_2Ar), 1.80 (q, 2H, CH_2), 1.15 (s, 6H, $2\times CH_3$)	$C_{15}H_{22}NO_3$
3 (a,c)	oil	1700 (CO), 1600 (C=C, Ar)	7.60 (s, 5H, Ar), 4.10 (t, 2H, CH_2), 3.30–2.70 (2t, 4H, $2\times CH_2$), 2.50 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 1.80 (t, 2H, CH_2)	$C_{15}H_{19}NO_2$
4 (b,c)	72	1700 (C=O), 1586 (C=C, Ar)	7.35–6.65 (m, 4H, Ar), 3.82 (t, 2H, CH_2), 3.04 (t, 2H, CH_2)	$C_{12}H_{10}NO_2Cl$
5 (b,c)	126	1726, 1670 (CO), 1618 (C=C, Ar)	7.31–7.20 (m, 5H, Ar), 3.83 (t, 2H, NCH_2), 2.92 (t, 2H, CH_2)	$C_{12}H_9NO_2Cl_2$
6 (b,c)	90–92	1705 (CO), 1600 (C=C, Ar)	7.55–7.40 (m, 5H, Ar), 4.10 (t, 2H, CH_2), 3.95 (dd, 1H, CH), 3.80–3.70 (m, 2H, CH_2), 3.30 (t, 2H, CH_2), 3.00–2.50 (m, 2H, CH_2), 1.20 (t, 2H, CH_2)	$C_{16}H_{20}N_2O_3$

Table 2
Analgesic effects for cyclic imides given intraperitoneally, against acetic acid-induced abdominal constriction in mice

Compound	ID_{50} (mg kg^{-1})	ID_{50} (μmol kg^{-1})	MI (%)
1	0.7 (0.3–1)	2.5 (1–3.5)	98 ± 1
2	3 (2–6)	11 (8–23)	100
3	>30	>123	31 ± 2
4	3 (1–6)	13 (4–25)	96 ± 3
5	1.2 (0.9–2)	4 (3–7)	100
6	10 (7–13)	35 (24–45)	92 ± 2
Aspirin	24 (13–44)	133 (73–247)	83 ± 1
Paracetamol	19 (16–23)	125 (104–250)	88 ± 1

ID_{50} values are accompanied by 95% confidence limits. MI indicates the maximal inhibition (%) of abdominal constrictions (at 30 mg/kg). Each group represents the mean of five to seven animals.

3.2. Pharmacological analysis: writhing test

Male Swiss mice, 25–30 g, were kept in a temperature controlled environment (23 ± 2°C) with a 12 h light–dark cycle. Food and water were freely available. The abdominal constriction resulting from intraperitoneal injection of acetic acid (0.6%), consisting of a contraction of the abdominal muscle together with a stretching of hind limbs, was carried out according to the procedures described previously [5,10]. Animals were pretreated with the compounds intraperitoneally (1–30 mg/kg) 30 min before acetic acid injection. Control animals received a similar volume of 0.9% NaCl (10 ml/kg). All experiments were carried out at 20–22°C. After challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions was cumulatively counted over a period of 20 min. The results are reported in Table 2.

3.3. Drugs

Acetic acid (Merck AG, Darmstadt, Germany) of high purity was dissolved in 0.9% of NaCl. All other compounds were dissolved in EtOH or $EtOH/H_2O$. The final concentration of solvents did not exceed 5% and did not cause any effect per se.

3.4. Statistical analysis

The results are presented as mean ± s.e.m., and statistical significance between groups was analyzed by means of analysis of variance followed by Dunnett's multiple comparison test. P values less than 0.05 were considered as indicative of significance. When appropriate, the ID_{50} values (the dose of compound that reduced responses by 50% relative to control value) were estimated by graphical interpolation from individual experiments.

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