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4-Nitro-1,8-naphthalimides exhibit antinociceptive properties

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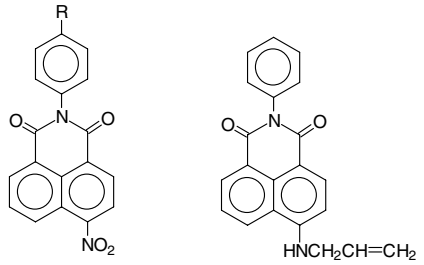
Cyclic imides are an important family of organic compounds which exhibit a great variety of biological activities [1]. Our research group has previously reported that some maleimides, succinimides, glutarimides, naphthalimides and related compounds exert pronounced analgesic or antinociceptive effects in mice [2–8]. One of the promising results were found with a series of analgesic naphthalimides or bis-naphthalimides [7]. Extending our previous investigations, we have now evaluated the analgesic or antinociceptive effects of the 4-nitro-1,8-naphthalimides **1**–**4** and a related compound, 4-N-allylamine-1,8-naphthalimide (**5**), using writhing and capsaicin models in mice. The results of some reference drugs were included for comparison. The molecular structures of the studied compounds are shown below:



R= H (**1**)
CH_3 (**2**)
OCH_3 (**3**)
Cl (**4**)

(**5**)

The two chemical models of pain were used here because both have been widely used for evaluation of the antinociceptive effects of synthetic and natural compounds [9]. As can be seen in Table 1, all the compounds showed potent and dose-related analgesic effects when analysed in the writhing test by the intraperitoneal route. They were more active than acetylsalycilic acid, acetaminophen and dypirone, well-known non-steroidal antiinflammatory and analgesic drugs. The most active compounds **2** and **4**, which contain a methyl group and a chloro atom attached in the position-4 of the phenyl moiety, respectively, were about 17 and 8-fold more active than standard drugs. This suggests that hydrophobic substituents improve the pharmacological action, but this hypothesis requires further investigation. Compound **5**, which is similar to compound **1**, with an allylamine group instead of a nitro group, was slightly more active. The analgesic effect of these compounds against the writhing test does not enable elucidation of its mechanism of action. However, recent studies carried out by Ribeiro et al. [10] have demonstrated that the nociceptive activity of acetic acid in the writhing model is due to the release of TNF-α (Tumour Necrosis Factor), interleukin 1 β and interleukin 8 by resident peritoneal macrophages and mast cells.

Table 1: ID_{50} values for 1,8-naphthalimides and reference drugs given intraperitoneally, against acetic acid-induced abdominal constriction in mice

Compd.	mg/kg	μmol/kg	MI (%)[a]
1	6.8 (4.4–9.3)	19.9 (12.9–27.2)	73 ± 2
2	2.8 (2.1–3.6)	7.9 (5.9–10.1)	81 ± 3
3	9.2 (6.7–12.2)	24.7 (18.0–32.8)	54 ± 4
4	6.0 (4.7–7.8)	15.9 (12.5–20.7)	74 ± 1.4
5	5.6 (4.1–7.3)	16.8 (12.3–21.9)	87 ± 1.2
ASA	24.0 (13.0–44.0)	133.0 (73–247)	35 ± 2
ACE	19.0 (16.0–23.0)	125.0 (140–250)	38 ± 1
DIP	59.0 (31.0–104.0)	162.0 (88–296)	33 ± 3.5

ID_{50} values are accompanied by 95% confidence limits. Each group represents the mean of six to eight animals. [a] Maximum inhibition at 10 mg/kg

Table 2: Antinociceptive effect of 1,8-naphthalimides against capsaicin induced-pain in mice

Compd.	Inhibition (%)
1	$40.3^{*} \pm 7.8$
2	$52.8^{**} \pm 5.3$
3	30.7 ± 3.6
4	$58.1^{**} \pm 5.9$
5	$73.2^{**} \pm 2.3$

Each group represents the mean of six to eight animals; * $P < 0.05$ and ** $P < 0.01$ compared with the respective control values. Compounds were administered intraperitoneally in a dose of 10 mg/kg

When evaluated on capsaicin induced-pain at 10 mg/kg administered intraperitoneally, all the compounds (except compound **3**) caused significant inhibition. These results show that some compounds act on neurogenic pain [9]. However, the mechanism by which these cyclic imides produce analgesia in the models of pain studied remains unclear.

In summary, our results suggest that the antinociceptive activity shown by the 1,8-naphthalimides studied is promising, and that these compounds may be used as leads to obtain new substances with analgesic potential.

Experimental

1. Synthesis of 1,8-naphthalimide derivatives: general procedures

4-Nitro-*N*-phenylsubstituted-1,8-naphthalimide derivatives **1**–**4** were obtained by reaction of 4-nitro-1,8-naphthalic anhidride with different aromatic amines, in boiling glacial acetic acid for 8 h. Compound **5** was obtained by nucleophilic substitution of the nitro group in compound **1** with a N-allylamino group by reaction with allylamine in *N,N*-dimethylformamide for 24 h at room temperature. Details of the synthesis are reported elsewhere [11].

2. Determination of antinociceptive activity

2.1. Writhing test

Male Swiss mice (25–35 g) were used. The abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%) was carried out according to the procedures described previously [8, 12] with minor modifications. The animals were pretreated with naphthalimides administered intraperitoneally, 30 min before the acetic acid injection. The control animals received a similar volume of 0.9% NaCl ($10\ ml \cdot kg^{-1}$, i.p.). All experiments were carried out at 23 ± 2 °C. After the challenge, pairs of mice were placed in separate boxes and the number of abdominal 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 between control animals and mice pretreated with the compound studied.

2.2. Capsaicin-induced pain

The procedure used was similar to that described previously [13]. The animals were placed individually in transparent glass cylinders. Following the adaptation period, 20 µl of capsaicin (1.6 µg/paw) was injected under the skin of the plantar surface of the right hindpaw, using a microsyringe. The animals were observed individually for 5 min following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. Animals were treated with the compounds (10 mg/kg, i.p.) or saline (10 ml/kg, i.p.) 1 h before administration of capsaicin. The control animals received a similar volume of 0.9% NaCl (10 ml/kg, i.p.).

3. Statistical analysis

The results are presented as mean ± s.e.m., and the statistical significance between the groups was analysed by means of an analysis of variance followed by Dunnett's multiple comparison test. *P* values less than 0.05 were considered as indicative of significance. The ID_{50} values (the dose of the compound that reduced responses by 50% in relation to the control values) were estimated by graphical interpolation from individual experiments. ID_{50}'s are presented as mean values and 95% confidence interval. MI is the maximum inhibition at higher dose used (10 mg/kg).

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Bioactive compounds from *Leycesteria formosa*

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As part of our research for natural products with anti-inflammatory potential, we investigated several plant extracts for their ability to inhibit *in vitro* cAMP-phosphodiesterase type IV (PDE4) activity. The control of inflammation is mediated in part by modulation of cAMP levels and PDE4 is currently considered as an intracellular target for new anti-inflammatory drugs [1, 2].

In a preliminary screening, we selected an ethanolic stem extract of *Leycesteria formosa* Wall. (Caprifoliaceae) for its promising PDE4 inhibition activity. This species, commonly known as "Himalayan honeysuckle", is a cultivated ornamental shrub native of East Asia, especially appreciated in gardens for its hardiness and its white flowers in pendent clusters [3]. Previous phytochemical studies on this species only reported the presence of coumarins [4] and monomeric flavonoids [5].

A bioactivity-directed fractionation led to the isolation of two polyphenolic constituents, identified by means of 1D and 2D NMR (H-H COSY, HMQC, HMBC) experiments and comparison with published data [6–7] as amentoflavone (3′–8″ biapigenin) and its 4‴ methyl derivative, podocarpusflavone A.

Biflavonoids are interesting taxonomic markers in Angiosperms because of their sporadic occurrence. In Caprifoliaceae family for example, only two genera are known for their biflavonoid content: *Viburnum* with amentoflavone exclusively [8] and *Lonicera* with both amentoflavone and ochnaflavone derivatives [9–11]. The occurence of this two biflavonoids in *Leycesteria* and their inhibitory effect on purified PDE4 are reported here for the first time.

Amentoflavone and to some extent, podocarpusflavone A, are good PDE4 inhibitors acting at an under micromolar range (Table). Thus, in view of the recently described correlation between *in vitro* PDE4 inhibition and *in vivo* topical anti-inflammatory properties [12], *Leycesteria formosa* may be considered of potential interest in treating cutaneous inflammation.

Experimental

1. Plant material

A sample of aerial parts was collected at the Botanical Garden of Metz (France) in January 1998. A voucher specimen was deposited at the corresponding Herbarium.

2. Extraction and isolation

The air-dried and powdered stems (52 g) were exhaustively extracted with boiling 95% EtOH. The alcoholic filtrated solution was concentrated *in*

Table: PDE4 inhibition of an ethanolic stem extract, a polyphenolic fraction and two biflavonoids isolated from *Leycesteria formosa*

Samples	CI_{50} (µg/ml)	CI_{50} (µM)
Ethanolic stem extract	62.10	–
Polyphenolic fraction	23.70	–
Podocarpusflavone A	2.26	4.10
Amentoflavone	0.145	0.27