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Synthesis and Analgesic Properties of Cyclic Imides: Naphthalimide and Bis-Naphthalimide Derivatives

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SUMMARY. In the last few years, the medicinal interest in cyclic imides has increased considerably. Since the isolation of phyllanthimide, a new alkaloid present in *Phyllanthus sellowianus*, which possesses moderate antispasmodic and antibacterial properties, several synthetic analogues have been reported to have antibacterial, antifungal, antiespasmodic and analgesic effects. In the present study, a series of new cyclic imides, Naphthalimide and bis-Naphthalimide derivatives were synthesized and evaluated against acetic acid-induced abdominal constriction in mice, given intraperitoneally at a dose of 10 mg kg^{-1}. The pharmacological results indicated that all compounds produced significant inhibition of acetic acid-induced abdominal constrictions in mice. Moreover, all compounds were more efficacious than some well-known drugs widely used in the therapeutic as analgesic. Such results confirm previous studies on biological activities of cyclic imides.

RESUMEN. "Síntesis y Propiedades Analgésicas de Imidas Cíclicas: Naftalimida y Bis-naftalimida". En los últimos años se ha incrementado considerablemente el interés medicinal en las imidas cíclicas. Desde el aislamiento de la filantimida, un nuevo alcaloide presente en *Phyllantus sellowianus*, que posee moderadas propiedades antiespasmódicas y antibacterianas, se ha informado que varios análogos sintéticos poseen efectos antibacterianos, antifúngicos, antiespasmódicos y analgésicos. En el presente estudio fueron sintetizadas una serie de nuevas imidas cíclicas, derivadas de la naftalimida y de la bis-naftalimida, y evaluadas contra la constricción abdominal inducida en ratas por aplicación de una dosis de 10 mg. kg^{-1} de ácido acético por vía intraperitoneal. Los resultados farmacológicos indicaron que todos los compuestos produjeron una inhibición significativa de las constricciones abdominales inducidas por ácido acético en ratas. Más aún, todos los compuestos fueron más eficaces que algunas drogas de reconocida acción terapéutica como analgésicas. Tales resultados confirman estudios previos sobre la actividad biológica de las imidas cíclicas.

INTRODUCTION

In medicinal chemistry, the chemist attempts to design and synthesize a medicine or a pharmaceutical agent which will benefit humanity. In this context, it should be emphasized that the organic synthesis represents the main source of new drugs [1]. However, these factors are partially related to active natural products,

KEY WORDS: Analgesic activity, cyclic imides, Naphthalimide and bis-Naphthalimide.
PALABRAS CLAVE: Actividad analgésica, Imidas cíclicas. Naftalimida y bis-naftalimida.

once a great number of naturally ocurring substances are used as model to synthesize more potent and selective derivatives or analogues [2].

Recently, our research group has studied the chemical and biological properties of cyclic imides, which were synthesized using phyllanthimide, an alkaloid extracted from *Phyllanthus sellowianus* [3], as a model for obtaining several analogues. Previous studies provide evidence that these compounds exerted different and important biological properties, such as antibacterial [4-8], antispasmodic [9], antifungal [10] and analgesic [11-14].

The aim of this study is not only to obtain new cyclic imides, particularly Naphthalimides and bis-Naphthalimides, but also analyse them as analgesic. Thus, we have prepared a series of N-arylnaphthalimides and bis-N-arylnaphthalimides from the appropriate aniline derivatives and respective naphthalic anhydride in acetic acid, and evaluated their analgesic effects by using writhing test in mice.

MATERIAL & METHODS

Chemistry

Melting points were determined with a Microquimica AP-300 apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. Infrared (IR) spectra were recorded with a FT Perkin Elmer 16PC spectrometer on KBr disks. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Brucker 200 MHz instrument with tetramethylsilane as an internal standard; chemical shifts are given on δ scale (ppm). Mass spectra were recorded on Shimadzu GC-MS-2000A mass spectrometer at 70 eV. Elemental analysis was determined with a Perkin Elmer 2400 elemental analyser. The purity of the sinthesized substances was monitored by thin-layer chromatography (TLC) using Merck silica pre-coated aluminum plates 200 µm in thickness with several solvent systems of different polarity. Spots were visualized by short-wave UV light and iodine vapour. The solvents and reagents were purified in a usual manner. All compounds were obtained in good yield (73-97%), and were characterized by spectral data (^{1}H NMR, MS, IR) and elemental analysis (CHN), which were in agreement with the proposal structures

General procedure. Compounds were prepared by adding the appropriate aniline or aniline derivative (1 mol) for each mol of anhydride in glacial acetic acid (Figure 1 and 2). The mixture was refluxed for 2 hours. The products were purified by recristallization from ethanol.

N-phenylnaphthalimide (**1**). m.p. 184.5-186 °C, yield 86%, IR (KBr, cm^{-1}): 1772, 1738 (C=O), 1584 (C=C Ar); MS 272 (M^+), 228, 180, 154, 126.
Anal. Calcd. for $C_{18}H_{11}NO_2$: C, 79.11; H, 4.06; N, 5.13. Found: C, 79.18; H, 4.11; N, 5.19.

N-4-methylphenylnaphthalimide (**2**). (290 °C dec.), yield 92%, IR (KBr, cm^{-1}): 1748, 1710 (C=O), 1580 (C=C Ar); M.S 287 (M^+), 243, 195, 126; ^{1}H NMR ($CDCl_3$) δ: 8.68 (dd, 4H, ArH), 8.64 (dd, 2H, ArH) 7.89 (dd, 2H, ArH), 7.35 (d, 2H, ArH), 7.28(d, 2H, ArH), 2.47 (s, 3H, $ArCH_3$).
Anal. Calcd. for $C_{19}H_{13}NO_2$: C, 79.43; H, 4.56; N, 4.87. Found: C, 79.57; H, 4.62; N, 4.90.

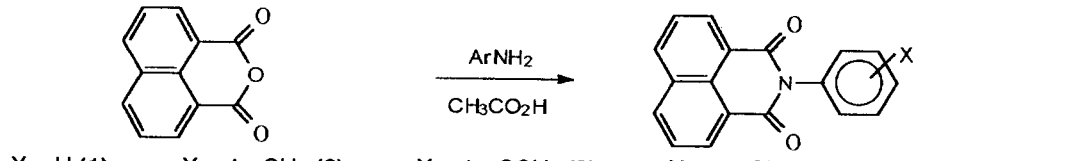

Figura 1. Synthesis of Naphthalimides.

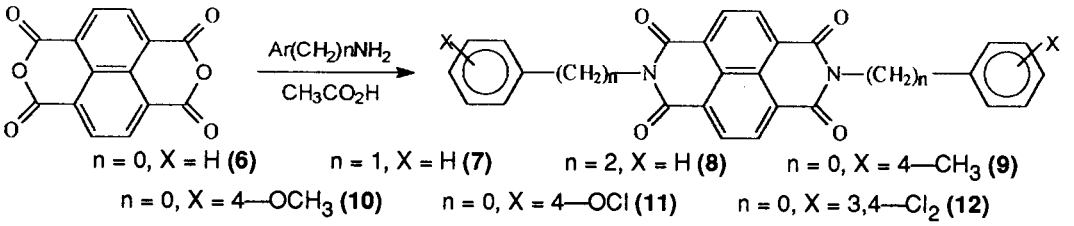

Figura 2. Synthesis of Bis-Naphthalimides.

N-4-methoxyphenylnaphthalimide (**3**). (290 °C dec.), yield 89%, IR (KBr, cm^{-1}): 1772, 1740 (C=O), 1586 (C=C Ar); M.S 303 (M^+), 154 (M^+—$C_8H_7NO_2$), 126; ^1H NMR ($CDCl_3$) δ: 8.55 (dd, 2H, ArH), 8.52 (dd, 2H, ArH), 7.90 (dd, 2H, ArH), 7.31 (d, 2H, ArH), 7.06 (d, 2H, ArH), 3.83 (s, 3H, OCH_3).
Anal. Calcd. for $C_{19}H_{13}NO_3$: C, 75.24; H, 4.32; N, 4.62. Found: C, 75.33; H, 4.41; N, 4.69.

N-4-chlorophenylnaphthalimide (**4**). (290 °C dec.), yield 78%, IR (KBr, cm^{-1}): 1772, 1738 (C=O), 1580 (C=C Ar); M.S 307, 309 (M^+), 154, 126.
Anal. Calcd. for $C_{18}H_{10}ClNO_2$: C, 70.25; H, 3.28; N, 4.55. Found: C, 70.34; H, 3.26; N, 4.62.

N-3,4-bis-chlorophenylnaphthalimide (**5**). (290 °C dec.), yield 78%, IR (KBr, cm^{-1}): 1770, 1736 (C=0), 1580 (C=C Ar); M.S 341, 343 (M+), 154, 126.
Anal. Calcd. for $C_{18}H_9Cl_2NO_2$: C, 63.18; H, 2.65; N, 4.09. Found: C, 63.26; H, 2.70; N, 4.13.

Bis-N-phenylnaphthalimide (**6**). m.p > 360 °C, yield 97 %, IR (KBr, cm^{-1}): 1716, 1702 (C=O), 1580 (C=C Ar); M.S 418 (M^+), 325, 227, 77.
Anal. Calcd. for $C_{26}H_{14}N_2O_4$: C, 74.64; H, 3.37; N, 6.70. Found: C, 74.71; H, 3.41; N, 6.72.

Bis-N-benzyllnaphthalimide (**7**). m.p > 360 °C, yield 91%, IR (KBr, cm^{-1}): 1782, 1740 (C=O), 1580 (C=C Ar); M.S 446 (M^+), 340, 212, 91. ^1H NMR (DMSO-d_6) δ: 8.74 (d, 4H, ArH), 7.56-7.24 (m, 10H, ArH), 5.37 (s, 4H, 2xCH_2); M.S 446 (M^+), 340, 312, 91
Anal. Calcd. for $C_{28}H_{18}N_2O_4$: C, 75.33;.H, 4.06; N, 6.27. Found: C, 75.39; H, 4.10; N, 6.26.

Bis-N-phenethylnaphthalimide (**8**). m.p > 360 °C, yield 86 %, IR (KBr, cm^{-1}): 1782, 1744 (C=O), 1582 (C=C Ar); M.S 474 (M^+), 249, 104. ^1H NMR (DMSO-d_6) δ: 8.78 (d, 4H, ArH), 7.33-7.21 (m, 10H, ArH), 4.43 (t, 4H, 2xNCH_2), 3.05 (t, 4H, $ArCH_2$).
Anal. Calcd. for $C_{30}H_{22}N_2O_4$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.88; H, 4.70; N, 5.92.

Bis-N-4-methylphenylnaphthalimide (**9**). m.p > 360 °C, yield 86 %, IR (KBr, cm^{-1}): 1772, 1738 (C=O), 1582 (C=C Ar); M.S 446 (M^+), 339, 241; ^1H NMR (DMSO-d_6) δ: 8.71 (d, 4H, ArH), 7.33 (d, 2H, ArH), 7.20 (d, 2H, ArH), 2.41 (s, 3H, $ArCH_3$).
Anal. Calcd. for $C_{28}H_{18}N_2O_4$: C, 75.33; H, 4.06; N, 6.27. Found: C, 75.44; H, 4.12; N, 6.22.

Bis-N-4-methoxylphenylnaphthalimide (**10**). m.p > 360 °C, yield 83 %, IR (Kbr, cm^{-1}): 1772, 1740 (C=O), 1586 (C=C Ar); M.S 478 (M^+), 355, 239; ^1H NMR (DMSO-d_6) δ: 8.71 (d, 4H, ArH), 7.38 (d, 2H, ArH), 7.11 (d, 2H, ArH), 3.84 (s, 3H, OCH_3).
Anal. Calcd. for $C_{28}H_{18}N_2O_6$: C, 70.29; H, 3.79; N, 5.85. Found: C, 70.33; H, 3.89; N, 5.89.

Bis-N-4-chlrophenylnaphthalimide (**11**). m.p > 360 °C, yield 76 %, IR (KBr, cm^{-1}): 1784, 1712 (C=O), 1578 (C=C Ar); M.S 486, 488 (M^+), 377, 250, 124.
Anal. Calcd. for $C_{26}H_{12}Cl_2N_2O_4$: C, 64.09; H, 2.48; N, 5.75. Found: C, 64.17; H, 2.53; N, 5.72.

Bis-N-3,4-bis-chlrophenylnaphthalimide (**12**). m.p > 360 °C, yield 73%, IR (KBr, cm^{-1}): 1784, 1714 (C=O), 1578 (C=C Ar). M.S 556, 558 (M^+), 411, 393, 250.
Anal. Calcd. for $C_{26}H_{10}Cl_4N_2O_4$: C, 56.15; H, 1.81; N, 5.04. Found: C, 56.21; H, 1.84; N, 5.09.

Pharmacology

Female Swiss mice, 25-30 g, were kept in an automatically controlled temperature (23 ± 2 °C) and 12 h light-dark cycles. Food and water were freely available. The abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%) was dissolved in 0.9% NaCl and then carried out according to the procedures described previously [15,16]. The animals were pretreated with compounds which were dissolved in Tween 80 (Merck) (10 mg Kg^{-1}), 30 min before the acetic acid injection. The final concentration of Tween 80 did not exceed 5% and did not cause effect by themselves. Control animals received a similar volume of 0.9% NaCl (10 mL Kg^{-1}, i.p.). All experiments were carried out at 20 ± 2 °C.

After challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions cumulatively counted over a period of 20 min. Analgesic activity was expressed as the reduction of the number of abdominal constrictions between control animals and mice pre-treated with the studied compound (see Table 1).

Compound	Inhibition (%)
1	84.0 ± 2.0
2	99.0 ± 1.0
3	91.0 ± 3.0
4	87.0 ± 2.0
5	87.0 ± 2.0
6	89.0 ± 1.0
7	98.0 ± 1.0
8	91.0 ± 2.0
9	90.0 ± 2.0
10	88.0 ± 2.0
11	90.0 ± 1.0
12	86.0 ± 1.0
Aspirin	35.0 ± 2.0
Paracetamol	38.0 ± 1.0

Table 1. Analgesic effect of Naphthalimides (**1-5**) and Bis-Naphthalimides (**6-12**) in comparison with aspirin and paracetamol against acetic acid-induced abdominal constriction in mice. Compounds were given intraperitoneally in a dose of 10 mg kg^{-1}.

RESULTS AND DISCUSSION

In previous studies, we have shown that several cyclic imides exhibit different biological activities, including analgesic action[11-14]. In order to obtain other pharmacologicaly active compounds, we have sinthesized new N-arylnaphthalimides (Figure 1) and bis-N-arylnaphthalimides (Figure 2) and evaluated their analgesic effects in mice.

The analgesic activity of the compounds sinthesized against acetic acid-induced abdominal constriction in mice is summarized in Table 1.

As can be seen, the pharmacological results show that all the tested compounds exerted marked analgesic action at 10 mg kg^{-1} by intraperitoneal route, confirming previous studies on biological activities of cyclic imides. They inhibited approximately 90% the abdominal constrictions whereas some well-known

drugs, ie, aspirin and paracetamol analysed under the same pharmacological procedure, caused inhibition of 35 and 38%, respectively.

The substituent group introduced in the aromatic ring of compounds **1-5** was selected according to the Topliss method [17,18], which permits to predict new more active compounds. Thus, it should be noted that compounds **2** and **3**, which have eletron-donor substituents (methyl and methoxy, respectively) were more efficacious, suggesting that electronic effects seem to be related to the reported analgesic activity. Other pharmacological studies are required to confirm this proposal.

Since compounds **1-5** exhibited high analgesic action, other symetric analogues, particularly bis-Naphthalimides, were pharmacologically analysed. However, the analgesic effects did not significantly charge, suggesting that the naphthyl moiety is acting as a pharmacophoric group in these molecules.

Although other pharmacological data are necessary to establish which structural parameters, *i.e.,* hydrophobicity, electronic or steric effects, are mainly responsible for the analgesic action of the naphthalimides and bis-naphthalimides studied, our findings suggest that compounds of this nature may represent an important class of analgesic agents.

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