Synthesis and Nootropic Activity of Some 2,3-Dihydro-1*H*-isoindol-1-one Derivatives Structurally Related with Piracetam

by **Adelfo Reyes***[a]), **Leticia Huerta**[a]), **Marisol Alfaro**[a]), and **Andrés Navarrete**[b])

[a]) Facultad de Estudios Superiores Zaragoza, Unidad Multidisciplinaria de Investigación Experimental, Universidad Nacional Autónoma de México, Batalla 5 de Mayo esquina Fuerte de Loreto, Ejército de Oriente, Iztapalapa 09230, México D. F., México (phone: +(55)56230794; fax: +(55)57736330; e-mail: adelfo@puma2.zaragoza.unam.mx)

[b]) Facultad de Química, Departamento de Farmacia. Universidad Nacional Autónoma de México, México D. F., 04510, México

Three 2,3-dihydro-1*H*-isoindol-1-ones structurally related with piracetam (=2-oxopyrrolidine-1-acetamide) have been synthesized and tested for their nootropic effects in the passive avoidance test in mice. Compounds (*RS*)-**2**, (*R*,*R*)-**3**, and (*R*,*S*)-**3** were obtained in good yields in only two steps starting from methyl DL-phthaloylalanine. Compound (*RS*)-**2** exhibited nootropic activity at lower doses than piracetam, used as reference drug, but it showed lower efficacy. Whereas diastereoisomers (*R*,*R*)-**3** and (*R*,*S*)-**3** were as potent as piracetam to revert amnesia induced by scopolamine, (*R*,*S*)-**3** showed lower efficacy than (*R*,*R*)-**3**. Only (*R*,*R*)-**3** showed myorelaxant effect at doses of 10 and 30 mg/kg; other compounds did not exhibit any anticonvulsant, sedative, myorelaxant, or impaired motor-coordination effect in mice. These synthesized 2,3-dihydro-1*H*-isoindol-1-one derivatives constitute a new kind of nootropic compounds.

Introduction. – Cognitive dysfunction is one of the clinical manifestations that often appears in age-related pathologies, head injury, and neurodegenerative diseases such as *Alzheimer*'s disease. Cognition enhancers, often referred as nootropics, are frequently used to treat such cognitive alterations. In some European countries, piracetam has been used for patients with a moderate degree of *Alzheimer*'s disease to increase the cognitive functions [1] with excellent tolerance and security [2]. Although the mode of action of piracetam in the nervous system is not well-defined, its efficacy has been documented in a wide range of clinical indications like cognitive disorders and dementia, vertigo, and dyslexia, as well as cortical myoclonus [3][4].

The properties of piracetam (=2-oxopyrrolidine-1-acetamide) were disclosed in 1967, and this stimulated the design and synthesis of a large number of structurally related molecules that were found to be endowed with a similar pharmacological profile [5]. Much like the cognition enhancers of the first generation, piracetam-like nootropics revert amnesia induced by scopolamine and other amnesing drugs, electroconvulsive shock, and hypoxia with an unknown mechanism [6]. In general, they show no affinity for the most important central receptors ($K_a > 10$ mM), but are able to modulate the action of most central neurotransmitters, in particular, acetylcholine and glutamate [7]. Several biochemical and behavioral findings have been presented for piracetam-like nootropics, but, so far, a common molecular mechanism of action has not been indicated [7]. The lack of a common mechanism at a molecular

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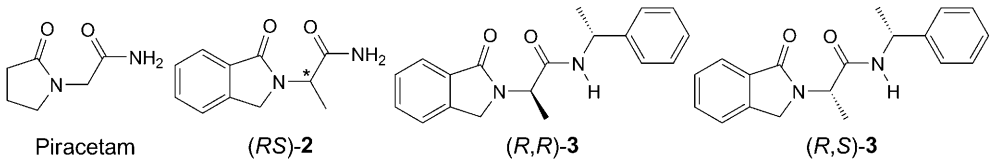
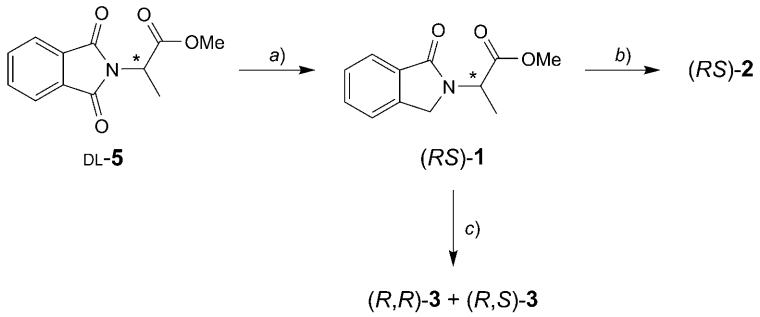
level allows sound structure–activity correlations only with *in vivo* behavioral assays, leading to frustrating consequences in drug design. However, most of the compounds containing a 2-oxopyrrolidine structure do present cognition-enhancing activity, suggesting that this feature plays a critical role [5][7].

On the other hand, the development of *N*-substituted isoindolinone (=2,3-dihydroisoindolone) derivatives has attracted considerable attention of chemists, because it has been found that these compounds exhibit diverse biological activities. Some isoindolinones have been described as anxiolytics and reverse transcriptase inhibitors [8][9], antipsychotics [10], tranquilizing agents and sedatives [11], antimicrobials [12], antitumor agents [13], and 5-HT_{2C} antagonists [14]. Therefore, taking in count that isoindolinone has the 2-oxopyrrolidine moiety in its structure, and that, among the large number of structurally related molecules to piracetam, there are no nootropic isoindolones, in the present work we synthesized three isoindolinones structurally related with piracetam and tested their ability to revert the scopolamine-induced amnesia in mice. In addition, a neuropharmacological profile was assessed for these compounds.



Results and Discussion. – *Chemistry.* The synthesis of isoindolinone derivatives (*RS*)-**2**, (*R*,*R*)-**3**, and (*R*,*S*)-**3** is outlined in *Scheme 1*. The *N*-substituted isoindolinone (*RS*)-**1** was obtained by the reduction of a racemic mixture of **5** with Zn in AcOH in the presence of HCl. Upon reacting (*RS*)-**1** with NH_3, (*RS*)-**2** was formed, whereas its reaction with (*R*)-α-phenylethylamine yielded (*R*,*R*)-**3** and (*R*,*S*)-**3**. These three amides were obtained with yields higher than 80% (see *Scheme 1*).

Scheme 1

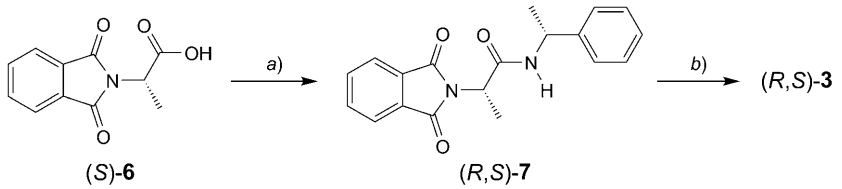


a) HCl (g), AcOH, Zn, reflux, 5 h; 88.7%. *b*) NH_3 (g), MeOH, room temperature, overnight; 96.8%. *c*) (*R*)-α-Phenylethylamine, 160°, 8 h; 80.0%.

The ammonolysis of (*RS*)-**1** at room temperature using MeOH saturated with NH_3 led to the racemic amide **2** in almost quantitative yield (96.8%), whereas, for the reaction with (*R*)-α-phenylethylamine, it was necessary to heat the mixture at 160° to give the diastereoisomeric compounds (*R*,*R*)-**3** and (*R*,*S*)-**3**, obtained in acceptable yields of 80.0 and 66.7% respectively. Compound (*RS*)-**2** has been described in a patent [11].

To establish the absolute configuration of the aliphatic stereogenic C-atoms of (*R*,*R*)-**3** and (*R*,*S*)-**3**, the latter was prepared by an independent method in three steps: the first one consisted of the reaction of L-alanine with phthalic anhydride to afford (*S*)-**6** (*Scheme 2*) [15]; the second step consisted of the reaction of (*S*)-**6** with $SOCl_2$ and then with (*R*)-α-phenylethylamine to give (*R*,*S*)-**7**; the final step was the reduction of (*R*,*S*)-**7** according to the modified *Brewster* procedure [16], employing Zn in AcOH in the presence of HCl to give (*R*,*S*)-**3** as the sole product. The configuration of the compounds (*R*,*R*)-**3** was established by comparison of its NMR data with those of the diastereoisomer (*R*,*S*)-**3** synthesized according the reaction outlined in *Scheme 2*. The main differences observed in the ^{1}H-NMR spectra between (*R*,*R*)-**3** and (*R*,*S*)-**3** were the presence of an *AB* system at δ(H) 4.47 (*d*, $J = 17.2$, 1 H) and δ(H) 4.60 (*d*, $J = 17.2$, 1 H) for the two related diastereotopic H-atoms ($-CH_2N$) in (*R*,*R*)-**3** compared to a single signal at δ(H) 4.35 (*s*, 2 H) for (*R*,*S*)-**3**.

Scheme 2



a) $SOCl_2$, toluene, room temperature, 20 h and then (*R*)-α-phenylethylamine, room temperature, 3 h; 78.4%. *b*) HCl (g), AcOH, Zn, 90°, 1 h; 92.2%.

Pharmacology. The ability of the synthesized compounds to revert scopolamine-induced amnesia is shown in *Fig. 1*. The *y*-axis expresses Δ avoidance-latency values (difference between initial and 24-h latency, expressed in s). In this test, the control group, treated only with saline solution, showed a Δ avoidance latency of 49.9 ± 11.8 s (*Fig. 1*). This value represents the normal behavior of mice in this test. Scopolamine induced amnesia in this test; the mice, treated with 1 mg/kg scopolamine, showed a Δ avoidance latency value of 14.3 ± 5.2 s (*Fig. 1*). Compound (*RS*)-**2** from 0.3 to 10 mg/kg reverted scopolamine-induced amnesia [2][17]. At doses higher than 10 mg/kg, (*RS*)-**2** showed toxicity signs in mice, such as ataxia and tremors; therefore, we did not continue tests with doses higher than 10 mg/kg for nootropic activity. Apparently, the absolute configuration of the stereogenic center is important for the activity, since (*R*,*R*)-**3** was more active as nootropic than (*R*,*S*)-**3** to inhibit the scopolamine-induced amnesia in the avoidance test in mice [2][17]. At a dose of 100 mg/kg, (*R*,*R*)-**3** led to the same level of Δ avoidance latency (185 ± 40.3 s) as piracetam (197.2 ± 57.1 s) used as positive nootropic drug, whereas (*R*,*S*)-**3** showed only one third of Δ avoidance latency

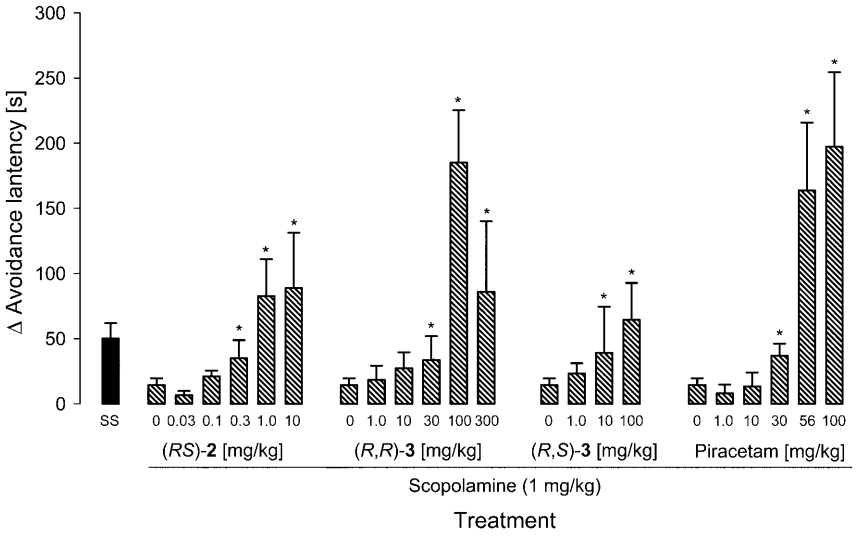


Fig. 1. *Δ Avoidance latency values* [s] *for test compounds and piracetam at different doses after intraperitoneal administration in mice.* Bars represent the mean ± SEM, $n = 10$. *: $P < 0.05$, *vs.* respective control; *Dunn*'s multiple comparison test after *Kruskal–Wallis* test.

(64.4 ± 28.3 s) of piracetam at the same dose (*Fig. 1*), however, it was sufficient to revert the scopolamine-induced amnesia. Although only three compounds were tested, they provide a clear indication of the structure–activity relationship with respect to the structural modification of piracetam: the presence of a Me group in the lateral chain of the isoindolinone, the introduction of the *N*-(1-phenylethyl) fragment, and the fusion of the benzo ring to the pyrrolidinone did not affect the nootropic effect in much as the absolute configuration of the stereogenic center. The difference between these compounds can be also explained by the fact that, in the *in vivo* studies, the biological activity is the consequence of both the pharmacokinetic and pharmacodynamic properties, which may be differently affected by structural modifications. It is necessary to perform additional experiments to find out if differences in the effect are due to their pharmacokinetic or pharmacodynamic properties.

In addition to nootropic activity, a neuropharmacological profile was performed for the studied compounds and piracetam; neither the synthesized compounds nor piracetam showed anticonvulsant (pentylenetetrazole (PTZ)-induced seizures), sedative (exploratory cylinder), or impaired motor-coordination (rotarod test) effect in mice (data not showed). Only compound (*R*,*R*)-**3** showed a myorelaxant effect at a dose of 10 and 30 mg/kg but not in a dose-dependent manner (*Fig. 2*). These results are in agreement with those previously reported for nootropic drugs that have low levels of neurotoxicity and do not induce any behavioral impairment, sedative, or stimulant effects [18][19].

In summary, in this work the racemic amide (*RS*)-**2**, and diastereoisomeric amides (*R*,*R*)-**3** and (*R*,*S*)-**3** were synthesized by a rapid and low-cost procedure. Compound (*RS*)-**2** exhibited nootropic activity at low doses but not at the same intensity level of

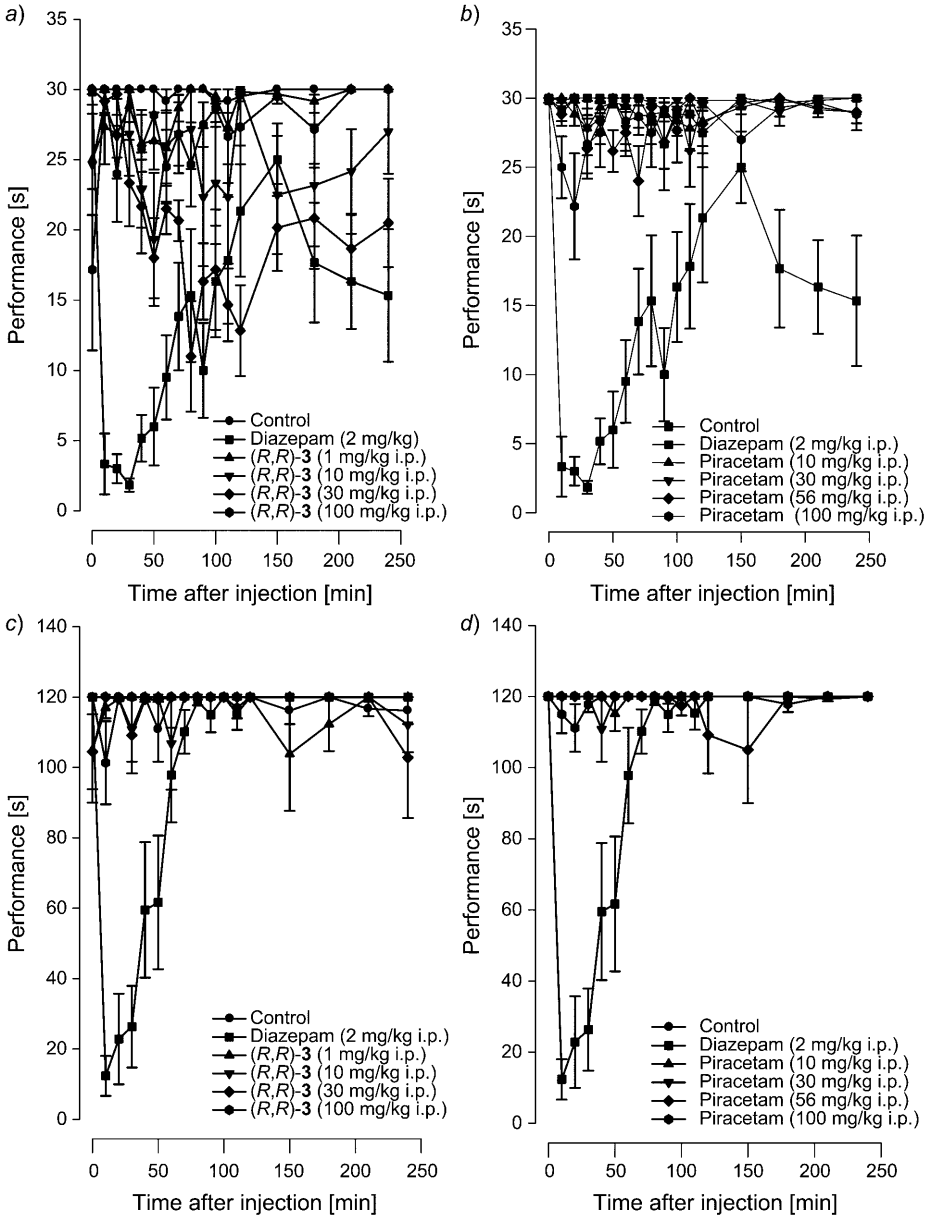


Fig. 2. *Myorelaxant effect in the traction test and effect on motor coordination in rotarod test for* (*R*,*R*)-**3** (*a* and *c*) *and piracetam* (*b* and *d*), *respectively.* Each point represents the mean ± SEM of six animals. Diazepam (2 mg/kg, i.p.) was used as positive myorelaxant and impaired coordination control drug.

piracetam in the passive avoidance test in mice. On the other hand, (*R*,*R*)-**3** showed nootropic activity with the same potency as piracetam, and (*R*,*S*)-**3** showed lower

nootropic activity than piracetam, highlighting the importance of the stereogenic center. The anticonvulsive, sedative, motor-coordination, or myorelaxant effects were absent or not related with the doses for these compounds. These compounds constitute a new kind of a nootropic drug.

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Experimental Part

General. Solvents were distilled from the appropriate drying agents prior to use. All reactions were monitored by TLC (sheets coated with silica gel *60 F_{254}* (*Merck*); spots were visualized with UV light). Products were purified by open column chromatography (CC) on silica gel *60* (SiO_2, 230–400 mesh ASTM; *Merck*). M.p.: Electrothermal *Digital IA9100* melting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter. IR Spectra: *Perkin-Elmer* model *599* spectrophotometer, in KBr; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Varian VXR-300S* spectrometer, in $CDCl_3$; chemical shifts (δ) in ppm, with TMS as internal reference; coupling constants (J) in Hz. MS: *Hewlett-Packard* model *5890* spectrometer.

Methyl N-*Phthaloyl*-DL-*alaninate* (=*(±)-Methyl 2-(1,3-Dihydro-1,3-dioxo-2*H-*isoindol-2-yl)propanoate*; DL-**5**). This racemic ester was prepared according to the procedure that we reported in [20]. M.p. 65–67° ([20]: m.p. 66–67°).

*Methyl (*RS*)-2-(1,3-Dihydro-1-oxo-2*H-*isoindol-2-yl)propanoate* ((*RS*)-**1**). In a modification of the procedure reported by *Brewster et al.* [16], HCl was bubbled at r.t. into a stirred mixture of DL-**5** (13.1 g, 56.13 mmol) and Zn dust (24.0 g, 0.367 mol) in glacial AcOH (175 ml). The mixture was heated at reflux for 5 h, filtered, and evaporated under reduced pressure to give crude product (11.8 g), which was purified by open CC (AcOEt/hexane 1:1) to yield (*RS*)-**1** (10.9 g, 88.7%). Colorless oil. R_f (AcOEt/hexane 1:1) 0.37. IR (KBr): 2952, 1745, 1693, 1681. ^1H-NMR (400 MHz, $CDCl_3$): 1.59 (*d*, *J* = 5.7, 3 H); 3.73 (*s*, 3 H); 4.43 (*d*, *J* = 12.3, 1 H); 4.59 (*d*, *J* = 12.3, 1 H); 5.22 (*q*, *J* = 5.7, 1 H); 7.40–7.50 (*m*, 2 H); 7.50–7.60 (*m*, 1 H); 7.80–7.90 (*m*, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 15.8; 46.7; 49.0; 52.3; 122.8; 123.9; 128.0; 131.6; 132.0; 141.6; 168.6; 172.2. EI-MS: 219 (M^+).

*(*RS*)-2-(1,3-Dihydro-1-oxo-2*H-*isoindol-2-yl)propanamide* ((*RS*)-**2**). A soln. of (*RS*)-**1** (3.0 g, 13.7 mmol) in MeOH (60 ml) saturated with NH_3 was stirred at r.t. overnight. The solvent was removed *in vacuo*, and the remaining residue (3.0 g) was crystallized from MeOH to afford (*RS*)-**2** (2.7 g, 96.8%). White solid. M.p. 188–189° ([11]: m.p. 184–187°). R_f (AcOEt/hexane/$CHCl_3$ 15:6:4) 0.32. IR (KBr): 3320, 3168, 1693, 1658. ^1H-NMR (300 MHz, $CDCl_3$): 1.54 (*d*, *J* = 7.2, 3 H); 4.49 (*s*, 2 H); 5.04 (*q*, *J* = 7.2, 1 H); 5.40 (br. *s*, 1 H); 6.40 (br. *s*, 1 H); 7.45–7.60 (*m*, 3 H); 7.83–7.87 (*m*, 1 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 14.4; 47.0; 49.9; 123.0; 123.8; 128.2; 131.8; 131.9; 141.5; 169.1; 172.7. EI-MS: 204 (M^+).

*(2*R*)- and (2*S*)-2-(1,3-Dihydro-1-oxo-2*H-*isoindol-2-yl)*-N-*[(1*R*)-1-phenylethyl]propanamide* ((*R*,*R*)-**3** and (*R*,*S*)-**3**, resp.). A stirred mixture of (*RS*)-**1** (3.2 g, 14.6 mmol) and (*R*)-α-phenylethylamine (2.1 ml, 16.5 mmol) was heated to 160° for 8 h. After cooling to r.t., the resulting mixture was dissolved in a minimum of hot AcOEt and then kept for 12 h at 0°. The resulting precipitate was collected by filtration to give (*R*,*R*)-**3** (1.8 g, 80.0%). White solid. The diastereoisomer (*R*,*S*)-**3** was obtained by evaporation of the mother liquor, followed by purification by open CC (AcOEt/hexane/CH_2Cl_2 1:1:1) to afford pure (*R*,*S*)-**3** (1.5 g, 66.7%).

Diastereoisomer (*R*,*R*)-**3**. M.p. 174–176°. R_f (AcOEt/hexane/CH_2Cl_2 1:1:1) 0.32. $[\alpha]_D = +101.68$ (*c* = 0.53, MeOH). IR (KBr): 3322, 1686, 1658. ^1H-NMR (300 MHz, $CDCl_3$): 1.38 (*d*, *J* = 7.2, 3 H); 1.52 (*d*, *J* = 7.2, 3 H); 4.47 (*d*, *J* = 17.2, 1 H); 4.60 (*d*, *J* = 17.2, 1 H); 5.023 (*q*, *J* = 7.2, 1 H); 5.024 (*q*, *J* = 7.2, 1 H); 6.92 (br. *d*, *J* = 7.2, 1 H); 7.22–7.36 (*m*, 5 H); 7.47 (*t*, *J* = 7.5, 2 H); 7.57 (*dt*, *J* = 7.5, 1.6, 1 H); 7.83 (*dd*, *J* = 7.5, 1.6, 1 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 14.8; 22.0; 47.1; 49.0; 50.5; 122.9; 123.7; 126.0; 127.3; 128.1; 128.6; 131.7; 132.0; 141.7; 143.1; 169.0; 169.8. EI-MS: 308 (M^+).

Diastereoisomer (*R*,*S*)-**3**: M.p. 95–96°. R_f (AcOEt/hexane/CH_2Cl_2 1:1:1) 0.24. $[\alpha]_D = +64.73$ (c = 0.52, MeOH). IR (KBr): 3303, 2927, 1664. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.46 (*d*, *J* = 7.2, 3 H); 1.49 (*d*, *J* = 7.2, 3 H); 4.35 (*s*, 2 H); 5.05 (*q*, *J* = 7.2, 1 H); 5.12 (*q*, *J* = 7.2, 1 H); 7.02–7.08 (*m*, 3 H); 7.12–7.18 (*m*, 2 H); 7.24–7.38 (*m*, 2 H); 7.47 (*dt*, *J* = 7.2, 1.2, 1 H); 7.56 (br. *d*, *J* = 7.2, 1 H); 7.65 (*dd*, *J* = 7.2, 1.2, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 14.3; 22.3; 46.9; 49.1; 50.3; 122.9; 123.7; 125.6; 127.0; 128.1; 128.4; 131.7; 131.9; 141.5; 143.4; 169.2; 169.6. EI-MS: 308 (M^+).

*(2*S*)-2-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propanoic Acid* ((*S*)-**6**). This acid was prepared according to a modification of the procedure described by *Zeng et al.* [15]. Briefly, a stirred mixture of phthalic anhydride (4.1 g, 27.7 mmol) and L-alanine (2.22 g, 25 mmol) was heated at 135–140° at atmospheric pressure for 30 min. The resulting product was crystallized from EtOH/H_2O to give (*S*)-**6** (4.5 g, 82.2%). White solid. M.p. 145–146°. $[\alpha]_D = -21.82$ (c = 0.82, EtOH) ([15]: m.p. 149–151°; $[\alpha]_D = -23.0$ (c = 0.8, EtOH)).

*(2*S*)-2-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)*-N-*[(1*R*)-1-phenylethyl]propanamide* ((*R*,*S*)-**7**). $SOCl_2$ (1.5 ml) was added dropwise to a suspension of (*S*)-**6** (2.25 g, 10.3 mmol) in toluene (40 ml) at r.t., and the mixture was stirred for 20 h. The solvent was removed *in vacuo*, and the remaining residue was dissolved in dry THF (30 ml). The resulting soln. was then treated with (*R*)-α-phenylethylamine (1.4 ml, 11.0 mmol) and pyridine (0.6 ml) at r.t. and stirred for further 3 h. The solvent was removed *in vacuo*, and the remaining residue was partitioned with AcOEt (20 ml) and 1M HCl (20 ml). The org. phase was washed with H_2O (2 × 20 ml), dried (Na_2SO_4), and evaporated to give the crude product (3.7 g). Crystallization from MeOH afforded (*R*,*S*)-**7** (2.6 g, 78.4%). White solid. M.p. 160–161°. $[\alpha]_D = +54.33$ (c = 0.52, $CHCl_3$). IR (KBr): 3350, 1718, 1661. ^{1}H-NMR (400 MHz, $CDCl_3$): 1.49 (*d*, *J* = 7.2, 3 H); 1.69 (*d*, *J* = 7.2, 3 H); 4.93 (*q*, *J* = 7.2, 1 H); 5.13 (*q*, *J* = 7.2, 1 H); 6.38 (br. *d*, *J* = 7.2, 1 H); 7.22–7.36 (*m*, 5 H); 7.70–7.75 (*m*, 2 H); 7.81–7.86 (*m*, 2 H). ^{13}C-NMR (75.5 MHz, $CDCl_3$): 15.4; 21.5; 49.1; 49.6; 123.5; 126.1; 127.3; 128.6; 131.8; 134.2; 142.7; 167.9; 168.2. EI-MS: 322 (M^+).

*(2*S*)-2-(1,3-Dihydro-1-oxo-2*H*-isoindol-2-yl)*-N-*[(1*R*)-1-phenylethyl]propanamide* ((*R*,*S*)-**3**). HCl was bubbled at r.t. into a stirred mixture of (*R*,*S*)-**7** (0.644 g, 2.0 mmol), Zn dust (0.85 g, 13.0 mmol), and glacial AcOH (10 ml). The mixture was heated at 90° for 1 h, filtered, and evaporated under reduced pressure to give a crude product (0.598 g), which was purified by open CC (AcOEt/hexane/CH_2Cl_2 1:1:1) to give (*R*,*S*)-**3** (0.568 g, 92.2% yield). M.p. 95–96°. R_f (AcOEt/hexane/CH_2Cl_2 1:1:1) 0.24. $[\alpha]_D = +64.73$ (c = 0.52, MeOH).

Animals. All experiments were performed on adult male ICR (Institute for Cancer Research) mice weighing 25–34 g purchased from *Centro UNAM-Harlan* (*Harlan México, S.A. de C.V.*). Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999) currently adopted in our laboratory, and in compliance with international rules on care and use of laboratory animals. During the experiment, the animals had free access to water and food, with a 12 h light-dark cycle at r.t. (22 ± 2°).

Drugs and Dosage. Compounds (*RS*)-**2** (0.03 to 10 mg/kg), (*R*,*R*)-**3** (1.0 to 300 mg/kg), (*R*,*S*)-**3** (1.0 to 100 mg/kg), and diazepam (*Roche S.A.*; 2 mg/kg) were suspended in 0.5% *Tween 80* in saline soln. Piracetam (*Sigma Co.*; 1.0 to 100 mg/kg), pentylenetetrazole (PTZ; *Sigma Co.*; 80 mg/kg), and scopolamine (*Fluka Co.*; 1.0 mg/kg) were prepared in saline soln. (0.9%). The drugs were freshly prepared each time and intraperitoneally injected in a volume of 0.1 ml/10 g body weight. Control animals received the same volume of vehicle (0.5% *Tween 80* in saline or saline soln. only).

Passive Avoidance Test. The passive avoidance apparatus (*Ugo Basile 7550*) consists of two compartments: a white and illuminated compartment (of 18 × 9.5 × 16 cm) separated by a guillotine door from the second dark compartment (18 × 9.5 × 16 cm). The floor of the dark compartment is a grid with steel bars (0.3 cm of diameter separated by 1.2 cm) [21]. The training session started by placing one mouse in the illuminated compartment. After 10 s, the sliding door was automatically opened, leaving free access to the dark compartment. As soon as the mouse entered the dark compartment with its four paws, the door was automatically closed, and an electrical shock (0.3 mA, with 2 s of duration) was delivered. The time required for each mouse to enter the dark compartment was recorded (time of initial latency in s). Immediately afterwards, the animals were returned to their home cage and injected with scopolamine (1 mg/kg) to induce amnesia. The animals that needed more than 100 s to enter the dark compartment were eliminated from the test [22]. The same procedure was repeated 24 h after (latency at

24 h), but without the electrical shock and without scopolamine administration. The test session is finished when the animal enters the dark compartment, or remains in the illuminated compartment for more than 420 s. The test compounds or vehicle were intraperitoneally administered 20 min before training session. A control group treated exclusively with saline and without scopolamine injection was included to evaluate the normal behavior of the mice in this test.

PTZ-Induced Seizures. The mice were injected with PTZ (80 mg/kg) and immediately placed in individual plastic boxes, and observed for at least 30 min to record the occurrence of the first episode of clonic or tonic seizures and mortality. Mice living longer than 30 min following PTZ administration were considered to be protected [23]. The drugs were administered in different doses 30 min before PTZ-induced seizures.

Exploratory Cylinder Test [24][25]. The apparatus consisted of a glass cylinder (30 cm in height, 11 cm in diameter, with a wall of 3 mm). The cylinder was placed on filter paper in a room with constant lighting and isolated from external noise [23][26]. An individual naïve mouse was put on the filter paper-covered floor of the glass cylinder, and the number of rearings performed over a 5-min period was recorded. The inner side of the apparatus and floor were cleaned with alcoholic soln. (10% *v*/*v* in H_2O), and filter paper was changed between each animal test session [23][24]. The drugs were administered 30 min before testing in different doses. During observation, the experimenter stood next to the apparatus always in the same place. The observations were made without prior knowledge of the experimental conditions applied to the animal. Reduced exploratory rearing shown by naïve mice after placement in an unfamiliar environment reveals a sedative effect [23][24][26][27].

Rotarod Test. Mice remaining for at least 2 min on the rod (Rotarod Treadmills for mice, constant speed model *7600*, *Ugo Basile*; 4-cm diameter, 16 rpm) were selected and allocated to a group of six animals each. Immediately after administration of the test compound, reference drug or vehicle mice were placed on the rod for a maximum of 120 s, and the length of time each mouse remained there was recorded ('time on rod'). The time on the rod was registered each 10 min for the first 2 h and each 30 min for the following 2 h, for a total recording time of 4 h. The effect on motor coordination was considered when animals remained on the rod less than 120 s [28].

Traction Performance. The traction test was conducted with a stainless bar (diameter 1.5 mm, length 35 cm) set horizontally at an elevation of 40 cm. The mice were hung with their forelimbs on the horizontal bar. A 30-s performance time was considered normal. Only animals that fulfill this criterion were included in the experiment. The performance time was registered each 10 min for the first 2 h and each 30 min for the following 2 h, for a total recording time of 4 h [23].

Statistical analysis. The results are presented as mean ± SEM. Data were analyzed using one-way ANOVA, followed by *Dunnett*'s *t*-test for neuropharmacological tests. For nootropic activity evaluation, the statistically significant differences between the treatments were tested by *Kruskal–Wallis* test (non-parametric one-way analysis of variance), followed by *Dunn*'s multiple comparison test. A value of $P < 0.05$ was considered significant.

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