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Research Article

Anti-Anxiety and Sedative Profile Evaluation of Imidazo[1,2-*a*]Pyridine Derivatives

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Strategy, Management and Health Policy				
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ABSTRACT Three imidazo[1,2-*a*]pyridine-3-nitrosated (L-1, L-2, L-3) and a 3-formyl imidazo[1,2-*a*]-pyridine thiosemicarbazone (L-4) were synthesized and evaluated for their effects in the elevated plus maze, burying behavior test, rotarod performance, the horizontal wire test, and locomotor activity. L-2 and L-3 increased the percent time spent in the open arms of the plus maze at doses of 1 and 2 mg/kg without modifying the number of total entries. In addition, L-2 and L-3 (1 mg/kg) increased the number of open arm entries indicating anxiolytic-like activity at this dose. In the burying behavioral test, L-1 (2–8 mg/kg), L-2 (8 mg/kg), and L-3 (4 and 8 mg/kg), induced a clear reduction in cumulative burying behavior, without modifying burying behavior latency, thus reducing experimental anxiety. In the rotarod test, L-1 and L-2 impaired rotarod performance only at the highest evaluated dose (64 mg/kg) at which reduction of motor activity was observed and thereby no conclusions about myorelaxant effects can be proposed. All compounds showed a clear sedative effect and corresponding ED_{50} values were obtained. Results indicate that compounds L-1, L-2, and L-3 show a sedative and an anxiolytic profile. Drug Dev Res 71:371–381, 2010.

Key words: anxiety; sedation; imidazo[1,2-*a*]pyridines; behavioral tests

INTRODUCTION

Sleep behavior and anxiety are two major recurrent problems treated by the general medical physician. Sleep problems like insomnia are frequently associated with anxiety disorders [Wittchen and Hoyer, 2001; Ressler and Mayber, 2007] while anxiety is a common emotional phenomenon in humans. "Normal" anxiety can be defined as occurring as an adaptive response to various stressors (physiological, psychological, sociological). When anxiety becomes pathological, with disproportionate or unjustified reactions, it can result in disturbance of daily life and cause suffering in patients.

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The pharmacotherapy of anxiety and sleep disorders involves the use of benzodiazepines (BZs) that have potent anxiolytic, sedative-hypnotic and anticonvulsant activity, acting as indirect agonists of the $GABA_A$ (γ-aminobutyric acid-A) receptor. However, a great tendency to produce tolerance, addiction, ataxia, sleep suppression REM (rapid eye movements), and amnesia are factors associated with BZs [Feldman et al., 1997]. Therefore, treatment for anxiety and associated pathologies require more selective drugs with less collateral effects [Kent et al., 2002; Davidson, 2002].

Imidazo[1,2-*a*]pyridines, such as zolpidem (sedative-hypnotic agent) and alpidem (anxiolytic agent), are chemically non-related to BZs and show more selective pharmacological action. However, amnesia and insomnia rebound effects following the use of zolpidem have been reported while alpidem showed hepatotoxicity [Berson, 2001; Depoortere et al., 1986].

The imidazo[1,2-*a*]pyridine is an attractive core that has been evaluated in connection to several biological activities. In particular 3-nitrosoimidazo[1,2-*a*]pyridines have been evaluated as antiretroviral [Chaouni-Benabdallah et al., 2001] and anti-parasitic [Srivastava et al., 1998] agents and have shown antifungal activity [Yu et al., 2008]. In the present study, three 2-aryl-3-nitrosoimidazo[1,2-*a*]pyridines (L-1-3) and the 3-formyl imidazo[1,2-*a*]pyridine thiosemicarbazone (L-4) were selected from a previous round of preliminary tests and evaluated for their action on anxiety behavioral tests.

MATERIALS AND METHODS

Chemistry

Melting points were measured on an Electrothermal melting point apparatus and are uncorrected. ^1H and ^{13}C nmr spectral data were recorded at 300 and 75 MHz, respectively, using a Bruker DPX 300 MHz NMR spectrometer. Chemical shifts (δ) are given in parts per million downfield from TMS (δ = 0). Synthesis of the compounds under investigation was achieved as depicted in Figure 1. The starting 2-arylimidazo[1,2-*a*]pyridines were prepared by condensation of 2-aminopyridine with the corresponding phenacyl bromide according to the literature protocol [Lombardino, 1965]. The nitroso functional group was introduced by treating the 2-arylimidazopyridine with nitrous acid generated from sodium nitrite and acetic acid at low temperature [Chaouni-Benabdallah et al., 2001]. The carboxaldehyde functional group was introduced through a Vilsmeier Haack reaction, using dry *N,N*-dimethyl formamide and phosphorous oxychloride, and heating at 80°C for 12 h, followed by treatment with aqueous sodium hydroxide [Fuentes and Paudler, 1975]. The thiosemicarbazone L-4 was

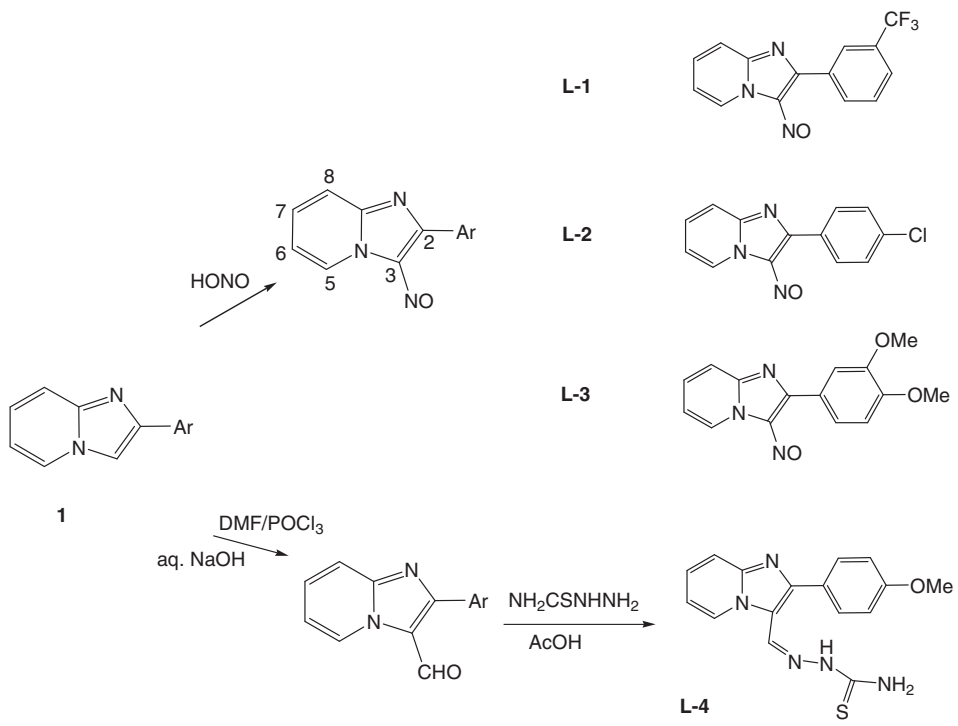

Fig. 1. Synthesis and chemical structures of imidazo[1,2-*a*]pyridine derivatives.

prepared from imidazo[1,2-*a*]pyridine-3-carboxaldehyde and thiosemicarbazide in acetic acid [West et al., 1993]. All products were purified and characterized by conventional spectroscopic methods and combustion analysis. Compounds L-1, L-3, and L-4 have not been previously described in the literature, therefore the ^{1}H and ^{13}CNMR data are provided.

2-(3′-Trifluoromethylphenyl)-3-Nitroso Imidazo [1,2-*a*]Pyridine (L-1)

Isolated as a light green solid, in 90% yield, mp 124–125°C. ^{1}H NMR ($CDCl_3$) δ H_5 9.9 (dd, $J_{5,6} = 8.8$, $J_{5,8} = 1.0$, 1H), $H_{5',6'}$ 8.9 (m, 2H), $H_{8,2',4'}$ 7.9–7.2 (m, 3H), H_7 7.7 (t, $J = 7.6$, 1H), H_6 7.0 (td, $J_{5,6} = 8.8$, $J_{6,7} = 7.7$, $J_{6,8} = 2.4$, 1H). ^{13}C NMR δ 117.7, 120.0, 125.9, 126.4, 127.2, 127.3, 127.8, 127.9, 129.3, 132.4, 134.1, 136.3, 145.5, 153.3. Anal. Calcd. for $C_{14}H_8N_3F_3O$: C, 57.73; H, 2.75. Found: C, 57.61; H, 2.90.

2-(4′-Chlorophenyl)-3-Nitroso Imidazo[1,2-*a*] Pyridine (L-2)

Isolated as a green powder in 75%, mp 223–224°C, lit mp 222–224 [Salgado-Zamora et al., 2008].

2-(3′,4′-Dimethoxyphenyl)-3-Nitrosoimidazo [1,2-*a*]Pyridine (L-3)

Isolated as a dark yellow solid, mp 196–198°C. ^{1}H NMR ($CDCl_3$) δ H_5 9.9 (d, $J_{5,6} = 6.0$, 1H), H_8 8.4 (d, $J_{8,7} = 8.0$, 1H), Ar-H 8.2 (s, 1H), Ar-H 7.8 (s, 2H), Ar-H +H_7 7.2 (m, 2H), H_6 7.0 (d, $J_{6,7} = 8.0$, 1H), MeO-, 4.0 (s, 3H), -OMe 3.98 (s, 3H). ^{13}CNMR 56.0, 56.04, 111.1, 112.8, 117.1, 119.0, 124.3, 125.0, 126.6, 136.2, 146.0, 149.2, 152.5, 153.3, 159.5. Anal. Calcd. for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.59. Found: C, 63.74; H, 4.68.

2-(4′-Methoxyphenyl)-3-Formylimidazo [1,2-*a*]Pyridine Thiosemicarbazone (L-4)

Isolated as a light brown crystalline powder in 98% yield, mp 223–224°C. ^{1}H NMR ($DMSO_{d6}$) δ H_5 9.5 (d, $J_{5,6} = 6.7$. 1H), NH 8.7 (s, 1H), Ar-H 8.2 (bs, 1H), Ar-H 7.8–7.6 (m, 4H), H_7, 7.51 (t, $J_{7,6} = 7.3$ 1H), Ar-H +H_6, 7.13–7.06 (m, 3H), MeO-3.8 (s, 3H). ^{13}CNMR 55.9, 114.7, 114.9,117.0,126.0, 128.2, 130.2, 131.1, 126.4, 146.6, 150.8, 160.3, 177.3. Anal. Calcd. for $C_{16}H_{15}N_5SO$: C, 59.07; H, 4.61. Found: C, 59.23; H, 4.26.

Drugs

Diazepam was obtained from Roche and Zolpidem tartrate from Lab Auylacem. and suspended in 0.3% Tween 80 (Sigma-Aldrich, St. Louis, MO)/saline solution. For in vivo pharmacological studies, all compounds were prepared as suspensions in a 10% aqueous solution of Tween 80 and Polietilenglicol 400 (Merck-Schuchardt, Hohenbrunn, Germany) freshly prepared on the experimental day. All drugs were administered intraperitoneally (i.p.) in a volume of 10 mL/kg in mouse and 1 mL/kg in rat, 30 min prior to testing at a single dose.

Animals

Male ICR mice, weighing 18–22 g; male Wistar rats weighing 180–200 g were purchased from UAM-Xochimilco (Mexico City) and Harlan-México (Mexico City). Mice and rats were housed in groups of five per cage, allowed access to water and food ad libitum, and maintained under a constant temperature (23 ± 1°C) and humidity (60 ± 10%). The rats used for the Elevated plus-maze (EPM) test were housed under a normal 12-hour light/dark cycle. In other evaluation experiments, animals were kept under a 12:12 h inverted light-dark cycle conditions (lights on 9:00–21:00). Animals were allowed to acclimate for 1–2 weeks prior to use and were transported to the test room at least 1 h prior to testing. All behavioral evaluations were carried out between 10 a.m. and 3 p.m. Care and handling of the animals were in agreement with internationally accepted procedures and approved by our own institutional Committee following the recommendation indicated in the Mexican Technical Specifications for the Production, Care, and Use of Laboratory Animals (Secretaría de Agricultura, Ganaderìa, Desarrollo Rural, Pesca y Alimentación).

BEHAVIOURAL TESTING

Elevated Plus-Maze (EPM)

The test apparatus based on that described by Pellow and colleagues [1985] is widely used for studying anxiolytic drugs and neurobiological mechanisms of anxiety in rodents due to the natural aversion of rodents to heights and open spaces. The EPM consisted of two open arms, $50 \times 10 \times 40$ cm, and two enclosed arms, $50 \times 10 \times 40$ cm, with an open top, arranged so that the arms of the same type were opposite each other, connected by an open central area (10×10 cm). The maze was elevated to a height of 50 cm. Thirty minutes after i.p. administration of the test drug, the rat ($N = 5$ and 7) was placed in the center of the maze, facing one of the enclosed arms. EPM behavior was videotaped during a 5-min test period. The following were recorded: the number of entries and time spent in the open and closed arms, and the total number of arm entries.

Conditioned Defensive Burying

The test apparatus is based on that described by Pinel and Treit [1978]. The method is useful for testing physiological and pharmacological changes in experimental anxiety [Treit et al.,1980; Treit, 1985]. An

electrified prod (7 cm long) projecting 2 cm above the floor from one wall of an acrylic cage (27 × 16 × 23) was covered with fine sawdust. When the animal touched the prod, it received an electric shock of 0.3 mA supplied by a constant-current shocker. In this test, the mouse ($N = 6$ and 7) was introduced into the cage and its behavior was recorded for 10 min. During this period, two parameters were measured: (1) cumulative burying behavior, i.e., the time the mouse spent burying the prod and (2) burying behavior latency, i.e., the tine elapsed from the first shock to the defensive behavior display. In this model, cumulative burying has been directly linked to experimental anxiety levels, while burying behavior latency may represent an index of the animal coping ability stimulus response reactivity [Treit et al., 1981; Picazo and Fernández-Guasti, 1995].

Rotarod Test

The rotating rod ("rotarod") test is widely used to assess "minimal neurological deficit" such as impaired motor function (i.e., ataxia) and coordination in rodents. An Ugo Basile accelerating rotarod for rats (model 7750) was used in order to evaluate possible motor impairment. A modification of the method described by Dunham and Miya [1956] was adapted. Two hours before the experiment, rats were selected for their ability to maintain themselves for at least 2 min on the rod (diameter, 7 cm) which rotated slowly at a constant speed (10 r.p.m). Rats satisfying this criterion received i.p. injections of test compounds ($N = 5$ and 7). Then after 30 min, they were retested for their ability to remain on the rotarod. The number of total falls was recorded.

The Horizontal Wire Test

The horizontal wire test was used to assess compund effects on muscle tone in mice. The test apparatus was based on that described by Bonetti [1982]. In this test, the animals were lifted by the tail, allowed to grasp a horizontal strung wire (for mice 25 cm high, 2 mm diameter, 30 cm long) with their forepaws and then released. The number of animals ($N = 6,7$) that were unable to grasp the horizontal wire, with either the forepaws, or at least with one hindpaw within 10 sec (cut-off: 60 sec) was recorded. In untreated control animals, this number was consistently zero.

Spontaneous Locomotor Activity Measurement

Exploratory locomotor activity of rats can be evaluated in open field tests. Rats were placed in a squared open field (60 × 60 × 30 cm). The floor and the wall of the compartment were painted in black. The test was started by placing the rat (one rat per time) in the center of the open field to evaluate distance traveled, time spent in ambulation, stereotypic movements, and resting by a video-recording system (Videomex V. Columbus Instruments, Columbus, OH) during 2 repetitions of 3 min each per animal. After removing the rat, the open field was cleaned with a solution with damp and dry cloths to remove any residue or odors.

Locomotor activity in mice was recorded individually in an automatic activity counter (Opto-Varimex; Columbus Instruments) that consists of an acrylic cage measuring 51.1 × 9.5 × 69.2 cm with two arrays of 15 infrared emitting photocells placed perpendicular to each other. The photocells are spaced 2.5 cm apart. The interruption of each infrared beam generated an electric impulse, which was processed and presented as a count. This test was carried out immediately after evaluating the animals in the burying behavior paradigm and sedative effect tests, registering horizontal activity (ambulation) and vertical activity (rearing) over a 5-min period.

Statistical Analysis

The one-way variance analysis (ANOVA), followed by post-hoc Student-Newman-Keuls was applied to compare statistical significance between the treated group and the vehicle control. The Fisher's exact test was applied to evaluate statistical significance in the horizontal Wire test. $P < 0.05$ value was considered statistically significant. Analysis was done using the Sigma Stat version 3.5 and Sigma Plot 10 for windows.

RESULTS

Spontaneous Locomotor Activity

The effects of each of the four imidazopyridine derivatives evaluated at various doses on total activity during the first hour of testing are depicted in Table 1. A significant difference was observed on locomotor activity between individual dose groups and the vehicle control. One-way ANOVA revealed that doses of 4 and 8 mg/kg decreased both the distance traveled (cm) and time ambulatory (sec) of L-1 [$F(5,26) = 28.46$; $P < 0.001$], [$F(5,26) = 11.80$; $P < 0.001$], L-2[$F(4,26) = 69.653$; $P < 0.001$] [$F(4,22) = 18.794$; $P < 0.001$], and L-3[$F(5,30) = 15.644$; $P < 0.001$], [$F(5,30) = 11.711$; $P < 0.001$], respectively, while L-4 causes a decrease in these parameters, starting at 8 mg/kg [$F(4,22) = 19.519$; $P < 0.001$] and [$F(4,22) = 4.868$; $P < 0.006$]. Diazepam decreased both the distance traveled [$F(4,22) = 14.151$, $P < 0.001$] and ambulatory time [$F(4,22) = 3.711$; $P = 0.019$] at 3.5 mg/kg.

The four imidazopyridine derivatives had significant effects on spontaneous locomotor activity in mice on both ambulatory and vertical activity, as seen in Table 2. One-way ANOVA revealed the following: L-1 decreased ambulatory [$F(11,62) = 7.841$; $P < 0.001$]

TABLE 1. Average Results Obtained on Spontaneous Locomotor Activity Parameters in Rat Treated With Imidazopyridine Derivatives[a]

Compound	Dose (mg/kg, ip)	Locomotor activity: Distance traveled (cm)	Locomotor activity: Time ambulatory (sec)
Vehicle	0	662.5±50.5	31.0±2.3
Diazepam	0.5	609.8±26.1	31.2±2.3
	1	591.9±31.4	30.0±4.0
	2	581.2±14.9	30.0±3.1
	3.5	354.4±16.0*	18.8±1.7***
L-1	0.5	544.8±21.5	32.6±1.5
	1	630.0±30.2	25.6±3.7
	2	573.7±22.6	27.4±4.5
	4	350.3±28.7*	14.3±2.3*
	8	226.2±19.5*	9.5±0.3*
L-2	1	651.7±31.9	28.1±2.2
	2	609.3±12.3	27.7±2.9
	4	232.2±27.2*	10.8±1.2*
	8	68.7±36.7*	9.6±3.3*
L-3	0.5	683.5±37.8	28.8±2.6
	1	628.6±25.6	31.7±2.0
	2	608.0±21.9	31.5±2.8
	4	391.6±60.5*	16.0±1.8*
	8	331.0±17.9*	15.2±0.5*
L-4	1	595.2±13.2	35.0±2.7
	2	580.2±28.3	29.0±3.8
	4	567.6±3.9	29.0±2.8
	8	328.8±18.6*	18.8±1.6**

[a]The effects of imidazopyridine derivatives on mean±SEM spontaneous locomotor activity during the first hour of testing (*Ns* = 5 and 7 groups), $^{*}P<0.001$,$^{**}P<0.006$,$^{***}P<0.01$ versus corresponding vehicle control.

(120–480 mg/kg) and vertical [$F(11,62) = 10.912$; $P<0.001$] (4–480 mg/kg) activity. L-2 decreased ambulatory [$F(11,61) = 9.577$; $P<0.001$](30–480 mg/kg) and vertical [$F(11,62) = 9.391$; $P<0.001$] (120–480 mg/kg) activity. L-3 decreased the ambulatory [$F(11,61) = 13$; $P<0.001$](120–480 mg/kg) and vertical [$F(11,61) = 6$; $P<0.001$] (120–480 mg/kg) activity. L-4 decreased the ambulatory [$F(11,61) = 14.161$; $P<0.001$] (60–480 mg/kg) and vertical [$F(11,61) = 11.885$; $P<0.001$] (30–480 mg/kg) activity.

Elevated Plus-Maze (EPM)

The results for various doses of the four imidazopyridine derivates in the EPM are shown in Figure 2. L-1 treatment showed a significant increase in the total arm, open arm, and closed arm entries [one-way ANOVA $F(5,26) = 2.804$, $P<0.037$; $F(5,26) = 3$, $P<0.029$, $F(5,26) = 2.833$; $P<0.036$, respectively]. Post-hoc analyses revealed that the significant effects were attributable to the 4- and 8-mg/kg doses. These reductions are most likely due to motor effects. Figure 2 also shows a significant decrease with derivative L-2 on total and closed arms entries. Significant effects were noted for closed arm entries [one-way ANOVA $F(4,22) = 10.236$; $P<0.001$; $F(4,22) = 12.444$, $P<0.001$, respectively] at 4 and 8 mg/kg while L-2 had significant increments in open arm entries [$F(4,22) = 5.062$; $P<0.005$] only at 1 mg/kg. As shown in Figure 2, L-3 had significant effects on total arm, open arm, and closed arm entries [one-way ANOVA $F(5,30) = 4.998$, $P<0.002$; $F(5,30) = 7.398$, $P<0.002$; $F(5,30) = 6.66$; $P<0.001$, respectively]. Subsequent post-hoc analyses revealed that L-3 caused a significant decrease in total arm (4 and 8 mg/kg) and closed arm entries (1–8 mg/kg). L-3 significantly increased the total open arm entries only at 1 mg/kg. Figure 2 shows a significant decrease in total arm and closed arm entries [one-way ANOVA $F(4,22) = 5.584$, $P<0.003$; $F(4,22) = 11.448$, $P<0.001$] at 8 mg/kg. No effects of treatment with L-4 were found for open arm entries [$F(4,22) = 2.429$; n.s.]. Diazepam had significant effects on the number of total arm, open arm, and closed arm entries [one-way ANOVA, $F(4,22) = 8.217$, $P<0.001$; $F(4,22) = 20.516$, $P<0.001$, $F(4,22) = 14.668$, $P<0.001$] as indicated in Figure 2. Newman-Keuls post-hoc test revealed that diazepam had a significant increase in open arm and a significant decrease in closed arm entries at 1 and 2 mg/kg in the absence of any change in total arm entries while reduction in total arm and closed arm entries at 3.5 mg/kg was observed. Zolpidem significantly decreased closed arm entries [Fig. 3; one-way ANOVA $F(3,18) = 8.55$, $P<0.001$] at 0.75 and 1 mg/kg. Furthermore, 1 mg/kg significantly reduced total arm [$F(3,18) = 5.806$, $P<0.006$] and open arm entries [$F(3,18) = 4.467$; $P<0.016$].

Figure 3 shows the effects of the four imidazopyridine derivatives at various doses on the percent of time in the open arms in the elevated plus maze. No statistically significant differences with L-1 [one-way ANOVA $F(5,26) = 1.867$, n.s] between individual dose groups and the vehicle control were found over a range of doses (1–8 mg/kg). One-way ANOVA revealed a significant effect of the L-2 derivate [$F(4,26) = 19.042$; $P<0.001$] with both 1 and 2 mg/kg increasing the percent time spent in the open arms. L-3 also had a significant effect on percent time spent in the open arms [one-way ANOVA $F(5,28) = 15.767$, $P<0.001$]. A Newman-Keuls post hoc test, confirmed that L-3 (1 and 2 mg/kg) caused significant increases on the percent of time spent in the open arms. No effect for L-4 [$F(4,22) = 2.052$; $P<0.122$] was found.

Diazepam produced a significant and selective increase on time spent [Fig. 3; one-way ANOVA $F(4,23) = 34.33$; $P<0.001$] at 1- and 2-mg/kg dose. No reliable differences [one-way ANOVA $F(3,18) = 2.788$; $P = 0.07$] between individual dose groups of zolpidem and the vehicle control were found.

TABLE 2. Average Results Obtained on Spontaneous Locomotor Activity Parameters in Mice Treated With Imidazopyridine Derivatives[a]

			Locomotor activity				Locomotor activity	
Dose (mg/kg)	Groups	ED_{50}	Ambulatory activity (counts)	Vertical activity (counts)	Groups	ED_{50}	Ambulatory activity (counts)	Vertical activity (counts)
0	Vehicle		1,078.9±38.1	105.1±9.9				
0.5	L-1	7.43	1,202.8±64.8	105.1±9.9	L-3	30.88	1,254.8±215.9	87.0±22.9
1		(5.7–9.6)	1,195.5±70.6	97.5±5.8		(27.8–34.4)	1,449.7±240.1	126.3±28.8
2			1,050.3±32.4	91.5±9.3			1,080.3±40.3	114.5±7.6
4			797.3±198.8	85.0±15.2*			1,063.2±94.2	105.6±19.5
8			761.0±211.4	41.8±7.2*			1,024.4±93.9	102.2±26.4
15			684.1±140.3	32.9±6.9*			912.0±146.8	64.4±14.9
30			626.6±113.6	32.3±9.0*			818.5±58.8	76.5±23.7
60			595.3±176.8	31.6±6.1*			777.5±114.0	70.0±22.5
120			466.8±204.4*	21.1±7.9*			467.0±32.9*	7.0±3.5*
240			153.0±59.5*	18.0±4.6*			67.5±4.3*	5.5±0.3*
480			98.1±33.9*	11.1±5.4*			183.5±19.3*	0.0±0.0*
0.5	L-2	19.07	1,080.3±40.3	114.5±7.6	L-4	17.70	1,059.0±171.2	87.8±14.4
1		(15.9–22.8)	1,063.2±94.2	105.6±19.5		(11.4–27.6)	1,066.2±34.2	119.0±7.4
2			924.0±57.2	87.5±6.6			1,063.2±94.2	105.6±19.5
4			910.2±104.9	63.0±12.7			1,070.3±135.9	85.1±11.9
8			817.4±58.7	52.4±8.4			926.6±77.7	85.2±14.7
15			759.3±145.7	85.2±17.4			849.3±78.6	78.0±7.4
30			692.8±119.2*	62.5±9.2			829.3±45.4	58.9±8.0*
60			712.7±186.6*	60.2±12.4			542.0±53.1*	27.0±4.0*
120			666.0±145.5*	37.5±13.1*			409.5±23.4*	24.0±3.5*
240			203.2±52.9*	16.9±4.5*			465.8±114.7*	29.3±4.0*
480			140.0±10.0*	7.7±0.3*			122.0±23.7*	12.0±0.0*
0	Diazepam	0.95		Zolpidem		3.0		
		(0.8–1.1)				(1.5–6.1)		

[a]The effects of imidazopyridine derivatives on mean±SEM spontaneous locomotor activity during the first hour of testing (*Ns* = 6 and 7 groups), *$P<0.001$ versus the corresponding vehicle control.

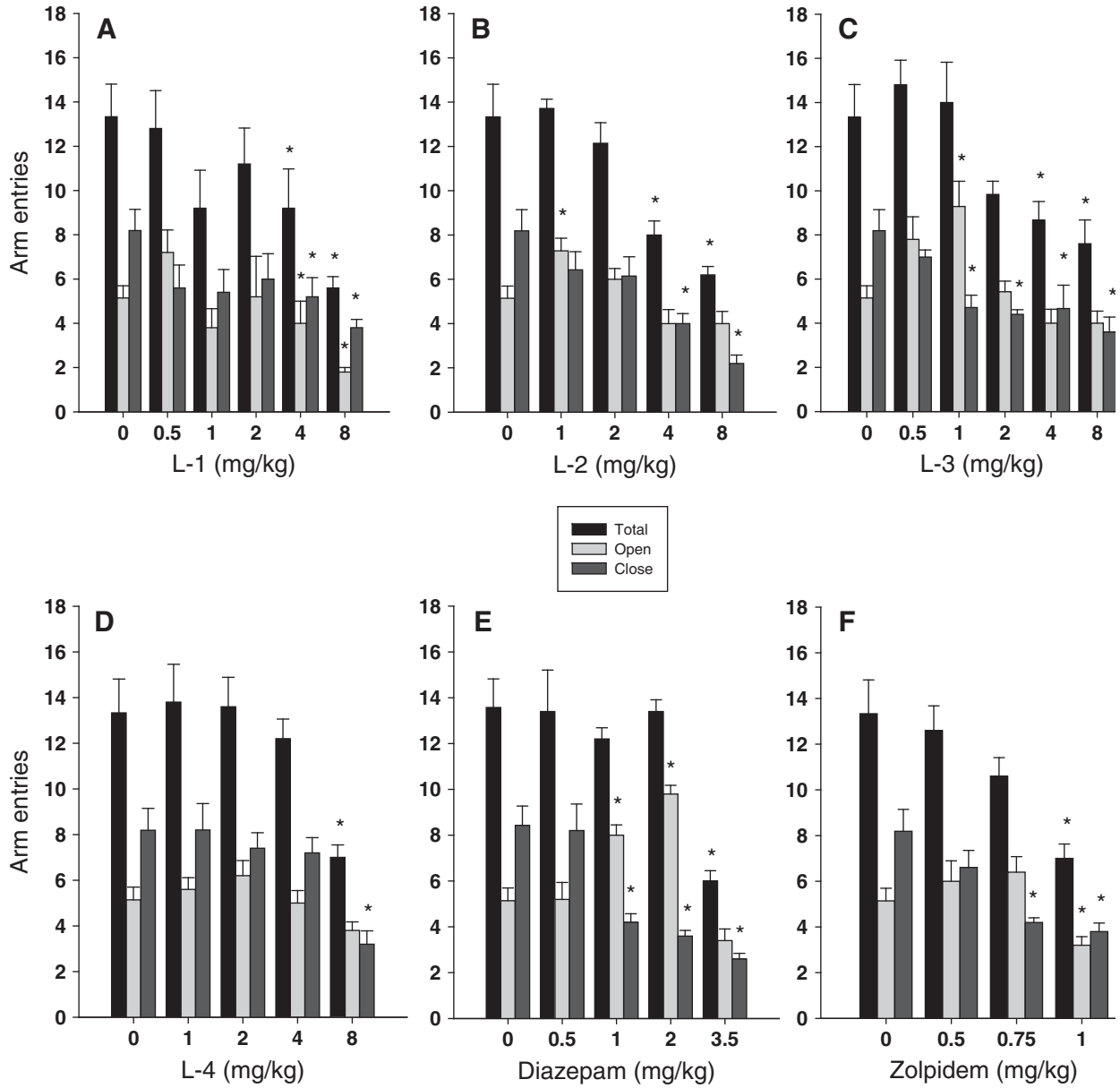

Fig. 2. The effects of various doses of the four imidazopyridine derivatives (**A**, **B**, **C**, **D**), diazepam (**E**) and zolpidem (**F**) on the means ± SEM of total, open and closed arms entries of the plus maze (*Ns* = 5 and 7 groups). *$P \leq 0.05$ versus vehicle control.

Conditioned Defensive Burying

The burying behavior latency and the cumulative burying behavior of the four imidazopyridines at various doses (Fig. 4A–D), diazepam (Fig. 4E), and zolpidem (Fig. 4F) on male mice are shown in Figure 4. In Figure 4A, conditioned defensive burying was significantly suppressed [$F(6,36) = 7.347$, $P < 0.001$] by L-1 over a dose range of 2–15 mg/kg, whereas significantly increased effects [$F(6,36) = 4.443$, $P < 0.002$] on the burying behavior latency at the highest dose of 15 mg/kg was observed, an effect likely due to motor effects. Treatment with L-2 also had an increased effect on cumulative burying behavior and burying behavior latency [Fig. 4B, one-way ANOVA $F(6,37) = 5.893$; $P < 0.001$, $F(6,37) = 3.771$; $P < 0.005$]. A Newman-Keuls post-hoc test confirmed that L-2 at 8 and 15 mg/kg suppresses conditioned burying while it caused an increase on burying behavior latency at 15 mg/kg. L-3 caused significant differences in cumulative burying behavior [$F(6,36) = 6.307$, $P < 0.001$] with no change in burying behavior latency [$F(6,36) = 2.237$; n.s.] observed (Fig. 4C). No change in burying behavior latency was found [Fig. 4D, one-way ANOVA [$F(6,37) = 1.762$, n.s.] for L-4 at any doses evaluated while a decreased effect

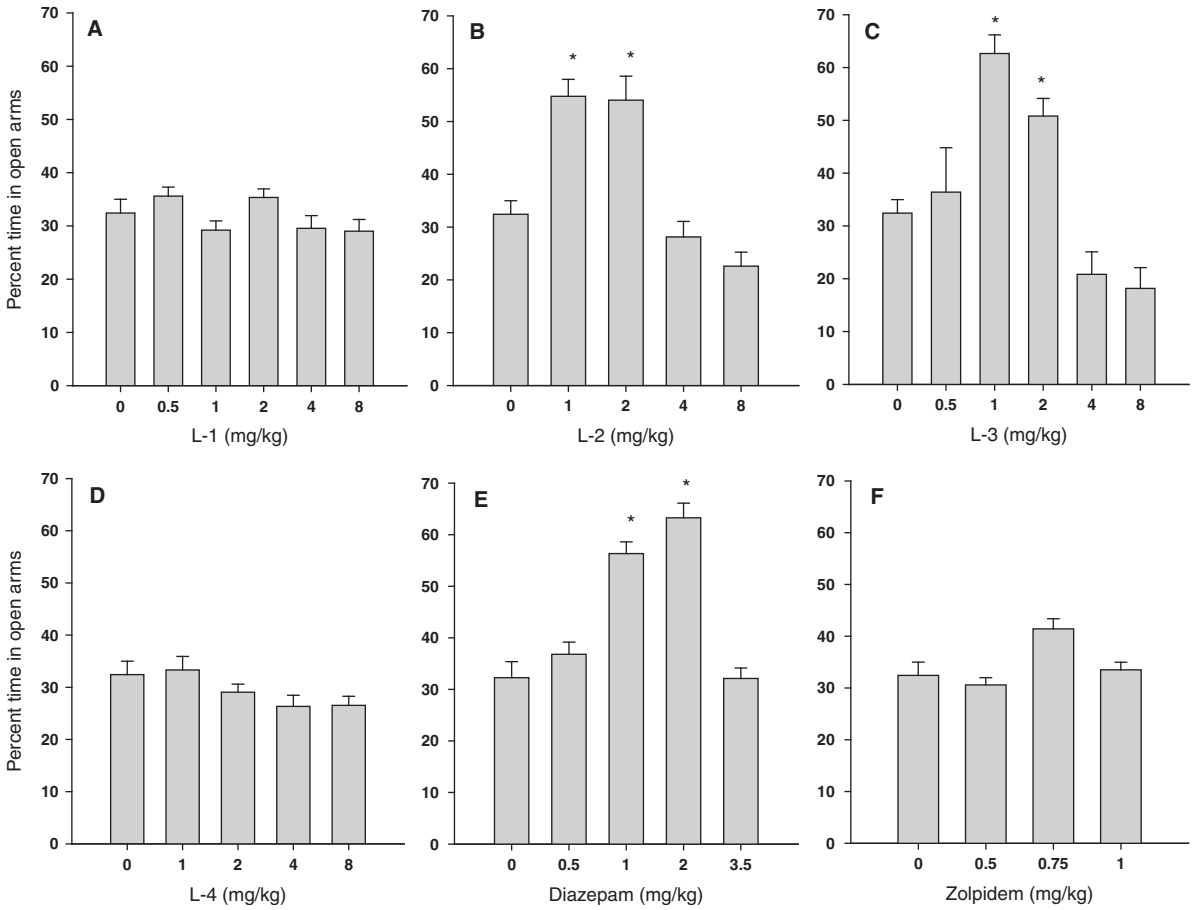

Fig. 3. The effects of various doses of the four derivates of imidazopyridines (**A**, **B**, **C**, **D**), diazepam (**E**), and zolpidem (**F**) on the mean ± SEM percent of time spent in the open arms of the plus maze *Ns* = 5 and 7 groups). $^{*}P<0.05$ versus vehicle control.

$[F(6,37) = 3.648,\ P<0.006]$ on cumulative burying behavior was found.

Diazepam (Fig. 4E) had a significant effect $[F(6,38) = 12.024;\ P<0.001]$ on cumulative burying behavior. Student-Newman-Keuls post-hoc comparisons demonstrated a predictable decrease in the time of cumulative burying behavior with diazepam at doses of 0.75–1.5 mg/kg, with an increase in burying behavior latency occurring at 1.5 mg/kg $[F(6,38) = 8.424;\ P<0.0.001$; Fig. 4E]. Zolpidem decreased cumulative burying behavior $[F(4,26) = 19.69;\ P<0.001]$ at 3 and 10 mg/kg and burying behavior latency increased at the same doses $[F(4,26) = 41.351;\ P<0.001$; Fig. 4F].

Motor Coordination (Rotarod and the Horizontal Wire) Tests

The effects of each of the four imidazopyridines in rats on total numbers of falls on the rotarod (2–64 mg/kg) and the percent of mice with impaired grasping reflex in the horizontal wire test (4–60 mg/kg) was determined. A statistically reliable effect was observed on total numbers of falls for L-1 $[F(6,35) = 2.929;\ P<0.02]$ and L-2 $[F(6,35) = 6.436;\ P<0.001]$ only at the highest dose (64 mg/kg) effects, most likely due to motor effects. The imidazopyridines had no effect on grasping reflex in the horizontal wire test at any doses evaluated while diazepam impaired rotarod performance at 2.75 mg/kg and prevented grasping reflex in 71% of the mice at 1.5 mg/kg (Table 3).

DISCUSSION

In this study a series of imidazo[1,2-*a*]pyridines were synthesized and evaluated for behavioral responses in comparison to zolpidem and the BZ diazepam based on structural analogy with the former and a qualitatively similar performance with the latter.

The results obtained in the rotarod test determined that the imidazo[1,2-*a*]pyridine derivatives L-1 and L-2 impaired rotarod performance only at the highest evaluated dose (64 mg/kg) at which reduction of motor activity was observed (the results are not shown) and thereby no conclusions about myorelaxant effects can be proposed. In contrast, derivates did not exhibit effects on

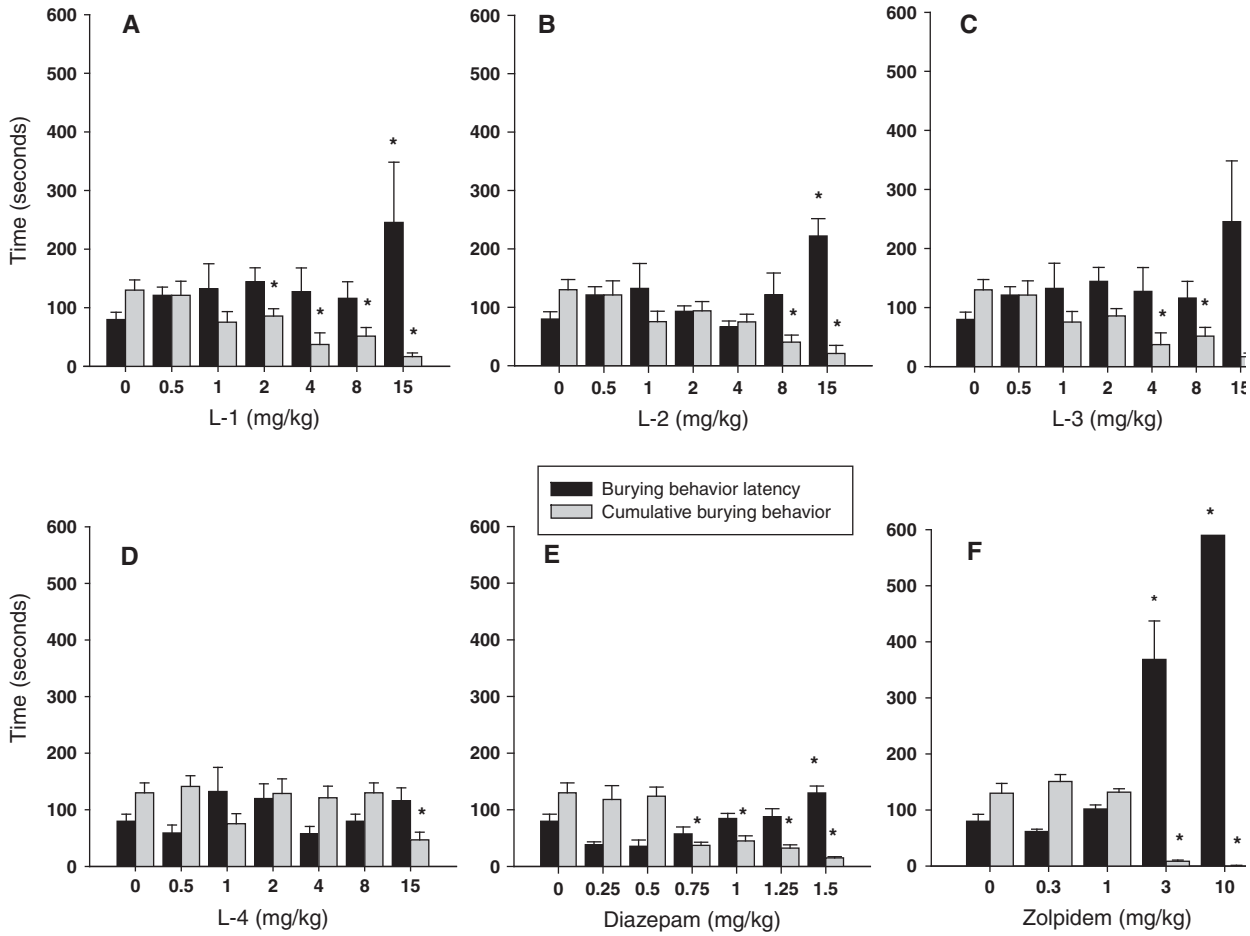

Fig. 4. The effects of the four imidazopyridine derivatives (**A**, **B**, **C**, **D**), diazepam (**E**) and zolpidem (**F**) at various doses on the means ± SEM of cumulative burying behavior and burying behavior latency of male mice (*Ns* = 6 and 7 groups). $^{*}P < 0.05$ versus vehicle control.

the horizontal wire test even at the highest evaluated doses (Table 3). Diazepam impaired the motor ability at minimum doses of 2.75 mg/kg, similar to previous reports [Soderpalm et al., 1988].

Anxiolytic-like behavior was determined for derivatives L-2 and L-3 based on the following facts: Treatment with L-2 or L-3 significantly increased the percent of time spent in the open arms of the plus maze at doses of 1 and 2 mg/kg without modifying the number of total entries. In addition, L-2 and L-3 at 1 mg/kg increased the number of entries to open arms. It must be emphasized that the previous results are not mediated by motor actions (Table 1).

The total number of arm entries is directly related to locomotor activity [Pellow et al., 1986], although this parameter cannot be considered independently of the anxiety state. In agreement with the previous statement, L-1, L-2, and L-3 at higher doses (4 and 8 mg/kg) decreased the number of total entries and the number of entries to open and closed arms. L-4 showed a similar effect at a dose of 8 mg/kg. Results also agree with those obtained in locomotor activity measured on the open field test. As expected, Diazepam significantly increased the open arms entries and percent of time spent in the open arms of the plus maze at doses of 1 and 2 mg/kg without modifying the number of total entries [Soderpalm et al., 1988; Frankowska et al., 2007].

The most widely used behavioral tests of anxiolytic properties of drugs are conflict models. Since the drug effects in conflict models (the Conditioned Defensive Burying test) are positively correlated with BZ receptor binding [Lippa et al., 1978], the effects produced by the imidazopyridines during performance on the plus maze test were compared to those generated from treatment with aolpidem and diazepam on Conditioned Defensive Burying test. Derivatives L-1 (2–15 mg/kg), L-2 (8 and 15 mg/kg), L-3 (4–15 mg/kg), and L-4 (15 mg/kg) induced a clear reduction in cumulative burying behavior. As a consequence, an effect in the reduction in experimental anxiety was obtained [Treit et al., 1980; Treit, 1985]. L-1 and L-2 modified the burying behavior latency only at a dose of 15 mg/kg. This would indicate that the

TABLE 3. Average Results Obtained With Imidazopiridine Treatment on Rotarod (Rat) and Horizontal Wire (Mice) Tests to Evaluated Myorelaxant Effect[a]

	Rotarod		Horizontal Wire test		
Compound	Dose (mg/Kg)	Total falls (5 min)	Dose (mg/Kg)	Total falls (1 min)	Mice with impaired grasping reflex (%)
Tween 80 (10%)	0	1.8±0.3	0	0.33±0.21	0
Diazepam	2.75	5.43±0.61*	1.5	6.0±0.4*	71*
L-1	2	2.0±0.6			
	4	1.3±0.4	4	0.5±0.3	0
	8	1.3±0.2	8	0.3±0.3	0
	16	2.3±0.6	15	2.2±1.4	33
	32	2.6±0.5	30	1.6±0.5	33
	64	4.0±0.9**	60	2.6±0.9	33
L-2	2	1.2±0.4			
	4	2.0±0.3	4	0.7±0.4	0
	8	2.0±0.4	8	1.7±0.4	17
	16	1.7±0.4	15	1.3±0.7	17
	32	2.0±0.4	30	1.6±0.6	17
	64	2.2±0.7	60	1.9±0.6	50
L-3	2	2.7±0.5			
	4	2.0±0.6	4	0.8±0.4	0
	8	2.3±0.6	8	1.0±0.4	0
	16	2.0±0.6	15	2.1±0.7	50
	32	2.3±0.5	30	2.3±0.6	50
	64	3.0±0.6	60	2.2±0.6	50
L-4	2	1.3±0.3			
	4	1.5±0.4	4	0.3±0.2	0
	8	1.5±0.4	8	0.8±0.4	0
	16	2.6±0.5	15	0.3±0.2	0
	32	3.2±0.6	30	0.4±0.4	0
	64	4.2±0.3*	60	1.8±0.6	20

[a]The effects of imidazopyridine derivatives on mean±SEM myorelaxant activity in rat ($N = 6$ and 7 groups) and mice ($Ns = 6,7$/group), *$P < 0.001$, **$P < 0.02$ versus vehicle control.

reactivity of the animal is decreased. However, it is important to note that at such a dose, two out of six animals were sedated. As expected, diazepam reduced conditioned defensive burying at experimental doses 0.75–1.5 mg/kg and increased significantly the burying behavior latency but at sedative doses higher than 1.5 mg/kg. On its own, zolpidem (3 mg/kg) showed significant reduction on both parameters but at the same dose reduced motor activity and, therefore, no conclusions about reactivity or anxiety-like effects can be proposed.

To confirm sedative effects of the drugs, the motor activity was evaluated. The ED_{50} values in mice were calculated using a probit analysis (Table 2). The sedative effect consists of significant reduction in ambulatory and vertical activity. The ED_{50} doses found were: 7.43 mg/kg for L-1, 19.07 mg/kg for L-2, 30.88 mg/kg for L-3, and 17.70 mg/kg for L-4.

All compounds decreased motor activity in mice and rats; thus, the evaluated imidazopyridines had a sedative effect at higher doses than diazepam (0.95 mg/kg) and zolpidem (3 mg/kg). At high doses, mice moved very slowly and showed long pauses with minimum movement and partially closed eyes. These signs were variably present beginning at dose levels above 8 mg/kg for L-1 and 15 mg/kg for L-2, L-3, L-4, with larger standard errors often seen at these borderline doses. Diazepam and zolpiden begin a sedative effect at minimum doses of 1 and 3 mg/kg, respectively.

It has been suggested that imidazopyridines such as zolpidem and alpidem have preferential affinity for the type ω-1 $GABAA_A$/BZ receptor complex (a structure that contains α_1 subunits) [Langer et al., 1985; Depoortere et al., 1986; Benavides et al., 1988], which may suggest that the tested imidazopyridines exert their effect through a gabaergic mechanism.

Different reports [Rudolph et al., 1999; McKernan et al., 2000] indicate that benzodiazepine receptor-active agents produce sedation through interactions with α_1-containing $GABA_A$ BZ receptor complex, and that those compounds without this activity may act as anxyolytic agents (possibly by complexation to other α-subunits of the receptor) with diminished sedative and motor-impairing side effects. In light of the present findings, further investigation of the specificity of imidazopyridines evaluated for $GABA_A$ benzodiazepine receptor subtypes is warranted.

It was concluded that the tested imidazopyridines showed sedative properties at minimum doses of 8 and 15 mg/kg (mice) and 4 and 8 mg/kg (rats). In addition, L-1, L-2, and L-3 produced anxiolytic-like behavior in tested animals. Ongoing efforts are focused on whether the differential anxiolytic and sedative potencies of the four imidazopyridines observed in this study could be attributed to partial agonist actions.

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