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General Pharmacology of LB71350, a New HIV-1 Protease Inhibitor

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Abstract–Safety evaluation of LB71350, a new HIV-1 protease inhibitor, was performed in mice, rats and dogs. For the general behavior of mice, LB71350 at an oral dose of 200 mg/kg did not show any significant effects on muscle tone and locomotor activity. In terms of central nervous system, at oral doses of 200 mg/kg and 1000 mg/kg, LB71350 inhibited acetic acid-induced pain response approximately 41% and 83% of control, respectively. At oral doses of 200 mg/kg and 500 mg/kg, it reduced the rectal body temperature in rats. Pentylenetetrazole-induced seizure in mice was slightly potentiated by oral administration of LB71350 at doses ranging from 200 mg/kg to 1000 mg/kg. Single or five day treatment of LB71350 doubled the hexobarbital-induced sleeping time in mice at oral doses ranging from 50 mg/kg to 500 mg/kg. It did not cause any effects on gastric secretion and acidity in rat at oral doses of 200 mg/kg and 1000 mg/kg and also it did not change intestinal motility in mice up to 1000 mg/kg. Blood coagulation indices such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT) in rats were not affected by the treatment of LB71350 up to 500 mg/kg. LB71350 caused no significant effects on the cardiac output, stroke volume, heart rate, and mean blood pressure when infused intravenously to the anesthetized rats and dogs. Taken together, LB71350 at high oral doses caused significant pharmacological effects on the central nervous system and the hexobarbital-induced sleeping time.

Keywords ☐ HIV-1 protease inhibitor, LB71350, general pharmacology

LB71350 is a newly synthesized, irreversible HIV-1 protease inhibitor and it showed reliable antiviral activities in a time-dependent manner. Fifty percent effective concentration (EC_{50}) was estimated to be 15-20 nM in the MT-2 cells with NL43 when assessed by the p24 and MTT assays (unpublished results). The compound is now under preclinical test in order to evaluate its efficacy as therapeutics to treat acquired immune deficiency syndrome (AIDS) which is caused by the human immuno- deficiency virus transmitted primarily by blood products and sexual interaction (Baker, 1994; Ren and Lien, 1998). The present study was carried out to evaluate the safety pharmacology of LB71350 and to predict potential adverse effects in clinical use. For this, the effects of LB71350 on the general behavior, the central nervous system, the gastrointestinal system, the cardiovascular system, and the blood coagulation system were assessed in experimental animals after oral or intravenous application.

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MATERIALS AND METHODS

Animals

ICR mice (male, 23 g-26 g) and Sprague Dawley rats (male, 240-260 g) supplied by LG Chem animal facility were used for the experiments. Male beagle dogs (8-12 kg) were purchased from Hazleton Research product Inc.(Calamazoo, MI). All the animals were housed and fed with the standard commercial food at LG Chem animal facility where its environment was well controlled (temperature; 20-22°C, 12 hours light and 12 hours dark). All the animals for the oral application of drug were fasted overnight.

Test substance

In order to prepare test article for administration, LB71350 (lot number 05R1P1, purity: 97%) was dissolved in ethanol : propylene glycol : tween 20=10 : 85 : 5 (v/v). Doses of 50, 200, 500 or 1000 mg/kg were applied *per oral* to mice and rats, respectively. Doses of 390 μg/min/kg and 140 μg/min/kg were applied by infusion to anesthetized rats and dogs,

respectively. Equal volume of vehicle was applied to control animals.

Statistics

All the data were expressed as mean standard error of the mean and the statistical analysis was performed with *students t-test* at the significance level of $p<0.05$ and $p<0.01$.

General behavior

According to the method of Irwin (Irwin, 1968), behavioral profiles including spontaneous motor activity, posture, muscle tone, equilibrium and gait were observed in mice prior to and at 0.5, 1, 2, and 4h after oral administration of 200 mg/kg of LB71350.

Effects on central nervous system

Effects on acetic acid-induced writhing response

Experiments were performed according to the method of Koster (Koster, 1959). At sixty minutes after oral administration of 50, 100, 200, and 1000 mg/kg of LB71350, the mice were treated with an intraperitoneal injection of 10 ml/kg of 0.6% acetic acid solution. The onset time of the first writhing response was recorded and the number of writhing was measured during the following 10 minutes.

Rota-rod test

Experiments were carried out as described in the method of Dunham (Dunham, 1957). One day before experiment, the mice underwent five minutes of learning process by placing them on the rota-rod in opposite direction to the rotation of the rota-rod. Just before experiment, the mice underwent the aforementioned process and the animals that does not fall down within 3 minutes were used for the experiment. The test was carried out prior to and at 30, 60, 120, and 240 minutes after oral administration of 200 mg/kg and 1000 mg/kg of LB71350. Chlorpromazine (CPZ) treatment (10 mg/kg) was set up as a reference. The number of mice falling down within 2 minutes when subjected to rotation were counted to indicate impairment of motor coordination.

Effects on hexobarbital induced-hypnosis

Single dose treatment

At sixty minutes after oral administration of 200 mg/kg and 500 mg/kg of LB71350, mice were injected intraperitoneally with 70 mg/kg of sodium hexobarbital. Then, sleeping time was measured during the time interval between the loss and the recovery of the righting reflex.

Five day of multi-dose treatment

Every morning animals were treated with oral doses of 50 mg/kg and 200 mg/kg for 5 days. At sixty minutes after the last treatment, the mice were injected intraperitoneally with 70 mg/kg of sodium hexobarbital. Then, sleeping time was measured during the time interval between the loss and the recovery of the righting reflex.

Effects on rectal body temperature

Rectal body temperature was recorded in each animal with an electric rectal thermometer (YSI 400 series, YSI Incorporated, Yellow Spring Instruments Co., Inc. USA) prior to and at 30, 60, 120, and 240 minutes after oral administration of 200mg/kg and 500mg/kg of LB71350, respectively.

Effects on pentylenetetrazole-induced seizure

At sixty minutes after oral administration of 100, 200, 500, 1000 mg/kg of LB71350, mice were treated with the intraperitoneal injection of 65 mg/kg of pentylenetetrazole. The presence or absence of tonic extension, flexion of the hind paws, and death were observed (Williams, P.D., *et. al.*, 1988).

Effects on gastrointestinal system

Effects on intestinal propulsion

Experiments were performed as described by Takemori *et al.* (1969). At sixty minutes after oral administration of 200, 500, 1000 mg/kg of LB71350, respectively, mice were treated with 0.25 ml of suspension of 5% charcoal in 0.5% CMC by gavage. At thirty minutes after sacrificing the mice, the gastrointestinal tract was removed and the distance traveled by the charcoal was measured. The passage rate(%) was calculated by the following formula: passage rate(%)= (length of charcoal passage from pylorus)/(total length of intestine from pylorus to cecum) $\times$ 100.

Effects on gastric acid secretion

At sixty minutes after oral administration of 50, 200, 1000 mg/kg of LB71350 to the rats, the abdominal region was dissected and pylorus ligated. Four hours later, the rats were sacrificed, stomach removed, and then volume and pH of gastric juice were measured.

Effects on blood coagulation system

At sixty minutes after oral application of 250 mg/kg and 500 mg/kg of LB71350 to rats, blood was drawn through heart puncture. Prothrombin time (PT), thrombin time (TT) and activated partial thromboplastin time (aPTT) were measured with simplastin (Organon Teknika Corporation), thrombin time reagent (sigma) and platelin LS (Organon Teknika Corporation) kit, respectively using an autocoagulometer (Coag-A-Mate RA4, Organon Teknika Corporation,USA).

Effects on cardiovascular system

Effects on cardiovascular system in rats

Rats were anesthetized with an intraperitoneal injection of urethan (1.25 g/kg). One cannula was inserted through left

jugular vein to measure cardiac output (CO) and stroke volume (SV). Another cannula was inserted through the left femoral artery to measure mean blood pressure (BP) and heart rate (HR). Rats were treated with an infusion of the 390 g/min/kg of LB71350 dissolved in 25% hydroxypropyl--cyclodextrin (HPCD) via right femoral vein for 2 hours. Control group animals were treated with vehicle solution (25% HPCD). All the cardiovascular parameters were automatically measured with Cardio MaxII (Colombus Instruments, Inc, Ohio) at every 10 minutes for 2 hours during infusion period . All the obtained data were expressed by the mean values of the percent changes. The percent changes were calculated by the following formula; the percent change(%)=(value of each time point after infusion-value prior to infusion)/(value prior to infusion)×100.

Effects on cardiovascular system in dogs

Dogs were anesthetized with an intravenous injection of sodium pentobarbital (50 mg/kg). The right jugular vein was isolated and thermal dilution balloon catheter (Electrocatheter corporation, NJ) was introduced to pulmonary artery to measure cardiac output (CO) and stroke volume (SV). Cannula was inserted into the left femoral artery to measure mean blood pressure (BP) and heart rate (HR). LB71350 dissolved in 25% hydroxypropyl-β-cyclodextrin (HPCD) was applied to the dog via the infusion of 140 μg/min/kg through the left femoral vein for 2 hours. All cardiovascular parameters were automatically measured every 10 minutes for 2 hours during infusion period with CardioMaxII (Colomus Instruments, Inc, Ohio). All the obtained data were expressed by the mean values of the percent changes. The percent changes were calculated by the following formula; the percent change(%)=(value of each time point after infusion-value prior to infusion)/(value prior to infusion)×100.

RESULTS

Various pharmacological effects of LB71350 in mice, rats and dogs were observed following oral and intravenous administration.

Table I. Effects of LB71350 on the acetic acid-induced writhing response in mice (*p.o*)

Durg	Dose (mg/kg)	No of Animals	Writhing(%) Mean±S.E.
Vehicle	0	15	100±20.6
LB71350	50	9	90.7±10.4
LB71350	100	9	70.9±11.0
LB71350	200	19	38.6±8.0*
LB71350	1000	9	12.0±16.5**

Significant difference from the vehicle-treated group(*p<0.05, **p<0.01). Vehicle(Ethanol: Propyleneglycol: Tween 20=10:85:5). Writhing was induced by 0.6% acetic acid solution.

Effects on general behavior

The oral administration of 200 mg/kg of LB71350 did not produce any significant effects on muscle tone and spontaneous motor activities in mice (qualitative findings, data not shown).

Effects on central nervous system

Effects on acetic acid-induced writhing response

The oral administration of LB71350 at 50 mg/kg and 100 mg/kg did not produce any significant effects on the writhing and its onset time as compared with the control. At doses of 200 mg/kg and 1000 mg/kg of LB71350, however, significant inhibition of writhing was observed (41% and 83% of control, respectively) in mice (Table I).

Effects on rota-rod test

The oral administration of LB71350 at doses of 200 mg/kg and 1000 mg/kg caused no effect on rota-rod test throughout the observation period, whereas oral administration of 10mg/kg chlorpromazine used as positive control caused the significant inhibition of motor coordination in mice (Table II).

Effects on hexobarbital-induced hypnosis

Single dose treatment

Oral administration of LB71350 at 200 mg/kg and 500 mg/kg to mice prolonged the sleeping time by two folds as compared with the control group (Table III).

Five day multi-dose treatment

Table II. Effects of LB71350 on the rota-rod test in mice(*p.o.*)

Drug	Dose(mg/lg)	No. of Animals	No. of dropped animals			
			30 min	1 hr	2 h	4 h
Vehicle	0	10	0	0	0	1
LB71350	200	10	0	0	0	1
LB71350	1000	10	0	0	0	0
Chlorpromazin	10	11	10	11	7	5

Vehicle (Ethanol: Propyleneglycol: Tween 20=10:85:5)

Table III. Effects of LB71350 on sodium hexobarbital-induced sleeping time in mice at single dose treatment (*p.o.*)

Drug	Dose(mg/kg)	No. of Animals	Onset time (min) (Mean±S.E.)	Duration time (min) (Mean±S.E.)
Vehicle	0	10	2.13±0.17	39.22±4.70
LB71350	200	10	1.22±0.08	52.47±6.18*
LB71350	500	10	2.27±0.25	77.72±8.58**

Significant difference from the vehicle-treated group (*p<0.05, **p<0.01). Vehicle (Ethanol: Propyleneglycol: Tween 20=10:85:5).

Table IV. Effects of LB71350 on sodium hexobarbital-induced sleeping time in mice at 5 day multi-dose treatment (*p.o.*)

Drug	Dose (mg/kg)	No. of Animals	Onset time (min) (Mean±S.E.)	Duration time (min) (Mean±S.E.)
Vehicle	0	10	2.47±0.27	27.77±3.83
LB71350	50	10	2.05±0.32	43.82±5.43*
LB71350	100	10	1.87±0.18*	41.90±5.37*

Significant difference from the vehicle-treated group (*p<0.05). Vehicle (Ethanol: Propyleneglycol: Tween 20=10:85:5).

Table V. Effects of LB71350 on rectal body temperature in rats (*p.o.*)

Drug	Dose (mg/kg)	Rectal body temperature (°C), Mean±S.E.				
		0 min	30 min	60 min	120 min	240 min
Vehicle	0	34.5±0.18	35.0±0.34	34.3±0.22	33.9±0.23	34.6±0.17
LB71350	200	34.5±0.63	34.0±0.40	33.2±0.41	32.4±0.69	32.0±0.86*
LB71350	500	35.0±0.09	34.1±0.29	33.6±0.36	32.9±0.26*	32.8±0.44*

Significant difference from the vehicle-treated group (*p<0.05). Vehicle (Ethanol: Propyleneglycol: Tween 20=10:85:5). N=5 in each group.

Oral administration of LB71350 to mice for 5 days at doses of 50 mg/kg/day and 200 mg/kg/day prolonged the sleeping time by two folds as compared with the control group (Table IV).

Effects on rectal body temperature

For the first sixty min after oral treatment of LB71350 at doses of 200 mg/kg and 500 mg/kg, no significant changes in the rectal body temperature were observed in rats. However, at the same doses, LB71350 induced the significant decreases in the rectal body temperature throughout 120 min to 240 min of the observation periods (Table V).

Effects on pentylenetetrazole-induced seizure

No alterations in the pentylenetetrazole-induced clonic seizure were observed in mice following oral administration of LB71350 at dose of 100 mg/kg. However, LB71350 administered orally at doses of 200 mg/kg, 500 mg/kg and 1000 mg/kg, respectively changed clonic convulsion into tonic convulsion in the frequencies of 3 out of 9, 2 out of 11, and 3 out of 9, respectively, and death was observed in all of these groups (Table VI).

Effects on gastrointestinal system

Effects on intestinal propulsion

The oral administration of LB71350 at doses of 200 mg/kg, 500 mg/kg, and 1000 mg/kg caused no observable effects on the intestinal motility in mice (Table VII).

Table VII. Effects of LB71350 on the intestinal propulsion in mice (*p.o.*)

Drug	Dose (mg/kg)(	No. of Animals	Assage rate (%) Mean±S.E.
Vehicle	0	15	60.78±2.33
LB71350	200	7	62.45±2.81
LB71350	500	9	63.08±3.19
LB71350	1000	8	63.67±4.24

Passage rate (%)=(length of charcoal passage from pylorus) divided by (total length of intestine from pylorus to cecum)×100.

Table VI. Effects of LB71350 on the pentylenetetrazole-induced seizure in mice (*p.o.*)

Drug	Dose (mg/kg)	No. of Animals	Clonic Convulsion	Tonic Convulsion	No. of Death
Vehicle	0	9	9	0	0
LB71350	100	11	11	0	0
LB71350	200	9	9	3	1
LB71350	500	11	11	2	2
LB71350	1000	9	9	2	3

Convulsion was induced by pentylenetetrazole (65 mg/kg)

Table VIII. Effects of LB71350 on gastric acid secretion in rats (*p.o.*)

Drug	Dose (mg/kg)	No. of Animals	pH Mean±S.E.	Volume (ml) Mean±S.E.
Vehicle	0	9	1.20±0.04	7.24±0.42
LB71350	50	4	1.37±0.10	7.03±0.66
LB71350	2000	4	1.61±0.27	6.38±0.18
LB71350	1000	4	1.69±0.64	7.73±1.82

Effects on gastric acid secretion

No significant changes in the gastric secretion and pH were observed in rats following oral administration of LB71350 at doses of 50 mg/kg, 200 mg/kg, and 1000 mg/kg, respectively. Only slight increase in pH was shown at oral dose of 1000 mg/kg (Table VIII).

Effects on blood coagulation system

Prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT) which reflect the functionality of blood coagulation system were not affected in rats by the oral administration of LB71350 at doses of 250 mg/kg and 500 mg/kg (Table IX).

Effects on cardiovascular system

Effects on cardiovascular system in rats

No significant changes in the aforementioned cardiovascular parameters were observed in rats treated with a dose of 390 μg/min/kg of LB71350 via infusion for 2 hours as compared with vehicle-treated control (Fig. 1).

Effects on cardiovascular system in dogs

No significant changes in the aforementioned cardiovascular parameters, except slight reduction in stroke volume at

Table IX. Effects of LB71350 on the blood coagulation system (*p.o.*)

Drug	Dose (mg/kg)	No. of Animals	TT (sec) Mean±S.E.	aPPT (ase) Mean±S.E.	PT (sec) Mean±S.E.
Vehicle	0	5	18.2±1.0	16.3±0.7	11.4±0.2
LB71350	250	5	20.1±0.1	17.6±0.4	12.0±0.2
LB71350	250	5	20.1±0.8	18.2±0.9	11.3±0.3

TT, aPTT and PT were measured as described in "Materials and Methods"

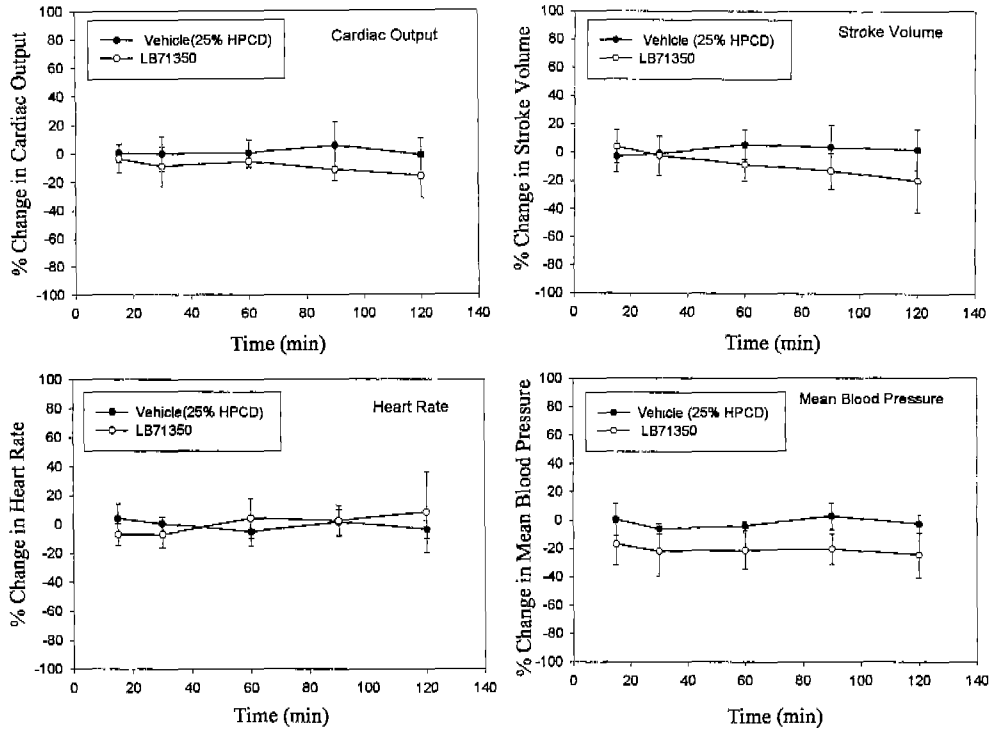

Fig. 1. Effects of LB71350 on the cardiovascular system in rats. Rats were treated with an infusion of the 390 μg/min/kg of LB71350 dissolved in 25% hydroxypropyl-β-cyclodextrin (HPCD) via right femoral vein for 2 hours. Control group animals were treated with 25% hydroxypropyl-cyclodextrin (HPCD) in the same manner. Cardiac output, Stroke volume, Heart rate, and Mean blood pressure were measured as described in "Materials and Methods".

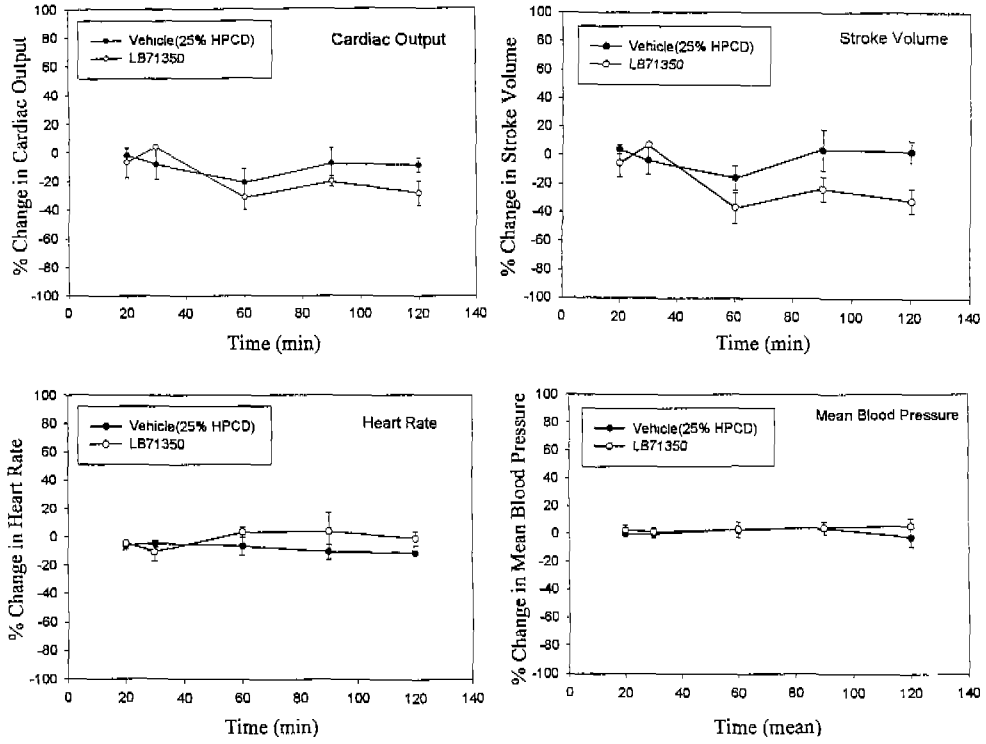

Fig. 2. Effects of LB71350 on the cardiovascular system in dogs. LB71350 dissolved in 25% hydroxypropyl-β-cyclodextrin (HPCD) was applied to the dog via the infusion of 140 μg/min/kg through the left femoral vein for 2 hours. Control group animals were treated with 25% hydroxypropyl-β-cyclodextrin (HPCD) in the same manner. Cardiac output, Stroke volume, Heart rate, and Mean blood pressure were measured as described in "Materials and Methods".

90 min and 120 min were observed in beagle dogs treated with a dose of 140 μg/min/kg of LB71350 via infusion for 2 hours as compared with the vehicle-treated control group (Fig. 2).

DISCUSSION

In order to evaluate and predict the safety of LB71350, general pharmacological studies were performed with high doses in terms of the effects on general behavior, central nervous system, gastrointestinal system, blood coagulation system, and cardiovascular system. It was shown that blood concentration of LB71350 was maintained at the level of approximately 8-12 μg/ml for 2-4 hours following oral administration of 200-500 mg/kg to rats (Investigators Report, LG Chemical, Ltd.). Therefore, experiments were performed at 30 min to 240 min after administration of test article. Results are summarized as follows; LB71350, at oral dose of 200 mg/kg induced analgesic action, potentiated the pentylenetetrazole-induced seizure, and prolonged hexobarbital-induced hypnosis in mice, respectively. In addition, LB71350, at oral doses of 200 mg/kg and 500 mg/kg caused decrease in body temperature of rats. However, no significant changes in general behavior, rota-rod test, gastrointestinal system, blood co-agulation system, and cardiovascular system were observed in mice, rats, and dogs with oral or intravenous application of high doses of LB71350. Although it does not show any significant effects on the spontaneous locomotor activities, LB71350 potentiated the pentylenetetrazole-induced seizure, indicating its stimulatory effects on central nervous system. In addition, oral administration of LB71350 significantly prolonged the hexobarbital-induced hypnosis, which might indicate that LB71350 inhibits the hepatic drug metabolizing enzymes. More studies are necessary to elucidate the effects of LB71350 on the liver cytochrome p450 enzyme system. In conclusion, LB71350, at high doses of oral application (200 mg/kg to mice and 1000 mg/kg to rats, respectively) exhibited significant pharmacological effects on the central nervous system and the hexobarbital-induced hypnosis. However, at clinically relevant dosages it is supposed to exert its anti-HIV activity with a little adverse effects.

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