Arch Pharm Res Vol 23, No 1, 72-78, 2000

General Pharmacological Properties of YJA20379-2, a New Antiulcer Agent

Eun Bang Lee[1], Sung Ig Cho[1], Seon Ah Cheon[1], Man Sik Chang[2], Kyu Bong Kim[2], Tae Wook Woo[2], and Young Kuk Chung[2]

[1]Natural Products Research Institute, Seoul National University, Seoul 110-460 and [2]Pharmacology and Toxicology Laboratory, Yung-Jin Pharmaceutical Co., Ltd., Kyunggi-do 445-850, Korea

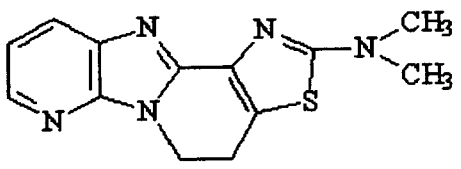
(Received October 8, 1999)

The general pharmacological properties of YJA20379-2 2-dimethylamino-4,5-dihydrothiazolo[4,5:3,4]pyridol[1,2-a]benzoimidazole, a novel proton pump inhibitor with antiulcer activities were investigated in mice, rats, guinea pigs and rabbits. YJA20379-2 at oral doses of 50, 100 and 200 mg/kg did not affect the general behaviour, hexobarbital hypnosis and motor coordination in mice. The drug did not have analgesic or anticonvulsant action at 200 mg/kg. Locomotor activity and body temperature were not influenced at 100 mg/kg. At a concentration up to 2×10^{-4} g/ml, YJA20379-2 did not produce any contraction or relaxation of isolated preparations, such as the rat fundus, the guinea pig ileum and the rat uterus, and did not antagonize the contractile response to several spasmogens, such as histamine, acetylcholine, serotonin and oxytocin. At dosages up to 200 mg/kg p.o. YJA20379-2 did not affect the pupil size of mice. Intestinal propulsion of mice was not affected up to 200 mg/kg p.o. and the drug did not affect urinary excretion at 100 mg/kg p.o. These results indicate that at dosages up to 100 mg/kg p.o. YJA20379 was found not to affect this pharmacological profile. However, at 200 mg/kg the drug lowered body temperature and showed decreases in locomotor activity and urine volume.

Key words: 2-Dimethylamino-4,5-dihydrothiazolo[4,5:3,4]pyridol[1,2-a]benzoimidazole,YJA 20379-2, Proton pump inhibitor

INTRODUCTION

YJA20379-2, 2-dimethylamino-4,5-dihydrothiazolo [4,5: 3,4] pyridol[1,2-a]benzomidazole is a newly synthesized proton pump inhibitor. Chung *et al.* (1998) reported that the compound showed eight times more potency than omeprazole in terms of proton pump inhibitory activity. This paper describes the results of an investigation of the general pharmacological properties of YJA20379-2. Its structural formula is as follows.



Chemical structure of YJA20379-2

Correspondence to: Eun Bang Lee, Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea
E-mail: eblee@snu.ac.kr

MATERIALS AND METHODS

Materials

YJA20379-2 synthesized by Yung-Jin Pharmaceutical Co., Ltd. was used for this study. For oral administration, the test compound was suspended in 1% CMC and the suspension was given in a volume of 10 ml/kg to mice or rats. For i.v. injection or for addition to the organ bath in the case of isolated organ preparations, it was dissolved in DMSO. The following drugs were obtained from Sigma Chemical Co. Ltd. (St. Louis, MO, USA) : histamine dihydrochloride, acetylcholine chloride and serotonin hydrochloride. The other drugs were either pharmaceutical or reagent grade.

Animals

Male ICR mice and Sprague-Dawley rats of either sex were supplied by the breeding facilities of this Institute. Male New Zealand White rabbits and male guinea pigs were supplied from Daehan Laboratory Animal Research Centre Co. Ltd., Seoul. All animals were

housed at a temperature of 22-25°C, humidity of 55 ± 10% and under 12 h on and off lighting. Solid food was supplied from Samyang Yooji Co., Ltd. (Won-Joo, Korea). The control and treated groups were divided at random.

Effects on the general behaviour in mice

Groups of 5 mice weighing 22-25 g received 50, 100 or 200 mg/kg p.o. of YJA20379-2 for the test. Their gross behaviors were observed 30, 60 and 120 min after treatment according to a modification (Lee, 1975) of the method described by Irwin (1968), that is, their behaviors were checked using the rating scales of -4~+4 for behavioral, neurological and auto-nomic profiles.

Effects on central nervous system

Spontaneous locomotor activity in mice : The test was conducted according to the method of Nahorski (1975). Male mice weighing 20-23 g were used in groups of 12. Two animals were kept in an activity cage (Ugo Basile, Italy) for 30 min. for adaptation pur-poses. The next day, 30 min after the oral administration of the drug at 50, 100 and 200 mg/kg, the locomotor activity of the two animals was measured for 30 min. Anhydrous caffeine was used as reference.

Motor coordination in mice : The test was conducted according to the method of Dunham *et al.* (1957). Male mice weighing 20-25 g were used in groups of 6. The mice which remained for more than 1 min cling-ing to the rotating rod were selected for the test. Thirty min after oral administration of the drug at 50, 100 and 200 mg/kg, the number of animals falling off from the rotarod within 1 min. was counted. Chlorpromazine hydrochloride was used as reference.

Hexobarbital-induced hypnosis in mice : Male mice weighing 22-25 g were used in groups of 7. Thirty min after the oral administration of the drug at 50, 100 and 200 mg/kg, 50 mg/kg of hexobarbital · Na was i.p. injected and the time between righting reflex loss and arousal was measured. Chlorpromazine hydrochloride was used as reference.

Body temperature in mice : Male mice weighing 22-26 g were used in groups of 7. Before dosing, the rectal temperature of each animal was measured using a digital rectal thermometer (Shibaura Eleconics Co., Japan). The temperature was measured before and at 30, 60, 90 and 120 min after oral administration of the drug at 50, 100 and 200 mg/kg. Aminopyrine was used as reference.

Acetic acid-induced writhing syndrome in mice : The test was conducted according to the method of Whittle (1957). Male mice weighing 22-26 g were used in groups of 7. Thirty min after oral administration of the drug at 50, 100 and 200 mg/kg, the animals were subjected to an i.p. injection of 0.7% acetic acid saline. Ten min. later, the number of writhings exhibited for 10 min was recorded. Aminopyrine was used as reference.

Strychnine-induced convulsion in mice : The test was conducted according to the method of Araki and Ueki (1972). Male mice weighing 22-26 g were used in groups of 6. Thirty min after the oral administration of the drug at 50, 100 and 200 mg/kg, the animals received strychnine at 1.5 mg/kg s.c. The number of which mice died within 30 min was counted. Diallyl-barbiturate was used as reference.

Pentetrazol-induced convulsion in mice : The test was conducted according to the method of Swinyard and Ueki(1952). Male mice weighing 22-26 g were used in groups of 6. Thirty min after the oral administration of the drug at 50, 100 and 200 mg/kg, 85 mg/kg of pentetrazol was injected s.c. Abolition of the hindleg tonic extensor phase of the seizure was used as the criterion for protection against convulsion. The number of protected mice was counted. Chlorpromazine was used as reference.

Maximal electroshock-induced convulsion in mice : The test was conducted according to the method of Araki and Ueki (1972). Male mice weighing 20-25 g were used in groups of 10. Thirty min after oral administration of the drug at 50, 100 and 200 mg/kg, the animals received a 60Hz alternating current of 50 mA delivered for 0.2 sec via corneal electrodes, and the number of mice which survived was counted. Phenobarbital · Na was used as reference.

Effects on the respiratory and cardiovascular systems in rabbits

The depth and rate of respiration of three rabbits weighing 1.8-2.2 kg anesthetized by urethane-saline (2 g/kg, s.c.) were measured using a pneumatic pulse transducer (Narco Biosystems Inc. Houston, Tex., U.S.A.) and the direct blood pressure was simultaneously measured, through a canula inserted into the left common carotid artery, using a blood pressure transducer (P-1000B). The drug was injected in the ear vein at a dose of 5, 10 or 20 mg/kg.

Effects on autonomic nervous system and isolated smooth muscles

Pupil size in mice : The diameters of the pupils of mice weighing 22-26 g in groups of 6 were measured with a micrometer before, and 30 and 90 min after the oral administration of the drug at 50, 100 and 200 mg/kg. Atropine sulfate was administered as reference.

Isolated guinea pig ileum : Male Hartley guinea pigs weighing 160-180 g were used in groups of 4. About 2 cm segments of ilea were dissected and suspended in an organ bath containing Locke-Ringer solution at

33, and bubbled with a mixture of 95% O_2 and 5% CO_2. The Locke-Ringer solution contained 9.0 g of NaCl, 0.42 g of KCl, 0.24 g of $CaCl_2$, 0.2 g of $MgCl_2$, 0.5 g of $NaHCO_3$, and 0.5 g of glucose per litre. The resting tension was 0.5 g. The contractile responses were recorded using the isometric force-displacement transducers of a physiograph (Narco Biosystems, Inc. Italy).

Isolated fundus strip of rats : Stomach fundus strips from male rats weighing 160-200 g which had fasted for 24 h before the experiment were made according to the method of Vane (1957). The fundus strips were suspended in an organ bath containing Locke-Ringer solution which was maintained at 33°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The resting tension was 1 g. Isometric contractile responses were monitored on a recorder as described in the experiment on the guinea pig ileum.

Isolated rat uterus : Virgin rats weighing 170-200 g were primed with a s.c. injection of estradiol (1.0 mg/kg) 24 h before use. The animals, in groups of 4, were sacrificed and the uterine horns were dissected and then suspended in an organ bath containing Locke-Ringer solution at 34°C and bubbled with a mixture of 95% O_2 and 5% CO_2. The isometric contractile responses in the presence of 1 g of resting tension were monitored on a recorder, as described in the experiment on the guinea pig ileum.

Effects on gastrointestinal system

Intestinal propulsion in mice : The test was conducted according to the method of Takagi and Lee (1972). Male mice weighing 22-26 g were used in groups of 8. Thirty min after the oral administration of the drug at 50, 100 and 200 mg/kg, 0.2 ml of $BaSO_4$ suspension (1 g/ml) was given orally and 30 min later, the animals were sacrificed. The intestine was removed and the distance to which $BaSO_4$ had been propelled was measured. This propelled distance was calculated as a ratio of the full length of the small intestine. Lidocaine hydrochloride was used as reference.

Diuretic effect in rats

Male rats weighing 150-170 g were used in groups of 6. The test was performed using a modification of the method of Gilman and Goodman (1937). Briefly, the rats were fasted for 24 h, with free access to water, and were orally administered at doses of 50, 100 and 200 mg/kg of the drug and 0.9% NaCl solu- tion at a volume which corresponded to 2.5% of the body weight of rats was given p.o. Three hr later the solution was again administered orally at a volume of 5% to the body weight. Five hr after this administration, the total volumes of urine were measured and the levels of sodium and potassium in urine were measured using an atomic absorption spectrophotometer (Perkin-Elmer, Wilton, CT, U.S.A.)

Statistical analysis

All data represent the mean ± S.E.M. Statistical analysis of the data was performed using an analysis of variance followed by Dunnett's *t*-test, unless otherwise specified, or *Chi*-square test as specified in each table. All data was evaluated at the $p<0.05$ level of significance.

RESULTS

Effects on the general behaviour in mice

YJA20379-2 at oral doses of 50, 100 and 200 mg/kg did not show any effects on the general behaviour of mice (the data not shown).

Effects on central nervous system

Spontaneous locomotor activity in mice : A single oral administration of YJA20379-2 at 50 or 100 mg/kg did not affect spontaneous activity. However, at a dose of 200 mg/kg, it significantly increased this activity (Table I). Anhydrous caffeine was found to increase markedly the activity.

Motor coordination in mice : YJA20379-2 at oral doses of 50, 100 and 200 mg/kg had no effect on motor coordination as measured by the rotating rod test in mice (Table I).

Hexobarbital-induced hypnosis in mice : YJA20379-2 at oral doses of 50, 100 and 200 mg/kg had no effect on the duration of hexobarbital-induced sleeping time in mice (Table I).

Body temperature in mice : YJA20379-2 at oral doses of 50 and 100 mg/kg did not influence the rectal temperature of mice. However, at the highest dose of 200 mg/kg, it significantly lowered the normal body temperature (Table I).

Acetic acid-induced writhing syndrome in mice : YJA20379-2 at oral doses of 50, 100 and 200 mg/kg had no significant effect on acetic acid-induced writhing syndromes in mice (Table I).

Strychnine-induced convulsion in mice : YJA20379-2 at oral doses of 50, 100 and 200 mg/kg had no effect on strychnine-induced death due to convulsion in mice (Table I).

Pentetrazol-induced convulsion in mice : YJA 20379-2 at oral doses of 50, 100 and 200 mg/kg had no effect on pentetrazol-induced convulsion in mice (Table I).

Maximal electroshock-induced convulsion in mice : YJA20379-2 at oral doses of 50, 100 and 200 mg/kg had no effect on the death rate induced by electroshock in mice. The 50% death rate of the control group was

Table I. Some results of pharmacological profile of YJA20379-2 in mice

Effect	Test	Drug	Dose (mg/kg, p.o.)	Response[a]
1. CNS	(1) Locomotor activity	YJA 20379-2	50	-
			100	-
			200	+
		Caffeine	10	+
	(2) Rotarod test	YJA 20379-2	50	-
			100	-
			200	-
		Chloropromazine	10	+
	(3) Hexobarbital sleeping time	YJA 20379-2	50	-
			100	-
			200	-
		Chloropromazine	5	+
2. Body temperature	(1) Rectal temperature	YJA 20379-2	50	-
			100	-
			200	+
		Aminopyrine	200	+
3. Analgesic	(1) Writhing test	YJA 20379-2	50	-
			100	-
			200	-
		Aminopyrine	50	+
4. Anticonvulsant	(1) Strychinine mortality test	YJA 20379-2	50	-
			100	-
			200	-
		Diallybarbiturate	60	+
	(2) Pentetrazol induced convulsion test	YJA20379-2	50	-
			100	-
			200	-
		Chloropromazine	60	+
	(3) Maximal electroshock test	YJA 20379-2	50	-
			100	-
			200	-
		Phenobarbital·Na	50	+

a) The significant differences at a level of $p<0.05$ in Dunett's *t*-test or *Chi*-square test.

shown by a group of mice which were given 50 mg/kg of phenobarbital sodium. However, the death rate of the treated group was not significantly different from that of the control group (Table I).

Effects on respiratory and cardiovascular systems in rabbits

The results are shown in Fig. 1. YJA20379-2 did not affect the respiration or blood pressure at an intravenous dose of 5 mg/kg. The heart and respiration rates were also not influenced at these doses (not shown in the figure). However, at 10 mg/kg i.v. the respiration depth was increased and the blood pressure decreased.

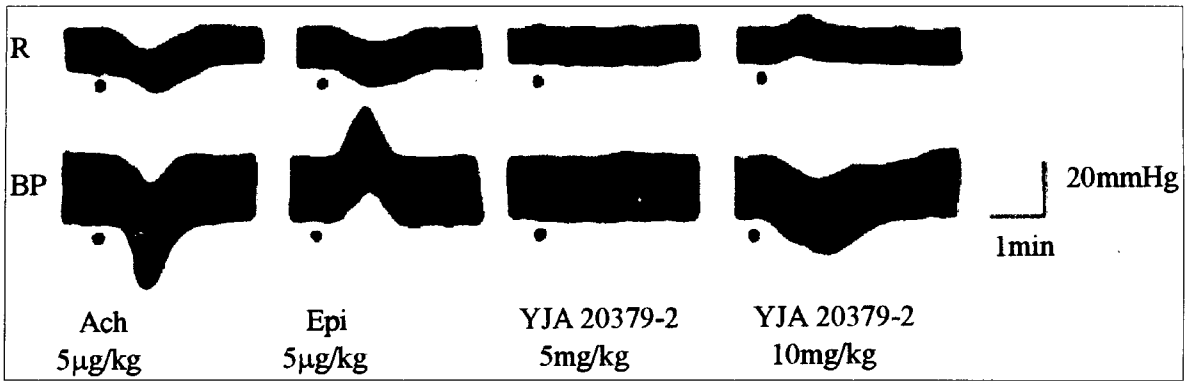


Fig. 1. Effect of YJA20379-2 on the respiration and blood pressure of anesthetized rabbits. Each black-filled circle represents the addition point of each sample. R: Respiration, BP: Blood pressure, ACh: Acetylcholine, Epi: Epinephrine

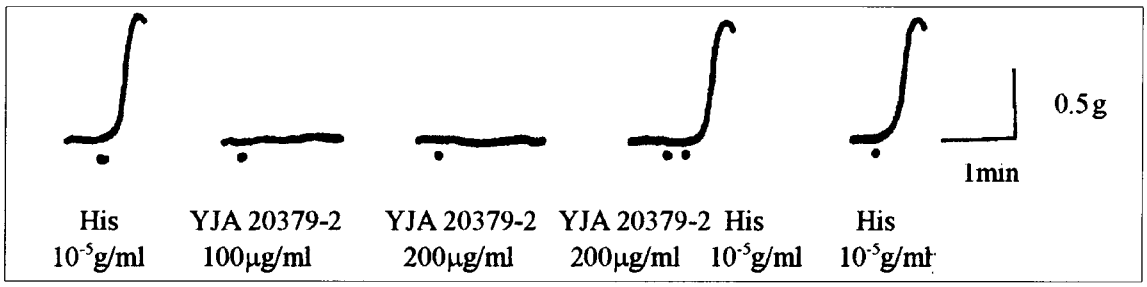


Fig. 2. Effect of YJA20379-2 on the isolated guinea pig ileum. Each black-filled circle represents the addition point of each sample. His: Histamine, ACh: Acetylcholine

Table II. Effect of YJA20379-2 on pupil size in mice

Treatment	Dose (mg/kg, p.o.)	No. of mice	Pupil size (mm)		
			0	30	90 min
Saline	-	6	0.653±0.013	0.661±0.017	0.660±0.017
YJA 20379-2	50	6	0.673±0.020	0.671±0.023	0.665±0.023
	100	6	0.663±0.025	0.666±0.029	0.666±0.028
	200	6	0.685±0.019	0.688±0.023	0.691±0.021
Atropine	2	6	0.680±0.018	1.265±0.057*	1.658±0.019*

All data represent the mean±S.E.M.
Significantly different from the control group (*$p<0.05$)

Effects on autonomic nervous system and isolated smooth muscles

Pupil size in mice : The oral administration of atropine at 2 mg/kg caused significant mydriasis in mice. However, YJA20379-2 at oral doses of 50, 100 and 200 mg/kg did not affect the mice pupil size (Table II).

Isolated guinea pig ileum : Histamine at a concentration of 110-5 g/ml produced a spasmogenic response of the isolated guinea pig ileum. YJA20379-2 at a final concentration of 1×10^{-4} g/ml or 2×10^{-4} g/ml exerted no influence on the resting tone and did not inhibit the spasmogenic response produced by histamine (Fig. 2).

Isolated fundus strip of rats : Acetylcholine and serotonin produced spasmogenic responses at a concentration of 5×10^{-6} and 1×10^{-6} g/ml, respectively. However, YJA 20379-2 at a concentration of 1×10^{-4} and 2×10^{-4} g/ml exerted no influence upon the resting tension, and did not inhibit the spasmogenic response produced by acetylcholine or serotonin (Fig. 3).

Isolated rat uterus : The presence of oxytocin at a concentration of 5×10^{-3} IU/ml produced uterotonicity of the estrogenized rat uterus. YJA20379-2 at a concentration of 1×10^{-4} g/ml or 2×10^{-4} g/ml had no effect on the resting tension of the rat uterus and did not inhibit the spasmogenic response produced by oxytocin (Fig. 4).

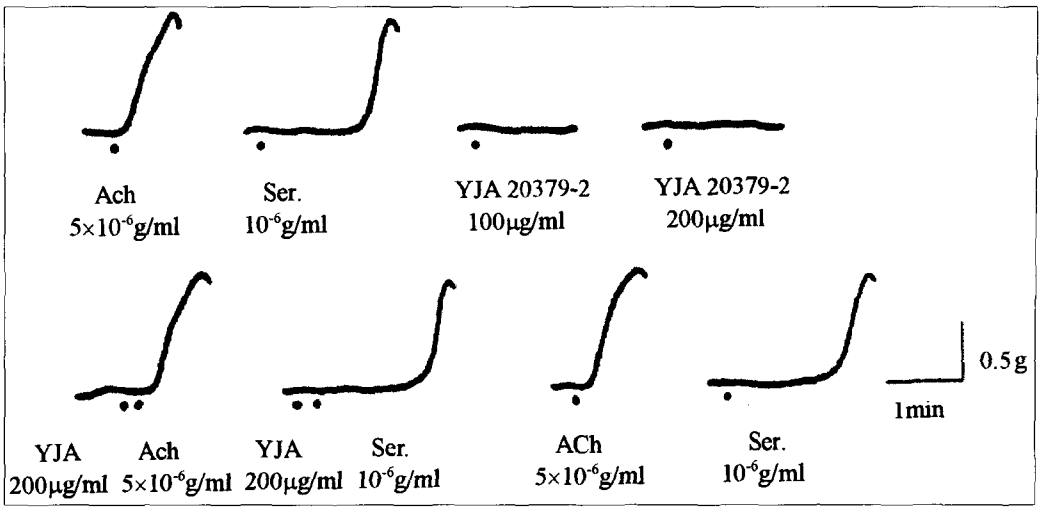


Fig. 3. Effect of YJA20379-2 on the isolated rat fundus strip. Each black-filled circle represents the addition point of each sample. ACh: Acetylcholine, Ser: Serotonin

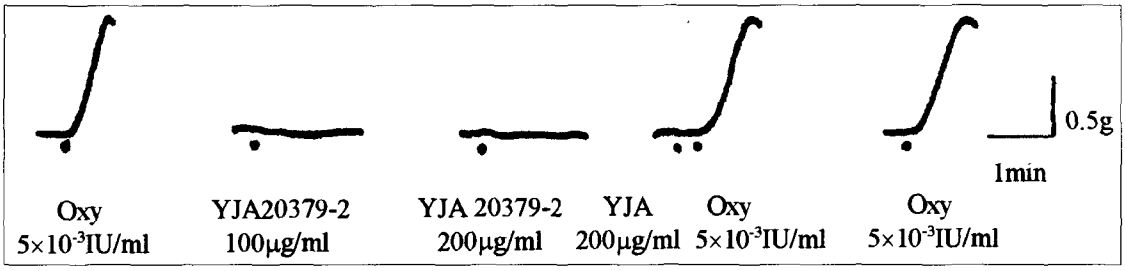


Fig. 4. Effect of YJA20379-2 on the isolated rat uterus. Each black-filled circle represents the addition point of each sample. Oxy: Oxytocin

Table III. Effect of YJA20379-2 on intestinal propulsion in mice

Treatment	Dose (mg/kg, p.o.)	No. of mice	Propulsion (%)	Inhibition (%)
Control	--	8	43.1±2.5	--
YJA 20379-2	50	8	45.2±4.7	-4.9
	100	8	33.2±6.0	23.0
	200	8	32.3±9.2	25.1
Lidocaine	100	8	33.0±3.9*	23.4

All data represent the mean±S.E.M.
Significantly different from the control group (*$p<0.05$)

Effects on gastrointestinal system

Effect on intestinal propulsion in mice : YJA20379-2 at doses of 50, 100 and 200 mg/kg p.o. exerted a tendency to inhibit the propulsion of barium sulfate meal. However, this was not significantly different from the control group. On the contrary, lidocaine at 100 mg/kg retarded the propulsion of the meal (Table III).

Diuretic effect in rats

As shown in Table IV, YJA20379-2 at oral doses of 50 and 100 mg/kg showed no significant effect on urine volume. However, at 200 mg/kg the urine volume decreased significantly compared with the control group. The excretion of sodium and potassium was not significantly altered.

DISCUSSION

The present study deals with the general pharmacological properties of YJA20379-2, which was examined in mice, rats, guinea pigs and rabbits. It has been reported that YJA20379-2 shows a potent antiulcer acti-

Table IV. Effect of YJA20379-2 on urine excretion in rats for 5 h

Treatment	Dose (mg/kg, p.o.)	No. of mice	Volume (ml/100 g)	Sodium (mEq/5 hr/100 g)	Potassium (mEq/5 hr/100 g)
Control	-	6	4.0±0.6	397.62±64.48	91.97±13.47
YJA 20379-2	50	6	4.2±0.3	375.89±68.82	94.50±9.49
	100	6	3.6±0.6	477.29±113.36	73.92±10.83
	200	6	1.8±0.3*	238.81±55.82	59.81±20.21

Significantly different from the control group(*; $p<0.01$)

vity in ulcer models of rats; its ED_{50} in gastric ulcers induced by absolute ethanol, indomethacin and water-immersion are 11.0, 21.0 and 0.5 mg/kg p.o., respectively, as given when administered 30 min before ulcer induction (Chung *et al.* 1998). The oral doses used in this experiment in mice and rats were 50, 100 and 200 mg/kg. The highest dose of 200 mg/kg corresponded to about 10 and 100 times the ED_{50} of indomethacin and water-immersion ulcers, respectively.

The highest oral doses of YJA20379-2 at 200 mg/kg did not affect the general behaviour, motor coordination or hexobarbital hypnosis. The locomotor activity and body temperature of mice were not influenced at a dose of 100 mg/kg. However, at 200 mg/kg the activity was increased and the body temperature was lowered significantly. Analgesic and anticonvulsant actions were not observed at the highest doses of 200 mg/kg. These results indicate that YJA20379-2 might not elicit any specific activity on the central nervous system at 100 mg/kg.

The intravenous administration of YJA20379-2 at 5 mg/kg in rabbits showed no effect on blood pressure or respiration, but at 10 mg/kg i.v. respiration was deepened and blood pressure decreased. YJA20379-2 at a concentration of 210-4 g/ml neither produced contraction nor relaxation of the rat fundus strip, rat uterus, or guinea pig ileum preparation. Pretreatment of the drug at a concentration of 2×10^{-4} g/ml did not antagonize the contractile response produced by the spasmogens, such as histamine, acetylcholine, serotonin and oxytocin. YJA20379-2 up to 200 mg/kg had neither mydriatic nor miotic action in mice. The intestinal propulsion in mice was not affected significantly up to 200 mg/kg. The urination was not affected at the doses of 50 and 100 mg/kg, as measured for 5 h, but at 200 mg/kg the volume of urine was significantly decreased. However, the contents of sodium and potassium in urine were not altered at the highest dose of 200 mg/kg.

ACKNOWLEDGEMENTS

This work was generously supported by a grant from the Ministry of Science and Technology (HAN project, 4-2-47, 1995-1996), Korea.

REFERENCES

Araki, Y. and Ueki, S., Changes in sensitivity to convulsion in mice with olfactory bulb ablation. *Jpn. J. Pharmacol.*, 22, 447-456 (1972).

Chung, Y. K., Chang, M. S., Kim, B. K., Sohn, S. K., Woo, T. W., Kim, S. G. and Choi, W. S., The biochemical and pharmacological properties of a newly synthesized H^+-K^+ ATPase inhibitor, 2-dimethylamino-4,5-dihy-drothiazolo [4,5:3,4]pyridol [1,2-a]benzimidazole. *Can. J. Physiol. Pharmacol.*, 76, 921-929 (1998).

Dunham, N. W., Miya, T. S. and Edwards, C. D., Pharmacological activity of a series of basic esters mono- and dialkyl malonic acid. *J. Am. Pharm. Assoc.*, 46, 208-209 (1957).

Gilman, A. and Goodman, L., The secretory response of the posterior pituitary to the need for water conservation. *J. Physiol.*, 90, 113-118 (1937).

Irwin, S., Comprehensive observational assessment: Ia. A systematic quantitative procedure for assessing the behavioural physiologic state of the mouse. *Psychopharmacology*, 12, 222-257 (1968).

Lee, E. B., Pharmacological approach of crude drugs. *Yakhak Hoeji*, 19, 53-59 (1975).

Nahorski, S. R., Behavioural supersensitivity to apomorphine following cerebral dopaminergic denervation by 6-hydroxydopamine. *Psychopharmacology*, 42, 159-162 (1975).

Swinyard, E. A., Brow, W. C. and Goodman, L. S., Comparative assays of antiepileptic drugs in mice and rats. *J. Pharmacol. Exp. Therap.*, 106, 319-330 (1952).

Takagi, K. and Lee, E. B., Pharmacological studies on *Platycodon grandiflorum* A.DC. III: Activities of crude platycodon on respiratory and circulatory systems and its other pharmacological activities. *Yakugaku Zasshi*, 92, 969-973 (1972).

Vane, J. R., A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmacol.*, 12, 344-349 (1957).

Whittle, B. A., The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *Br. J. Pharmacol.*, 22, 246-253 (1957).