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Synthesis and Analgesic and Anti-inflammatory Activities of 1,2-Benzothiazine Derivatives

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Three 1,2-benzothiazine derivatives were synthesized, and their analgesic/anti-inflammatory efficacy and their effects on gastric irritation were evaluated. Among the three compounds, **39** exhibited the most potent analgesic action, but the effect was weaker than that of piroxicam. Nonetheless, the compound showed 4 times more potent analgesic action with less gastric damage than did ibuprofen. These compounds did not show anti-inflammatory effect at an oral dose of 5 mg/kg.

Key words : 1,2-Benzothiazine derivatives, Analgesic action, Gastric mucosal damage

INTRODUCTION

Many of non-steroidal anti-inflammatory and analgesic drugs have been used in acute and chronic responses and in various pain syndromes. However, most of the agents have serious gastrointestinal adverse effects such as diarrhea, nausea, vomiting, abdominal pain and ulceration (Munson *et al.*, 1995).

1,2-Benzothiazine derivatives such as piroxicam (Hite, 1989) and meloxicam (Engelhardt *et al.*, 1995) have been developed and used in clinic. However, yet it is necessary to develop peripherally acting analgesic drugs with high degree of efficacy and least side effects.

New 1,2-benzothiazine derivatives with analgesic and anti-inflammatory activities have recently been synthesized (Kwon *et al.*, 1996). The present study is concerned with further syntheses of the three compounds and their analgesic and anti-inflammatory activities as well as their effects on gastric irritation.

MATERIALS AND METHODS

Chemicals and Instruments

Chemicals were purchased from Aldrich (Milwaukee, WI, USA), Sigma (St. Louis, MO, USA), E. Merck (Datmstadt, Germany), and Tokyo Kasei (Tokyo, Japan). Melting points were determined on a Büchi 535 melting point apparatus and were uncorrected. NMR spectra were recorded on 300 MHz Gemini Varian NMR spectrometer (Seoul, Korea) using TMS as an internal standard. IR spectra were recorded on a Perkin-Elmer 16F PC FT-IR spectrometer using KBr discs. The elemental analyses (C, H, N) were carried out with a Perkin Elmer Model CHN 2400 elemental analyzer (Seoul, Korea).

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General procedure for the synthesis of 1,2-benzothiazine derivatives

Three compounds of **24, 36** and **39** were newly synthesized according to the method as described in the previous report (Kwon *et al.*, 1996). In brief, 6-halogen (Cl, Br)-substituted saccharins were synthesized through the process of chlorosulfonation, ammonolysis and oxidation of p-halotoluene. Halogen-substituted 1, 2-benzothiazine derivatives were synthesized by Gabriel-Colman rearrangement reaction of N-substituted halosaccharin with methyl chloroacetate. On the other hand, 1-aminothiohydantoin derivative was synthesized by cyclization of isothiocyanates and hydrazine derivatives formed from hydrazine and ethyl chloroacetate. The compounds **24, 36** and **39** were synthesized by condensation of the afore-mentioned halogen substituted 1,2-benzothiazine derivatives and 1-aminothiohydantoin derivative. These compounds were 7-bromo-4-hydroxy-N-(3-allyl-2-thio-1-hydantoinyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide (Comp. **24**), 7-bromo-4-hydroxy-2-allyl-N-[3-(p-chlorophenyl)-2-thio-1-hydantoinyl]-2H-1,2-benzothiazine-3-carboxamide1, 1-dioxide (Comp. **36**), 7-chloro-4-hydroxy-N-(3-allyl-2-thio-1-hydantoinyl)-2H-1,2-benzothiazine-3-carboxamide1, 1-dioxide (Comp. **39**).

Pharmacological methods

Animals. Male ICR mice weighing 20~22 g and male Sprague-Dawley rats weighing 130~150 g were used. All animals were maintained under the condition of lights on from 07:00 to 19:00 hr and the temperature of 22~24°C for at least 1 week before the start of the experiment. Food and water were provided *ad libitum* unless otherwise noted. The test compounds suspended in 0.5% CMC were administered orally.

Analgesic activity. A modification by Swingle *et al.* (1971) of a method of Randall and Selitto (1957) was employed and groups of 8 rats were used for each dose. A saline suspension of brewer's yeast (20%) in a volume of 0.1 ml was injected into the subplantar region of the right hind paw. The test compound was orally administered 2 h after the yeast injection. The pain threshold was measured by applying pressure to the foot using an analgesy meter (Ugo Basile). The reaction thresholds to pressure of the inflamed (yeast-injected) and non-inflamed hind paws were measured. Rats in the drug-treated group were designed as "protected" if the individual reaction threshold to pressure exceeded the control group mean threshold by two standard deviations of that mean. The reaction thresholds to pressure were determined at two time intervals of 30 and 60 min after the administration. ED_{50} values were calculated by the method of Litchfield-Wilcoxon (1949).

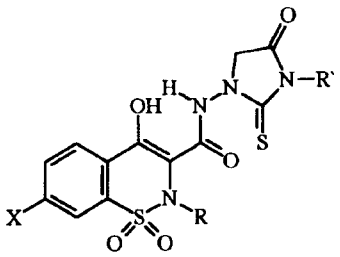
Anti-inflammatory activity. The method of Winter *et al.* (1962) was employed with a slight modification and groups of 8 rats were used. Carrageenan (Type IV, Sigma) solution (1%) in a volume of 0.1 ml was injected into the subplantar region of the left hind paw 0.5hr after the oral administration of the test compound. The paw volume was measured by means of a plethysmometer (Ugo Basile, Italy) before and 1, 2, 3 and 4 h after the carrageenan injection.

Gastric mucosal damage. A modification of a method by Melarange *et al.* (1994) was employed in a group of 8 rats. Rats fasted for 17 h were gavaged with the test compounds or vehicle. Four hrs later, the animals were euthenized and the stomachs were dessected, fixed by intraluminal irrigation with 10ml of 2% formalin, excised along its greater curvature and measured the length index in mm of gastric damage under microscope at × 10 magnification.

RESULTS AND DISCUSSION

Table I shows physical properties of the 1,2-benzothiazine derivatives which were readily synthesized using the general procedures as described in the method

Table I. Physical properties of synthetic compounds



Compd.	X	R	R'	Mp (°C)	Yield (%)	NMR (DMSO-d_6 δ, ppm)
24	Br	H	CH2=CH-CH2-	255~257 (dec.)	61	2.36 (s, 1H, CONH) 4.27 (s, 2H, CH_2) 4.28 (d, 2H, CH_2) 5.03 (m, 1H, $=CH_2$) 5.43 (m, 1H, CH) 7.56-7.80 (m, 3H, C_6H_3)
36	Br	CH_2=CH-CH_2-	p-Cl-phenyl	255~256 (dec.)	45	2.35 (s, 1H, CONH) 4.00 (d, 2H, CH_2) 4.51 (s, 2H, CH_2) 4.85 (m, 1H, CH) 5.03 (d, 2H, $=CH_2$) 7.13~7.56 (m, 4H, C_6H_4) 7.67~8.03 (m, 3H, C_6H_3)
39	Cl	H	CH_2=CH-CH_2-	(257~260)	95	2.91 (s, 1H, CONH) 4.79 (d, 2H, CH_2) 4.80 (s, 2H, CH_2) 5.46 (m, 1H, CH) 5.63 (m, 2H, CH_2) 7.92~8.68 (m, 3H, C_6H_3)

section. The compounds were purified on silica gel and the structures were identified by ^{1}H-NMR data as presented in Table I.

The compounds synthesized were subjected to the test of analgesic action by Randall-Selitto method, anti-inflammatory action in carrageenan edema, and production of gastric mucosal damage. The results are summarized in Table II~V.

As measured by Randall-Selitto's method, the pain thresholds were increased significantly at 30 min after the administration of compound **39** and piroxicam at 5 mg/kg *p.o.* and similar effect was shown at 60 min after the administration of compound **36** and piroxicam. However, compound **24** showed no significant increase in the pain threshold. The "protected" percentages after treatment with compound **36** and **39** were 12.5% in two determinations, and those of piroxicam were 50% and 25% in each determination (Table II).

The ED_{50} values of compound **36, 39**, ibuprofen and piroxicam determined at 30 min after the administration were estimated to be 79.9, 34.7, 120.1 and 5.2 mg/kg, respectively. The potency ratios for piroxicam, compound **39, 36** and ibuprofen were 1, 6.7, 15.4, and 23.1 (Table III). Here, it is noted that compound **39** exhibited 3.4 times more potent analgesic action than ibuprofen and 2.3 times more potent than compound **36**, but 6.7 times weaker action than piroxicam. All the compounds and reference drugs were estimated to be lower ED_{50} values at 30 min than 60 min after the administration.

In anti-inflammatory test, as presented in Table IV, the three compounds at the oral doses of 5 mg/kg did not inhibit the carrageenan edema, but piroxicam showed potent inhibitory action.

Inoue *et al.* (1994) reported that ED_{50} value of piroxicam in Randall-Selitto assay and ED_{30} value in anticarrageenin assay in their experimental condition were 1.94 and 1.31 mg/kg, respectively, which are not much different dose.

Compound **39** has no anticarrageenan activity at a dose of 5 mg/kg. For confirmation of anti-inflammatory activity, an assay at a dose of 34.0 mg/kg corresponding

Table II. Analgesic action of the compounds in the Randall-Selitto assay

Treatment	Dose (mg/kg, *po*)	No. of animals	30 min			60 min		
			Pain threshold (g)		Protected (%)	Pain threshold (g)		Protected (%)
			Inflamed	Non-inflamed		Inflamed	Non-inflamed	
Control	--	8	70.0±23.7#	129.4±35.7	--	80.9±20.7#	116.3±25.6	--
Compound 24	5	8	87.5±12.7	126.9±32.5	0.0	95.6±22.4	138.1±32.8	12.5
Compound 36	5	8	91.6±25.4	127.5±27.2	12.5	102.8±15.5*	123.8±30.4	12.5
Compound 39	5	8	101.3±19.6*	140.0±36.6	12.5	97.2±27.8	118.1±28.7	12.5
Piroxicam	5	8	102.5±27.7*	124.4±30.9	50.0	108.8±33.2*	122.5±18.4	25.0

All data represent the mean±S.E.M.
Significantly different from control group (*; $p<0.05$)
Significantly different from the non-inflamed paw of control group (#; $p<0.01$)
Pain threshold was determined at 30 min and 60 min after the drug treatment.

Table III. ED_{50} values of the compounds in the Randall-Selitto assay

Compound	ED_{50} (mg/kg, *p.o.*, 95% confidence limits)		Relative Potency (30 min)
	30 min	60 min	
Compound 36	79.9 (27.6~231.5)	215.9 (51.3~908.6)	15.4
Compound 39	34.7 (11.6~103.7)	944.5 (113.0~7893.1)	6.7
Ibuprofen	120.1 (57.3~251.8)	166.6 (99.7~278.6)	23.1
Piroxicam	5.2 (2.1~12.8)	37.5 (5.7~247.9)	1

Table IV. Effect of the compounds on the carrageenan-induced paw edema in rats

Treatment	Dose (mg/kg, *po*)	No. of animals	Increase percent of paw volume			
			1	2	3	4 (hr)
Control	--	8	56.9±6.1	57.8±6.1	75.3±10.6	79.2±7.5
Compound 24	5	8	64.1±9.5 (-12.7)	67.0±7.8 (-15.9)	82.8±6.8 (-10.0)	92.8±10.6 (-17.1)
Compound 36	5	8	71.5±2.4 (-25.7)	77.5±1.1 (-34.1)	80.1±1.9 (-6.4)	87.3±2.4 (-10.2)
Compound 39	5	8	69.0±5.8 (-21.2)	70.4±5.8 (-21.7)	73.0±5.9 (-3.0)	82.0±5.8 (-3.5)
Piroxicam	5	8	39.2±4.0* (31.2)	40.1±4.9* (30.5)	39.0±3.7** (48.3)	48.1±6.6** (39.2)

All data represent the mean±S.E.M.
Significantly different from control group (*; $p<0.05$, **; $p<0.01$).
The figures in parentheses indicate inhibition percents.

Table V. Induction of gastric mucosal damage by the compounds in rats

Treatment	Dose (mg/kg, *po*)	No. of animals	Lesion index (M.±S.E., mm)
Control	--	7	0
Compound 36	120	8	2.9±1.7
Compound 39	52	8	2.8±2.8
Ibuprofen	180	8	6.4±1.7*
Piroxicam 8	8	8	2.0±0.7

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

to ED_{50} in Randall-Selitto assay should be performed. Thus, at present, it is not confirmative that the compounds have no anti-inflammatory activity.

It is generally known that most non-steroidal anti-inflammatory and analgesic drugs produce severe gastric mucosal damage in experimental and clinical doses as adverse effect. Therefore, the samples were tested for production of gastric damage and the results are presented in Table V. The doses employed in this test were 1.5-fold of ED_{50} values of the compounds. The gastric lesion indices of compound **36, 39**, ibuprofen and piroxicam were estimated to be 2.9, 2.8, 6.4 and 2.0, respectively. These results obtained indicate that compound **36** and **39** have a little more gastric irritation than did piroxicam, but much less than ibuprofen.

In conclusion, it is revealed that compound **36** and **39** have more potent analgesic action with less side effect than ibuprofen, but less potent analgesic action than piroxicam, and compound **39** is more potent than compound **36**.

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REFERENCES CITED

Engelhardt, G., Homma, D., Schlegel, K., Utzmann, R. and Schnizler, C., Anti-inflammatory, analgesic, antipyretic and related properties of meloxicam, a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. *Inflamm. Res.* 44, 423-433 (1995).

Hite, G. J., Analgesics: An Overview, In Foye, W. O. (Eds). *Principles of Medicinal Chemistry.* Lea & Febiger, Philadelphia, pp.519-520, 1989.

Kwon, S. K. and Park, M. S., Some new 1,2-benzothiazine derivatives with analgesic and anti-inflammatory activities. *Arzneim.-Forsch.* 46, 966-971 (1996).

Inoue, K., Fujisawa, H., Motonaga, A., Inoue, Y., Kyoi, T., Ueda, F., and Kimura, K., Anti-inflammatory effects of etodolac; Comarison with other non-steroidal anti-inflammatory drugs. *Biol. Pharm. Bull.* 17, 1577-1583 (1994).

Litchfield, J. T. and Wilcoxon, F., A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 96, 99-113 (1949).

Melarange, R., Gentry, C., Durie, M., O'Connell, C. and Blower, P. R., Gastrointestinal irritancy, anti-inflammatory activity, and prostanoid inhibition in the rat. *Dig. Dis. Sci.* 39, 601-608 (1994)

Munson, P. L., Mueller, R. A. and Breese, G. R., *Principles of Pharmacology.* Chapman & Hall, New York, pp. 1177-1186, 1995.

Randall, L. O. and Selitto, J. J., A method for measurement of analgesic activity on inflamed tissue. *Arch. Int. Pharmacodyn. Ther.* 111, 409-419 (1957).

Swingle, K. F., Grant, T. I. and Kvam, D. C., Quantal responses in the Randall-Seitto assay. *Proc. Soc. Exp. Biol. Med.* 137, 536-538 (1971).

Winter, C. A., Risley, E. A. and Nuss, G. W., Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 111, 544-548 (1962).