Cytoprotective Effects of 4,6-Bis(1*H*-pyrazol-1-yl)pyrimidine and Related Compounds on HCl·Ethanol-Induced Gastric Lesions in Rats

Masazumi IKEDA,*[,a] Kazumi MARUYAMA,[a] Youichi NOBUHARA,[b] Toshihiro YAMADA,[b] and Susumu OKABE*[,a]

Kyoto Pharmaceutical University,[a] Misasagi, Yamashina, Kyoto 607, Japan and Nissin Food Products Co., Ltd.,[b] Central Research Institute, Noji, Kusatu, Shiga 525, Japan. Received September 2, 1996; accepted October 29, 1996

Bis(1*H*-pyrazol-1-yl)- and bis(1*H*-imidazol-1-yl)pyrimidines were synthesized and evaluated for cytoprotective effects. Among them, 4,6-bis(1*H*-pyrazol-1-yl)pyrimidine (3) showed a potent inhibitory effect on the HCl·ethanol-, ethanol-, and water immersion stress-induced gastric lesions in rats, and a very low acute toxicity. One of the major factors responsible for the cytoprotective effects of 3 is the increase in the bicarbonate secretion. This compound appears to be a promising cytoprotective drug for the treatment of gastric mucosal ulcers.

Key words 4,6-bis(1*H*-pyrazol-1-yl)pyrimidine; bicarbonate secretion; cytoprotective effect; mepirizole; dulcerozine

Previously,[1] we reported that some 2- or 4-(1*H*-pyrazol-1-yl)pyrimidine derivatives related to mepirizole (**1**)[2] and dulcerozine (**2**)[2] have a potent cytoprotective effect on HCl·ethanol- and stress-induced gastric lesions in rats. During the course of this study, we have found that 4,6-bis(1*H*-pyrazol-1-yl)pyrimidine (**3**)[3] exhibits a marked cytoprotective effect. Since compound **3** has a unique structure among known cytoprotective agents, we have studied the pharmacological effects of this compound and related ones in more detail.

Chemistry In general, 4,6-dichloro- and 2,4-dichloropyrimidines were treated with 2 molar eq of sodium pyrazolide or imidazolide in tetrahydrofuran (THF) at room temperature overnight. The crude material was purified by column chromatography or recrystallization to give the corresponding bis-substituted derivatives **3**—**6**.

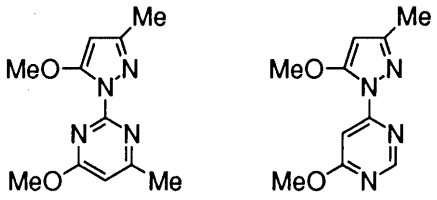
Biology Compounds **3**—**6** were tested for gastric cytoprotective effects in rats.[4] Test compounds (10 mg/kg) suspended in 1% carboxymethylcellulose (CMC) or the vehicle as a control were orally administered by gastric intubation 30 min before HCl·ethanol administration. The lengths of the lesions in the treated and control groups were compared and the inhibitory rates were calculated. 4,6-Bis(1*H*-pyrazol-1-yl)pyrimidine (**3**) exhibited a potent inhibitory activity (99% inhibition) against HCl·ethanol-induced lesions (HEL) (Fig. 1). Comparison with the related compounds **4** (24%), **5** (50%) and **6** (78%) indicated that the cytoprotective effects of compound **3** is extremely strong. Accordingly, compound **3** was selected for further evaluation of pharmacologic properties.

The oral cytoprotective ED_{50} values of compound **3**, irsogladine maleate, and cetraxate hydrochloride determined by the HEL test were 0.4, 3.2 and 38.0 mg/kg, respectively. The ED_{50} values of compound **3** for ethanol-induced lesions[5] and stress-induced gastric lesions[6] was 0.4 and 37.0 mg/kg, respectively. The LD_{50} value of **3** was found to be >3000 mg/kg when given orally to rats.

Compound **3**, given orally at 10 mg/kg, had no effect on gastric acid secretion (either the volume or the acid output) in pylorus-ligated rats. Consequently, it is clear that the cytoprotective effect of **3** is unrelated to gastric acid secretion. Preliminary studies suggested that the following four major factors are responsible for the cytoprotective effects of **3**.

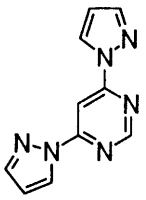
1) Increase in Bicarbonate Secretion[7]: The pH was markedly increased from 4.5 to 6.1 after exposure at 2 mg/ml in rats pretreated with omeprazole (30 mg/kg, i.p.). The levels remained elevated for about 2 h. A dose-dependent increase in the bicarbonate secretion was observed after exposure of the mucosa to concentrations of 60 μg/ml—2 mg/ml for 10 min. The output of HCO_3^- at 2 mg/ml was 1.2 ± 0.1 μeq/10 min (Fig. 2).

2) Increase in the Gastric Mucosal Blood Flow[7]: The flow increased from 3.1 to 22.3 ml/min immediately after exposure to 2 mg/ml, and was approximately 55% higher than the control value at 20 μg/ml (Fig. 3).

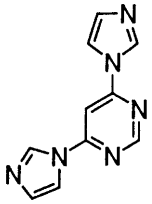


1

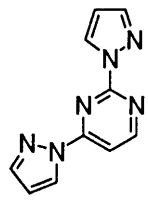
2



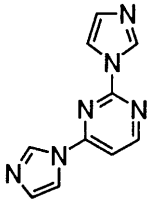
3



4



5



6

* To whom correspondence should be addressed.

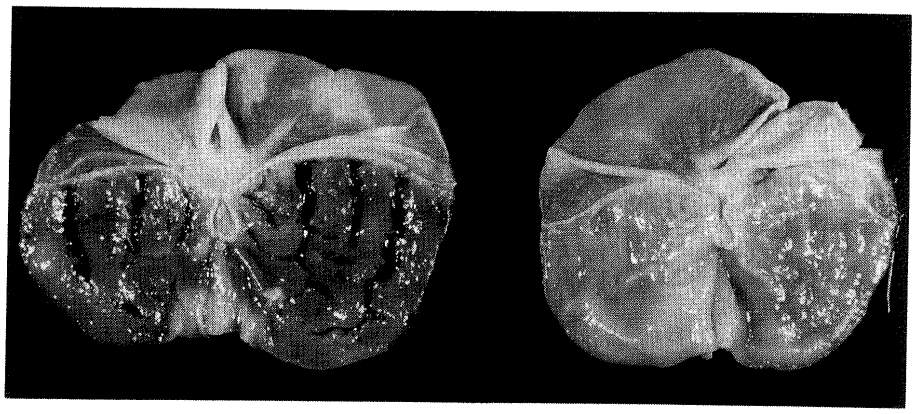


Fig. 1. Gross Appearances of HCl·Ethanol-Induced Gastric Lesions in Rats

The lesions were induced by giving 1 ml/200 g body wt of 60% ethanol in 150 mM HCl to rats which had been starved for 24 h. Animals were killed 1 h after HCl·ethanol administration. Left: stomach of a rat given the vehicle alone. Right: stomach of a rat given 10 mg/kg of compound **3** orally 0.5 h before HCl·ethanol administration. Note that pretreatment with compound **3** markedly inhibited lesion formation.

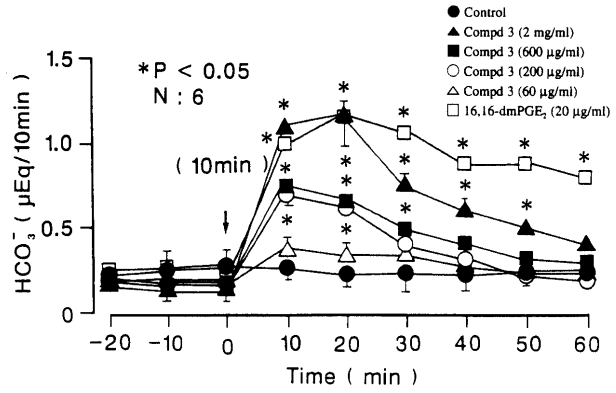


Fig. 2. Effects of Compound **3** and 16,16-dmPGE$_2$ on Gastric Bicarbonate Secretion in Anesthetized Rats

Rats were pretreated with omeprazole (60 mg/kg, i.p.) to inhibit gastric acid secretion. Using an *ex vivo* chamber, bicarbonate secretion was determined by perfusion of the mucosa with saline at a flow rate of 1 ml/min. Each compound was applied to the mucosal surface for 10 min and washed out with saline. Data are means ± S.E. of 6 rats.

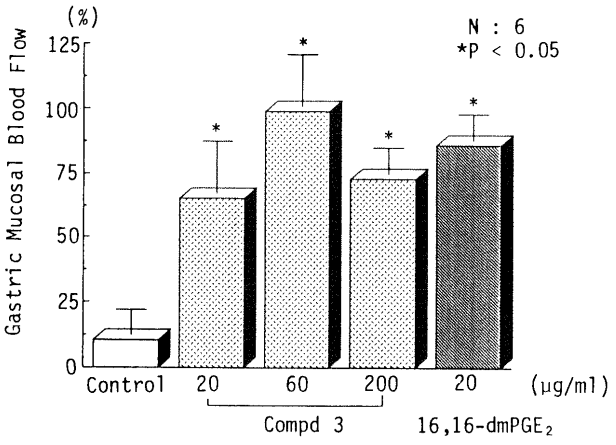


Fig. 3. Effects of Compound **3** and 16,16-dmPGE$_2$ on the Gastric Mucosal Blood Flow in Anesthetized Rats

The flow was determined by hydrogen gas clearance method. Note that the gastric mucosal blood flow was markedly increased by the topical application of these compounds. Data are means ± S.E. of 6 rats.

3) Increase in the Gastric Mucus Secretion[8]: The decrease of hexosamine content induced by HCl·ethanol in rats was fully restored at 10 mg/kg (see Table 1).

4) Increase in Potential Difference (PD) in the Gastric Mucosa: The PD in the mucosa was increased from −20 to −31 mV in rats after exposure to 200 μg/ml.[7]

Table 1. Effect of Compound **3** on Hexosamine Content in Rat Stomachs of HCl·Ethanol Treated Rats[a]

Treatments	*n*	Hexosamine content (μg/100 mg dried stomach)
Normal	9	5326.20 ± 410.04[b]
Control	8	3206.67 ± 425.76
3 (1.0 mg/kg)	7	4014.36 ± 445.21
3 (3.0 mg/kg)	8	4652.44 ± 921.39
3 (10.0 mg/kg)	8	6169.00 ± 613.34[b]

a) HCl·ethanol (1.0 ml, *p.o.*) was administered 0.5 h after administration of compound **3**. After 1 h, the rats were killed for determination of hexosamine content in the stomach. Data are means ± S.E. of 8 rats. *b*) Significantly different from the control ($p < 0.01$).

Finally, it is of interest to examine whether or not **3** is effective against *Helicobacter pylori*,[9] which is believed to be a major cause of acute and chronic superficial gastritis and peptic ulcer disease. Minimum inhibitory concentrations (MICs) for **3** and bismuth citrate against *H. pylori* 7795 were 50 and 6.25 μg/ml at 10^5 CFU/ml and 50 and 12.5 μg/ml at 10^7 CFU/ml, respectively.

In conclusion, we have found novel cytoprotective agents, bis(1*H*-pyrazol-1-yl)- and (1*H*-imidazol-1-yl)pyrimidines, among which compound **3** was considered to be a promising candidate as a cytoprotective agent. It was selected for clinical trials to evaluate its therapeutic usefulness against gastric injuries.

Experimental

Melting points are uncorrected. ^{1}H-NMR spectra were determined with a JEOL JNM-PMX 60 (60 MHz) spectrometer, using tetramethylsilane as an internal standard. Column chromatography was performed on Silica gel 60 PF_{254} (Merck) under pressure.

4,6-Bis(1*H*-pyrazol-1-yl)pyrimidine (3). General Procedure A solution of pyrazole (272 mg, 4.0 mmol) in THF (6 ml) was added to a suspension of NaH (160 mg, 60% in oil, washed with pentane) in THF (4 ml) under a nitrogen atmosphere at 0 °C and the mixture was stirred at room temperature until evolution of hydrogen gas ceased (20 min). A solution of 4,6-dichloropyrimidine (298 mg, 2.0 mmol) in THF (3 ml) was added to the above solution and the whole was stirred at room temperature overnight. The solvent was evaporated off, then the residue was diluted with water and extracted with dichloromethane. The extract was dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 30:1) to give **3** (334 mg,

79%), mp 133—133.5 °C (from hexane) (lit. mp 133—133.5 °C[1]; 122 °C[3]) as colorless needles.

The following compounds were also prepared similarly.

4,6-Bis(1*H*-imidazol-1-yl)pyrimidine (**4**) (69%), mp 243—244 °C (from isopropanol) (lit.,[1] mp 243—244 °C), was prepared from 4,6-dichloropyrimidine and imidazole.

2,4-Bis(1*H*-pyrazol-1-yl)pyrimidine (**5**) (84%), mp 152—153 °C (from hexane), was prepared from 2,4-dichloropyrimidine and pyrazole. ^{1}H-NMR ($CDCl_3$) δ: 6.4—6.7 (2H, m, H-4′), 7.65-8.0 (2H, m, H-3′), 7.71 (1H, d, J=5 Hz, H-5), 8.5—8.9 (2H, m, H-5′), 8.67 (1H, d, J=5 Hz, H-6). *Anal.* Calcd for $C_{10}H_8N_6$: C, 56.59; H, 3.80; N, 39.61. Found: C, 56.87; H, 3.47; N, 39.85.

2,4-Bis(1*H*-imidazol-1-yl)pyrimidine (**6**) (76%), mp 131—132 °C (from hexane–AcOEt), was prepared from 2,4-dichloropyrimidine and imidazole. ^{1}H-NMR ($CDCl_3$) δ: 7.15—7.4 (3H, m, H-5, H-4′), 7.72 (1H, br s, one of H-5′), 7.88 (1H, br, one of H-5′), 8.50 (1H, br, one of H-2′), 8.64 (1H, br, one of H-2′), 8.76 (1H, d, J=5 Hz, H-6). *Anal.* Calcd for $C_{10}H_8N_6$: C, 56.59; H, 3.80; N, 39.61. Found: C, 57.08; H, 3.30; N, 39.95.

Pharmacological Methods Male Sprague-Dawley rats (230—270 g, Nihon Charles-River, Kanagawa, Japan) were used in the experiments. The rats were deprived of food for 24 h beforehand. Drinking water was provided freely for the initial 22 h, but was withheld for 2 h before the start of the experiments. Eight rats were used in each group.

Effects on HCl·Ethanol- or Ethanol-Induced Gastric Lesions Gastric mucosal lesions were induced by giving 1 ml/200 g body wt of 60% ethanol (v/v) in 150 mM HCl or absolute ethanol orally, and 1 h later the animals were killed under ether anesthesia. The stomach was removed, and the gastric contents were expelled through the duodenum by gentle pressure on the gastric wall. Subsequently, the stomach was inflated by injecting 10 ml of 2% formalin to fix the gastric wall. The stomachs were incised along the greater curvature and examined for lesions. The length (mm) of each lesion was measured under a dissecting microscope (×10) with a square grid, and the lesion severity was expressed as the total length of lesions per stomach. A test compound (10 mg/kg) suspended in 1% CMC or vehicle as the control was orally administered 30 min before HCl·ethanol administration. The total lengths of the lesions of the treated and control groups were compared and the inhibitory rates were calculated. The statistical significance was evaluated by using Student's t-test ($p<0.05$).

Effect on Water-Immersion Stress-Induced Gastric Lesions Eight rats were individually immobilized in each compartment of Todai-Yakusaku type stress cages. The cage was immersed vertically in a water bath at 22 °C to the height of the xiphoid process of the rat. After 7 h, the rats were killed, and the stomachs were removed and incised along the greater curvature. The lengths of each lesion was measured under a dissecting microscope. The total length (mm) of each lesion was used as the lesion index. A test compound (30 mg/kg) suspended in 1% CMC or vehicle as the control was administered orally 5 min before immersion. The total length of the lesions of the treated and control groups were compared, and the inhibitory rates were calculated. The statistical significance was evaluated by means of Student's t-test ($p<0.05$).

Acute Toxicity in Rats A 0.3% CMC suspension of a test compound was orally administered to groups of Slc: Wistar/KY rats (male: 90—120 g), each group consisting of 10 rats. After 72 h, LD_{50} values were calculated by the probit method.[10]

References

1) Ikeda M., Maruyama K., Nobuhara Y., Yamada T., Okabe S., *Chem. Pharm. Bull.*, **44**, 1700—1706 (1996).
2) Tanaka H., Nakagawa M., Takeuchi K., Okabe S., *Dig. Dis. Sci.*, **34**, 238—245 (1989).
3) Steel P. J., Constable E. C., *J. Chem. Research (M)*, **1989**, 1601—1611.
4) Mizui T., Doteuchi M., *Jpn. J. Pharmacol.*, **33**, 939—945 (1983).
5) Roberts A., Nezamis J. E., Lancaster C., Hanchar A. J., *Gastroenterology*, **77**, 433—443 (1979).
6) Takagi K., Okabe S., *Jpn. J. Pharmacol.*, **18**, 9—18 (1968).
7) Takeuchi K., Ishihara Y., Okada M., Niida H., Okabe S., *Jpn. J. Pharmacol.*, **49**, 235—244 (1989).
8) Neuhaus O. W., *Anal. Chem.*, **29**, 1230—1233 (1957).
9) Mcgowan C., Cover T. L., Blaser M. J., *Gastroenterology*, **110**, 926—938 (1996).
10) Finney D. J., "Probit Analysis," Cambridge University Press, London, 1952, pp. 185—188.