Studies on Antiulcer Agents. I. Synthesis and Pharmacological Properties of Ethyl 2-[(1*H*-Benzimidazol-2-yl)sulfinylmethyl]-4-dimethylamino-5-pyrimidinecarboxylate, a New H^+/K^+-ATPase Inhibitor Possessing Mucosal Protective Activity

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Ethyl 2-[(1*H*-benzimidazol-2-yl)sulfinylmethyl]-4-dimethylamino-5-pyrimidinecarboxylate (2) has been synthesized and evaluated for antiulcer properties. Compound 2 is a H^+/K^+-ATPase inhibitor that affords mucosal protection against absolute ethanol-induced gastric lesions in rats after oral and parenteral administrations. On the other hand, omeprazole, a representative H^+/K^+-ATPase inhibitor, showed mucosal protective action only after oral administration, indicating that it required gastric acid secretion to generate activity. The antiulcer activity of 2 in animal models, such as water-immersion stress-induced gastric ulcer in rats and acidified aspirin-induced gastric ulcer in rats, was three times higher than that of cimetidine.

Keywords   pyrimidine derivative; H^+/K^+-ATPase inhibitor; mucosal protection; antisecretion; antiulcer agent

Peptic ulcer has been believed to result from an imbalance between gastric acid secretion and mucosal resistance in stomach and deuodenum.[1] Consequently, agents which possess both antisecretory and mucosal protective properties should be effective for therapy. In fact, recent clinical studies have demonstrated that the combined therapy with a histamine H_2-receptor antagonist and a mucosal protectant is greatly superior to either monotherapy.[2]

Our target was to find an antiulcer H^+/K^+-ATPase inhibitor with the ability to show mucosal protection after both oral and parenteral administrations. We selected omeprazole as a lead compound, since it is a potent inhibitor of H^+/K^+-ATPase involved in the final step of H^+ secretion in parietal cells. Mattsson *et al.* have reported that this drug exhibits a potent mucosal protective activity against ethanol-induced gastric lesions in rats when given orally, but not intravenously.[3] However, such a mucosal protective effect of omeprazole will probably not contribute to the healing of ulcers in patients, because the drug is usually administered orally as an enteric coated tablet to prevent chemical transformation under the acidic conditions in the stomach. Therefore, our chemical modification program was directed toward overcoming this problem.

We recently found that ethyl 4-dimethylamino-2-(2-methyl-propoxy)anilino-5-pyrimidinecarboxylate (1) had a potent mucosal protective effect after oral administration. On the basis of this finding, we designed ethyl 2-[(1*H*-benzimidazol-2-yl)sulfinylmethyl]-4-dimethyl-amino-5-pyrimidinecarboxylate (2) as a structural hybrid in which the pyridine nucleus of omeprazole is replaced by the ethyl 4-dimethylamino-5-pyrimidinecarboxylate function derived from 1.

We describe here the synthesis and antiulcer properties of 2.

Chemistry   The desired sulfinyl compound 2 was synthesized *via* the route illustrated in Chart 1. The starting material, ethyl 2-chloromethyl-1,6-dihydro-6-oxo-5-pyrimidinecarboxylate (3), was coupled with 2-mercapto-benzimidazole in the presence of sodium hydroxide in ethanol to give ethyl 2-[(1*H*-benzimidazol-2-yl)thiomethyl]-1,6-dihydro-6-oxo-5-pyrimidinecarboxylate (4) in good yield. The synthesized compound 4 was converted into the 4-chloro-5-pyrimidinecarboxylate (5) by treatment with phosphorus oxychloride in acetonitrile, followed

Chart 1
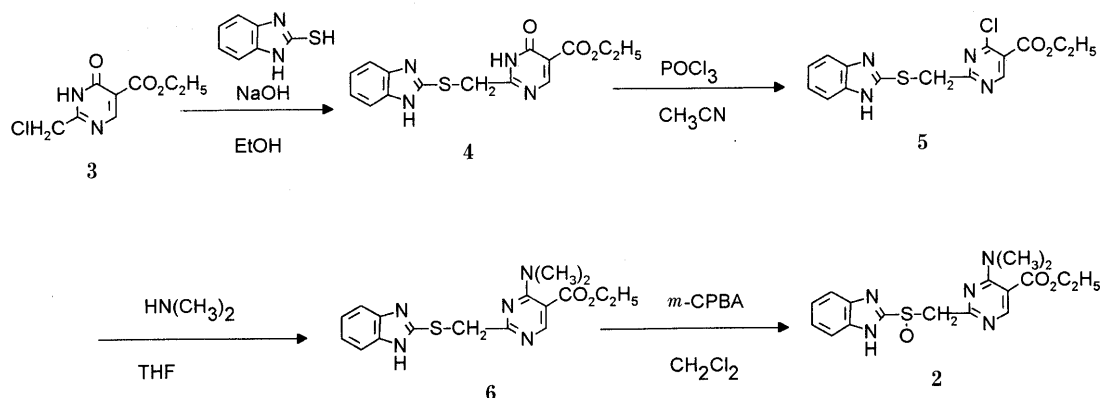
TABLE I. Antiulcer Properties of 2, Cimetidine and Omeprazole

Compd. No.	Antisecretory activity ED_{50} (mg/kg), i.d.		H^+/K^+-ATPase assay ED_{50} (M)	Mucosal protective activity				Antigastric ulcer activity ED_{50} (mg/kg), p.o.	
	Basal secretion	Histamine-stimulated secretion		ED_{50} (mg/kg)			Pretreatment with omeprazole inhibition (%) 10 mg/kg, p.o.	Stress ulcer	Acidified aspirin ulcer
				p.o.	i.p.	i.v.			
2	31.9	2.2	7.5×10^{-6}	1.8	18.8	7.5	66 $(95)^{a)}$	11.8	5.1
Cimetidine	10.2	38.6		>100	>100			32.0	14.7
Omeprazole	2.8	0.8	5.8×10^{-6}	3.9	>100	>20	$NE^{b)}$ (81)	4.1	3.9

a) Values in parentheses exhibit inhibitory activity without the pretreatment with omeprazole. b) NE shows no effect.

by substitution with dimethylamine in tetrahydrofuran to furnish the corresponding ethyl 4-dimethylamino-5-pyrimidinecarboxylate (6). The obtained compound 6 was oxidized with m-chloroperbenzoic acid (m-CPBA) in methylene chloride to yield the desired compound 2.

Results and Discussion

The antiulcer activity of 2 is summarized in Table I and compared with those of antisecretory standards, cimetidine and omeprazole. Initially, we measured antisecretory effect in the pylorus-ligated rat model by intraduodenal administration. The activity of 2 against the histamine-stimulated acid secretion predominated over its activity against basal acid secretion, which is similar to the inhibitory mode of omeprazole but opposite to that of cimetidine. Compound 2 also exhibited significant inhibitory activity, approximately equal to that of omeprazole, in in vitro H^+/K^+-ATPase assay according to Wolosin and Forte.[4] Subsequently, the mucosal protective effect was evaluated in gastric lesions induced by absolute ethanol in rats. When given orally, compound 2 showed excellent activity, as might be expected. Compound 2 also showed fairly good activity after intraperitoneal and intravenous administrations, although a considerable decrease of activity in comparison with the oral potency was observed. In the case of omeplazole, the drug exhibited great potency after oral administration but it had no effect after parenteral administration. The difference in parenteral mucosal protection activity between the two sulfinyl compounds prompted us to test them under conditions of inhibited gastric acid secretion. When 10 mg/kg of the test compounds was administered orally under normal conditions, compound 2 and omeprazole showed inhibitory values of 95% and 81%, respectively. However, after pretreatment by subcutaneous injection of omeprazole (30 mg/kg) 60 min before oral administration of the test compound,[5] compound 2 still gave an inhibitory value of 66%, while omeprazole showed a complete loss of activity. These observations indicated that compound 2, although more active under acidic conditions, afforded good mucosal protection regardless of the presence or absence of acid. On the other hand, omeplazole showed substantial mucosal protection only under acidic conditions, suggesting that the active substance was probably not omeprazole itself but a product derived from it by gastric acid in the stomach. Compound 2 was further evaluated in the water-immersion stress-induced gastric ulcer model (stress ulcer) and acidified aspirin-induced

gastric ulcer model (acidified aspirin ulcer). This compound was active in both ulcer models and its potency was three times that of cimetidine. In conclusion, it was found that compound 2 is a good H^+/K^+-ATPase inhibitor that affords mucosal protection after oral and parenteral administrations. To our knowledge, the present sulfinyl compound is the first omeprazole-like compound exhibiting mucosal protection after intravenous administration.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Infrared (IR) spectra were recorded in a Nujol mull on a Hitachi 270-30 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken in dimethyl sulfoxide-d_6 with a Bruker AC250 instrument using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a JEOL JMS-DX300 mass spectrometer using a direct inlet system.

Ethyl 2-Chloromethyl-1,6-dihydro-6-oxo-5-pyrimidinecarboxylate (3) Compound 3 was prepared by reaction of chloroacetamidine hydrochloride with diethyl ethoxymethylenemalonate in the presence of NaOH in EtOH, mp 177—178 °C.

Ethyl 2-[(1H-Benzimidazol-2-yl)thiomethyl]-1,6-dihydro-6-oxo-5-pyrimidinecarboxylate (4) Compound 3 (11.9 g, 55 mmol) was added portionwise to a mixture of 2-mercaptobenzimidazole (9.8 g, 65 mmol) and 40% aqueous NaOH solution (6.6 ml) in EtOH (200 ml). The mixture was stirred for 15 h at room temperature then heated at 60 °C for 2 h, and poured into H_2O (200 ml). The resulting solid was collected by filtration, washed with H_2O and recrystallized from N,N-dimethylformamide (DMF)–EtOH to give 4 (16.8 g, 93%) as pale brown needles, mp 205—206 °C. IR cm^{-1}: 3300—3100, 3250 (NH), 1740, 1660 (C=O). ^{1}H-NMR δ: 1.24 (3H, t, $J=7.0$ Hz, OCH$_2$CH$_3$), 4.21 (2H, q, $J=7.0$ Hz, OCH$_2$CH$_3$), 4.51 (2H, s, SCH$_2$), 7.10—7.17 (2H, m, Ar-H), 7.40—7.48 (2H, m, Ar-H), 8.43 (1H, s, pyrimidine-H), 13.10 (2H, br s, 2 × NH). MS m/z: 330 (M$^+$). Anal. Calcd for C$_{15}$H$_{14}$N$_4$O$_3$S: C, 54.53; H, 4.27; N, 16.96. Found: C, 54.70; H, 4.41; N, 16.64.

Ethyl 2-[(1H-Benzimidazol-2-yl)thiomethyl]-4-chloro-5-pyrimidinecarboxylate (5) Compound 4 (26 g, 79 mmol) was added portionwise to a solution of POCl$_3$ (24.6 g, 160 mmol) in CH$_3$CN (200 ml) and the mixture was heated under reflux for 1 h, then concentrated under reduced pressure. The residual oil was dissolved in CHCl$_3$ (300 ml) and the solution was added to H_2O (300 ml) with vigorous stirring. The pH was adjusted to 8 with 10% aqueous NaOH solution. The CHCl$_3$ layer was separated, washed with H_2O and dried over Na$_2$SO$_4$. After evaporation of the solvent, the residue was recrystallized from AcOEt–isopropyl ether to give 5 (24.5 g, 89%) as pale brown crystals, mp 133—134 °C. IR cm^{-1}: 3350 (NH), 1720 (C=O). ^{1}H-NMR δ: 1.32 (3H, t, $J=7.0$ Hz, OCH$_2$CH$_3$), 4.35 (2H, q, $J=7.0$ Hz, OCH$_2$CH$_3$), 4.85 (2H, s, SCH$_2$), 7.10—7.15 (2H, m, Ar-H), 7.40—7.45 (2H, m, Ar-H), 9.14 (1H, s, pyrimidine-H), 12.70 (1H, br s, NH). MS m/z: 348 (M$^+$). Anal. Calcd for C$_{15}$H$_{13}$ClN$_4$O$_2$S: C, 51.65; H, 3.76; N, 16.06. Found: C, 51.40; H, 3.83; N, 15.87.

Ethyl 2-[(1H-Benzimidazol-2-yl)thiomethyl]-4-dimethylamino-5-pyrimidinecarboxylate (6) A 50% aqueous dimethylamine solution (2.9 g, 32 mmol) was added to a stirred solution of 5 (3.0 g, 8.6 mmol) in tetrahydrofuran (THF) (50 ml) and the mixture was then stirred at room

temperature for 1 h. The solvent was removed under reduced pressure and the residue was extracted with CH_2Cl_2 (50 ml). The extract was washed with H_2O and dried over Na_2SO_4. After evaporation of solvent, the residue was recrystallized from diethyl ether–EtOH to give **6** (2.6 g, 85%) as white prisms, mp 128—130 °C. IR cm^{-1}: 2700—3450 (NH), 1720 (C=O). ^{1}H-NMR δ: 1.28 (3H, t, $J=7.1$ Hz, OCH$_2$CH$_3$), 2.99 (6H, s, N (CH$_3$)$_2$), 4.25 (2H, q, $J=7.1$ Hz, OCH$_2$CH$_3$), 4.61 (2H, s, SCH$_2$), 7.10—7.20 (2H, m, Ar-H), 7.45—7.55 (2H, m, Ar-H), 8.40 (1H, s, pyrimidine-H), 12.30 (1H, br s, NH). MS m/z: 357 (M$^+$). *Anal.* Calcd for C$_{17}$H$_{19}$N$_5$O$_2$S: C, 57.13; H, 5.36; N, 19.56. Found: C, 57.00; H, 5.50; N, 19.50.

Ethyl 2-[(1H-Benzimidazol-2-yl)sulfinylmethyl]-4-dimethylamino-5-pyrimidinecarboxylate (2) A solution of 80% m-CPBA (6.0 g, 28 mmol) in CH_2Cl_2 (120 ml) was added dropwise to a stirred solution of **6** (9.1 g, 25.5 mmol) at -20 °C over 1 h. When addition of m-CPBA was complete, saturated aqueous NaHCO$_3$ solution (100 ml) was added immediately and the mixture was stirred vigorously for several minutes. The CH_2Cl_2 layer was separated, washed with saturated aqueous NaHCO$_3$ solution and H_2O, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was recrystallized from AcOEt to give **2** (8.3 g, 87%) as pale yellow prisms, mp 133—134 °C. IR cm^{-1}: 3160 (NH), 1720 (C=O). ^{1}H-NMR δ: 1.29 (3H, t, $J=7.0$ Hz, OCH$_2$CH$_3$), 2.65 (6H, s, N(CH$_3$)$_2$), 4.28 (2H, q, $J=7.0$ Hz, OCH$_2$CH$_3$), 4.63 (1H, d, $J=13.5$ Hz, SOCH), 4.77 (1H, d, $J=13.5$ Hz, SOCH), 7.31 (2H, br s, Ar-H), 7.59 (1H, br s, Ar-H), 7.71 (1H, br s, Ar-H), 8.49 (1H, s, pyrimidine-H), 13.62 (1H, s, NH). MS m/z: 373 (M$^+$). *Anal.* Calcd for C$_{17}$H$_{19}$N$_5$O$_3$S: C, 54.68; H, 5.13; N, 18.75. Found: C, 54.51; H, 5.22; N, 19.09.

Basal Secretion in Pylorus-Ligated Rats The animals were anesthetized and the pylorus was ligated according to the method of Shay *et al.*[6] A suspension of test compound in 0.5 % aqueous carboxymethyl cellulose sodium salt (CMC) solution was administered into the duodenum immediately after pylorus ligation and the abdomen was closed by suturing. Four hours later, the animals were killed. The stomachs were removed and the gastric juice was collected by centrifugation at 3000 rpm for 10 min. The volume of each sample was measured and the acidity was determined using an autoburette. The total acid output was calculated.

Histamine-Stimulated Secretion in Pylorus-Ligated Rats A suspension of test compound in 0.5% aqueous CMC solution was administered intraduodenally to pylorus-ligated rats and, immediately, histamine (30 mg/kg) was injected subcutaneously. Two hours later, the animals were killed and the volume and acidity of gastric juice in the stomach were measured.

Ethanol-Induced Gastric Lesions[7] A suspension of test compound in 0.5% aqueous CMC solution was administered orally and, 30 min later, absolute ethanol (5 ml/kg) was given orally. One hour later, the animals were killed and the stomachs were isolated and fixed by treatment with 1 % aqueous formalin solution. The stomachs were cut open along the greater curvature and the length of lesions in the glandular portion was measured under a dissecting microscope with a square grid. The sum of the length of all lesions was employed as an ulcer index. In the case of parenteral examination, a 0.5 % aqueous CMC suspension or an aqueous solution of test compound was given by intraperitoneal or intravenous administration 30 min or just before, respectively, the oral administration of absolute ethanol.

H$^+$/K$^+$-ATPase Activity Dog gastric membrane protein containing H$^+$/K$^+$-ATPase was prepared according to the method of Saccomani *et al.*[8] The H$^+$/K$^+$-ATPase enzyme activity was measured in 1 ml of 70 mM Tris–HCl buffer (pH 7.4), 3 mM MgCl$_2$, and 2 mM Na$_2$ATP, with or without 20 mM KCl and a solution of test compound.[4] Membrane protein (20 μg) was added and the test tubes were preincubated with test compound at 37 °C for 30 min. The substrate, Na$_2$ATP, was then added and the test tubes were further incubated at 37 °C for 20 min. The reaction was stopped by addition of 14% trichloroacetic acid (1 ml) and the samples were centrifuged. The amount of inorganic phosphate in the supernatant was measured by the method of Fiske and Subbarow.[9]

Stress Ulcer[10] A suspension of test compound in 0.5% aqueous CMC solution was given orally 30 min before the immersion of rats into water. Each rat was placed in a stress cage and immersed to the level of the xiphoid process for 7 h in a water bath maintained at 23 °C. The animals were killed and the stomachs were examined for ulcers.

Acidified Aspirin Ulcer A suspension of test compound in 0.5% aqueous CMC solution was administered orally 30 min before the oral administration of a solution of aspirin (150 mg/kg) in 150 mM HCl. One hour later, the rats were killed and the stomachs were removed. The isolated stomachs were examined for ulcers.

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