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Benzylpiperazine Derivatives. VIII.[1] Syntheses, Antiulcer and Cytoprotective Activities of 1-(Aminocarbonylalkyl)-4-benzylpiperazine Derivatives and Related Compounds

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From a search for antiulcer agents with cytoprotective activity by random screening, 1-(pyrrolidinocarbonylmethyl)-4-(2,3,4-trimethoxybenzyl)piperazine dimaleate (**4h**) was found as a lead compound. Analogues of **4h** were synthesized and evaluated for antiulcer activity as well as toxicity. Four compounds (**4h**, **p**, **w**, **x**) exhibited potent antiulcer activity in several gastric ulcer models and low toxicity without any antisecretory activity. The antiulcer activity of these compounds was considered to be based on the cytoprotective activity.

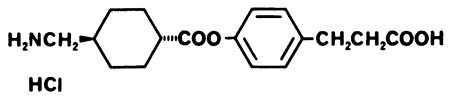
Keywords——benzylpiperazine; 1-piperazineacetamide; random screening; antiulcer activity; cytoprotective activity; antisecretory activity

It has been stated that peptic ulcers are induced by an imbalance between the aggressive factors such as acid or pepsin and the gastrointestinal mucosal resistance to the aggressive factors,[2] and that acid secretion is critical to the duodenal ulcer, while gastric ulcer is mainly induced by weakening of the defensive factors.[3] Thus, antiulcer agents are generally classified into two categories, antisecretory agents which suppress the aggressive factors and cytoprotective agents which strengthen the defensive mechanisms of the gastrointestinal mucosa. However, for the purpose of the treatment and prophylaxis of peptic ulcers, it is desirable to use an antiulcer agent with an excellent cytoprotective activity.[4]

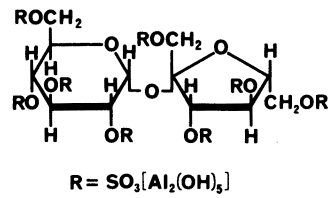
Strategy

There are various antiulcer agents which have cytoprotective activity, including cetraxate hydrochloride, sucralfate and teprenone. The structures of the known agents are quite diverse, as shown in Chart 1, but not all of these agents have sufficient activity, so that it does not seem likely to be fruitful to study derivatives of these agents in order to develop an antiulcer agent having more potent cytoprotective activity. Therefore, in order to find such an agent, random screening was considered to be appropriate.

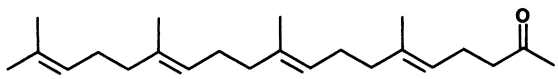
Experimental acute gastric ulcers are generally classified into four types; pylorus ligation-induced ulcer, drug-induced ulcer, stress-induced ulcer and necrotizing agent-induced ulcer. Among these models, pylorus ligation-induced ulcer was mainly used to identify antisecretory agents; other models are considered useful to find antiulcer agents with cytoprotective activity.[5] Thus, a facile indomethacin-induced ulcer model (a drug-induced ulcer model) was selected as a first screening method. A compound which showed a statistically significantly different result from the control at a dose of 200 mg/kg was judged to be active, and the oral toxicity (LD_{50}) was examined in mice. For active compounds with low toxicity, antisecretory activity and antiulcer activity against other ulcer models were examined and compared to those of known cytoprotective agents.



cetraxate hydrochloride



sucralfate



teprenone

Chart 1

Results and Discussion

Various biological activities have been reported for piperazine derivatives, so, benzylpiperazine derivatives which were originally designed as cerebral vasodilators but found to have low activity,[6] were screened, and 1-(pyrrolidinocarbonylmethyl)-4-(2,3,4-trimethoxybenzyl)piperazine dimaleate (**4h**) was found to possess potent antiulcer activity against indomethacin-induced gastric ulcer as well as ethanol-induced gastric ulcer, without any antisecretory activity. These facts prompted us to synthesize and test novel analogues of **4h**.

Most of the compounds were synthesized by three general methods according to Chart 2. In method A, a pyrrolidinocarbonylmethylpiperazine (**1**)[7] was alkylated or acylated by usual procedures. In method B, which was an application of the Leuckart–Wallach reaction, **1** was condensed reductively with a substituted benzaldehyde derivative by the use of formic acid without a solvent. In method C, a substituted benzylpiperazine[8] was allowed to react with a chloroalkylcarboxamide derivative (**2**)[9] in the presence of sodium bicarbonate in acetonitrile. Compound **4cc** was obtained from chloroethylpyrrolidine[10] and 2,3,4-trimethoxybenzylpiperazine. All compounds prepared were converted to acid addition salts and purified by recrystallization.

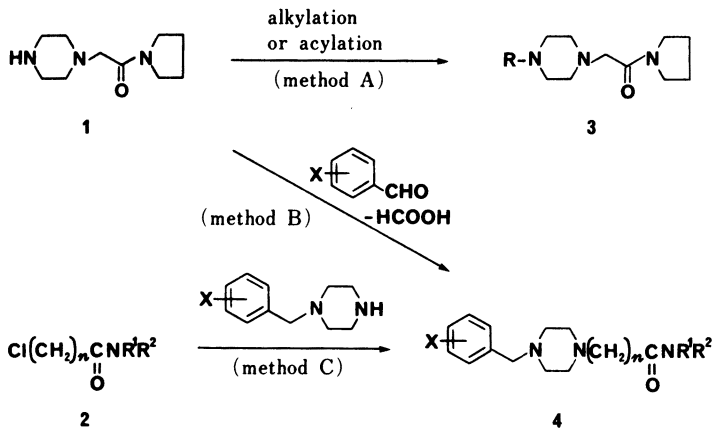
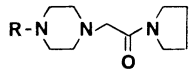


Chart 2

TABLE I. 1-Substituted-4-(pyrrolidinocarbonylmethyl)piperazine Salts (3) Prepared by Method A



No.	R	Yield	mp (°C) (Recryst. solvent)	Formula[a]	Analysis (%) Calcd (Found)			Antiulcer activity[a]
					C	H	N	
1[c]	H	75	149—151 (EtOH)	$C_{10}H_{19}N_3O \cdot 2MA$	50.35 (50.21	6.34 6.43	9.79 9.79)	—
3a	CH_3	73	186—190 (EtOH–MeOH)	$C_{11}H_{21}N_3O \cdot 2MA$	51.46 (51.52	6.59 6.67	9.48 9.53)	—
3b	$CH_2=CHCH_2$	43	166—168 (EtOH)	$C_{13}H_{23}N_3O \cdot 2MA$	53.84 (53.74	6.70 6.66	9.03 8.95)	—
3c	C_5H_{11}	38	188—189 (EtOH)	$C_{15}H_{29}N_3O \cdot 2MA$	55.08 (55.09	7.51 7.50	8.53 8.56)	—
3d	C_7H_{15}	59	184—186 (MeOH)	$C_{17}H_{33}N_3O \cdot 2MA$	56.91 (56.67	7.83 7.94	7.96 8.08)	—
3e	CH_3CO	36	140—145 (EtOH–AcOEt)	$C_{12}H_{21}N_3O_2 \cdot 1.5FA$	52.29 (52.45	6.58 6.69	10.16 10.21)	—
3f	TMB[d]	50	216—223 (EtOH–Et_2O)	$C_{20}H_{29}N_3O_5 \cdot HCl$	56.14 (55.98	7.07 7.03	9.82 9.92)	—
3g[e]	TMC[f]	67	127—129 (EtOH–Et_2O)	$C_{22}H_{31}N_3O_5 \cdot MA$	58.53 (58.46	6.61 6.45	7.88 7.97)	—

a) FA and MA stand for fumaric acid and maleic acid, respectively. *b*) Indomethacin-induced gastric ulcer (200 mg/kg). Statistically significant activity ($p<0.05$) is assessed on the following scale: +, active; −, inactive. *c*) See ref. 6. *d*) TMB: 2,3,4-trimethoxybenzoyl. *e*) See ref. 11. *f*) TMC: 3,4,5-trimethoxycinnamoyl.

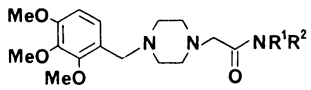
The compounds with various substituents in place of the 2,3,4-trimethoxybenzyl moiety of **4h** are summarized in Table I. Table I shows that the 2,3,4-trimethoxybenzyl moiety of **4h** can not be replaced by another alkyl or acyl moiety without considerable or complete loss of activity, so it plays a significant role in the antiulcer activity. For compound **3g**, which is a known cerebral vasodilator with the name of cinepazide maleate,[11] a weak antisecretory activity was reported, but no antiulcer activity was detected.

The compounds **4a**—**g** and **4i** modified at the amide moiety of **4h** are summarized in Table II. From these results, compounds with a less bulky amide group seemed preferable for antiulcer activity. Toxicity data (LD_{50}) revealed that **4h** is most preferable. Compound **4g** is a 2,3,4-trimethoxybenzyl derivative of hexaprazol, 1-cyclohexylaminocarbonylmethyl-piperazine, an antiulcer, antisecretory and cytoprotective agent.[12] However, **4g** did not exhibit any antiulcer activity.

Next, the effects of the substituent on the benzyl group were studied (Table III). The number and the locations of methoxy groups of **4h** were varied, and moreover, the substituents were examined according to Topliss' method.[13] Only the compounds with a 2,3,4-trimethoxy (**4h**), 3,4-dichloro (**4t**) or 3,4,5-trimethoxy (**4p**) group showed antiulcer activity. These results suggest that substitution at both the 3 and 4 positions is necessary for antiulcer activity, but the 3,4-dimethoxy compound (**4o**) is exceptional. From the LD_{50} values of active compounds, the hydrophilic 2,3,4-trimethoxy (**4h**) and 3,4,5-trimethoxy (**4p**) groups were preferable. The result with the homopiperazine analogue (**4v**) shows that the piperazine moiety is requisite for antiulcer activity.

For the final modification, the number of methylene groups between the piperazine 1-nitrogen and amide carbonyl was altered (Table IV). The compounds with short chains of one

TABLE II. 1-(Aminocarbonylmethyl)-4-(2,3,4-trimethoxybenzyl)piperazine Salts (**4a**—**i**) Prepared by Method C



No.	R^1	R^2	Yield	mp (°C) (Recryst. solvent)	Formula[a]	Analysis (%) Calcd (Found) C	H	N	Antiulcer[b] activity	Toxicity LD_{50} (mg/kg *p.o.*)
4a	H	H	92	134—136 (CH_3CN)	$C_{16}H_{25}N_3O_4 \cdot 2MA \cdot 0.75H_2O$	50.66 (50.74)	6.11 (6.18)	7.38 (7.68)	+	2250
4b	C_2H_5	C_2H_5	95	151—154 (CH_3CN)	$C_{20}H_{33}N_3O_4 \cdot 2MA$	54.71 (54.98)	6.67 (6.76)	6.97 (6.87)	+	1440
4c	C_3H_7	C_3H_7	80	164—167 (CH_3CN)	$C_{22}H_{37}N_3O_4 \cdot 2MA$	56.17 (56.33)	7.07 (7.09)	6.61 (6.57)	−	
4d	H	C_6H_5	68	161—164 (EtOH–MeOH)	$C_{22}H_{29}N_3O_4 \cdot 2MA \cdot 0.5H_2O$	54.96 (54.88)	6.87 (6.70)	8.68 (8.73)	+	<2500
4e	H	$C_6H_5CH_2$	76	148—150 (CH_3CN)	$C_{23}H_{31}N_3O_4 \cdot 2MA$	57.43 (57.67)	6.13 (6.09)	6.45 (6.51)	−	
4f	CH_3	C_6H_5	78	160—161 (EtOH)	$C_{23}H_{31}N_3O_4 \cdot 2MA$	57.65 (57.60)	6.19 (6.09)	6.59 (6.51)	−	
4g	H	cyclo-C_6H_{11}	44	131—135 (CH_3CN)	$C_{22}H_{35}N_3O_4 \cdot 2MA$	56.35 (56.51)	7.02 (6.80)	6.70 (6.59)	−	
4h	$-(CH_2)_4-$		98	147—148 (CH_3CN)	$C_{20}H_{31}N_3O_4 \cdot 2MA$	55.09 (55.16)	6.66 (6.45)	6.78 (6.89)	+	2500
4i	$-(CH_2)_5-$		58	159—160 (H_2O)	$C_{21}H_{33}N_3O_4 \cdot 2MA$	55.95 (55.85)	6.72 (6.63)	6.78 (6.74)	−	

a, *b*) See footnotes *a*) and *b*) in Table I, respectively.

or two methylenes showed antiulcer activity. The result of **4cc** suggests that the amide moiety is necessary for the activity.

From the standpoint of potent antiulcer activity and low toxicity, four structurally similar compounds (**4h**, **p**, **w**, **x**) were selected. Their ED_{50} values in various ulcer models and their antisecretory activities are summarized in Table V along with those of reference compounds. The selected compounds exhibited nearly equal potencies and their potencies were higher than those of the reference compounds.

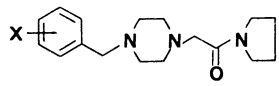
It should be noted that **4h**, **p**, **w** and **x** exhibited antiulcer activities against various model ulcers with no acid or pepsin antisecretory activity at a dose of 300 mg/kg. From these results, the antiulcer activity of these compounds was considered to be based on the cytoprotective activity. As the mechanism(s) of cytoprotection, mucosal blood flow increase, mucus excretion potentiation and bicarbonate ion excretion enhancing activity, *etc.* have been reported,[14] but the exact mechanism(s) in the cases of these compounds remain to be studied.

Experimental

Melting points were determined on a Yamato capillary melting point apparatus, model MP-21, and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Hitachi R-24B NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Silica gel 60 F_{254} (Merck) TLC plates were used for thin layer chromatography (TLC). For column chromatography, Silica gel 60 (Merck) was used.

Typical Procedures of Method A (Alkylation)——1-Pentyl-4-(pyrrolidinocarbonylmethyl)piperazine Maleate (**3c**): A mixture of pentyl iodide (3.5 g), **1** (3.0 g), K_2CO_3 (10.4 g) and *N*,*N*-dimethylformamide (DMF, 40 ml) was stirred at 70 °C for 2 h. The reaction mixture was poured into water and extracted with AcOEt (50 ml). The organic

TABLE III. 1-Benzyl-4-(pyrrolidinocarbonylmethyl)piperazine Salts (**4j**—**v**) Prepared by Method B



No.	X	Yield	mp (°C) (Recryst. solvent)	Formula[a)]	Analysis (%) Calcd (Found) C	H	N	Antiulcer activity	Toxicity LD_{50} (mg/kg *p.o.*)
4j	H	42	195—196.5 (EtOH)	$C_{17}H_{25}N_3O\cdot 2MA$	57.64 (57.80)	6.40 (6.40)	8.10 (8.09)	−	
4k	2-OMe	66	190—192 (EtOH)	$C_{18}H_{27}N_3O_2\cdot 2MA$	56.82 (56.94)	6.40 (6.43)	7.65 (7.72)	−	
4l	3-OMe	55	188—190 (dec.) (MeOH)	$C_{18}H_{27}N_3O_2\cdot 2MA$	56.82 (56.71)	6.40 (6.17)	7.65 (7.70)	−	
4m	4-OMe	35	190—192 (EtOH)	$C_{18}H_{27}N_3O_2\cdot 2MA$	56.82 (56.74)	6.40 (6.40)	7.65 (7.64)	−	
4n	2,4-$(OMe)_2$	67	168—169 (EtOH)	$C_{19}H_{29}N_3O_3\cdot 2MA$	55.92 (55.92)	6.43 (6.50)	7.25 (7.27)	−	
4o	3,4-$(OMe)_2$	47	179—180.5 (MeOH)	$C_{19}H_{29}N_3O_3\cdot 2MA$	55.92 (55.93)	6.43 (6.53)	7.25 (7.23)	−	
4p	3,4,5-$(OMe)_3$	52	204—207 (MeOH)	$C_{20}H_{31}N_3O_4\cdot 2FA$	55.17 (55.11)	6.45 (6.47)	6.89 (6.91)	+	4200
4q	2,4,6-$(OMe)_3$	50	156.5—158 (EtOH)	$C_{20}H_{31}N_3O_4\cdot 2MA$	55.17 (54.99)	6.45 (6.42)	6.89 (6.79)	−	
4r	4-Me	38	197.5—201 (EtOH)	$C_{18}H_{27}N_3O\cdot 2MA$	58.53 (58.30)	6.61 (6.50)	7.88 (7.90)	−	
4s	4-Cl	36	195—198 (dec.) (EtOH–MeOH)	$C_{17}H_{24}ClN_3O\cdot 2MA$	54.20 (54.07)	5.82 (5.61)	7.58 (7.58)	−	
4t	3,4-Cl_2	45	191.5—193.5 (EtOH)	$C_{17}H_{23}Cl_2N_3O\cdot 2MA$	51.03 (51.02)	5.31 (5.19)	7.14 (7.24)	+	<2500
4u	2,4-Cl_2	35	167.5—168.5 (H_2O)	$C_{17}H_{23}Cl_2N_3O\cdot 2MA$	51.03 (50.96)	5.31 (5.43)	7.14 (7.29)	−	
4v[c)]	2,3,4-$(OMe)_3$	35	147—151 (dec.) (EtOH)	$C_{21}H_{33}N_3O_4\cdot 2OA^{d)}\cdot 0.5H_2O$	51.72 (51.91)	6.60 (6.58)	7.24 (7.35)	−	

a, *b*) See footnotes *a*) and *b*) in Table I, respectively. *c*) Homopiperazine analogue of **4h**. *d*) OA stands for oxalic acid.

layer was washed with saline and dried over $MgSO_4$. After removal of the solvent, the residue was mixed with maleic acid (3.5 g) and EtOH (40 ml). The precipitated solid was collected and recrystallized from EtOH to give **3c** (2.8 g).

Compounds **3a**, **3b** and **3d** were obtained in the same manner as described for **3c**. The yield, melting point and elemental analysis data are given in Table I.

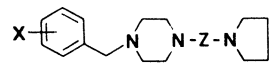
Typical Procedure of Method A (Acylation)——1-(Pyrrolidinocarbonylmethyl)-4-(2,3,4-trimethoxybenzoyl)-piperazine Hydrochloride (**3f**): A mixture of 2,3,4-trimethoxybenzoyl chloride (1.2 g). **1** (1.0 g), triethylamine (1.0 ml) and benzene (20 ml) was refluxed for 10 min. The reaction mixture was washed with water and dried over $MgSO_4$. After removal of the solvent, the residue was diluted with Et_2O (20 ml). Then HCl–MeOH was added and the precipitated solid was filtered off. Recrystallization from EtOH–Et_2O gave **3f** (1.1 g).

Compounds **3e** and **3g** were obtained in the same manner as described for **3f**. The yield, melting point and elemental analysis data are given in Table I.

Typical Procedures of Method B——1-(Pyrrolidinocarbonylmethyl)-4-(3,4,5-trimethoxybenzyl)piperazine Difumarate (**4p**): 3,4,5-Trimethoxybenzaldehyde (5.0 g) and **1** (5.0 g) were melted in an oil bath at 120 °C and formic acid (2.0 ml) was added dropwise. The mixture was stirred for 45 min under heat, and then allowed to cool to room temperature. The mixture was diluted with EtOH (40 ml) and fumaric acid (6.0 g) in EtOH (40 ml) was added to the solution. The precipitated solid was collected and recrystallized from MeOH to give **4p** (8.0 g).

Compounds **4j**—**o** and **4q**—**v** were obtained in the same manner as described for **4p**. The yield, melting point and elemental analysis data are given in Table III.

TABLE IV. 1-(Pyrrolidinocarbonylalkyl)-4-(2,3,4- or 3,4,5-trimethoxybenzyl)piperazine Salts (**4w**—**cc**) Prepared by Method C



No.	X	Z	Yield	mp (°C) (Recryst. solvent)	Formula[a]	Analysis (%) Calcd (Found) C	H	N	Antiulcer activity[b]	Toxicity LD_{50} (mg/kg *p.o.*)
4w	2,3,4-$(OMe)_3$	$(CH_2)_2CO$	32	137—141 (EtOH)	$C_{21}H_{33}N_3O_4\cdot$ 2MA	55.85 (55.84)	6.63 (6.73)	6.74 (6.78)	+	3400
4x	3,4,5-$(OMe)_3$	$(CH_2)_2CO$	41	175—180 (MeOH)	$C_{21}H_{33}N_3O_4\cdot$ 2MA	55.85 (55.82)	6.63 (6.72)	6.74 (6.65)	+	3600
4y	2,3,4-$(OMe)_3$	$(CH_2)_3CO$	38	145—148 (EtOH)	$C_{22}H_{35}N_3O_4\cdot$ 2MA	56.51 (56.51)	6.78 (6.80)	6.66 (6.59)	−	
4z	3,4,5-$(OMe)_3$	$(CH_2)_3CO$	26	149—155 (EtOH)	$C_{22}H_{35}N_3O_4\cdot$ 2MA·H_2O	54.76 (54.95)	6.67 (6.92)	6.24 (6.41)	−	
4aa	2,3,4-$(OMe)_3$	$(CH_2)_4CO$	35	132—135 (EtOH)	$C_{23}H_{37}N_3O_4\cdot$ 2MA·$0.75H_2O$	55.97 (55.94)	7.05 (6.87)	6.32 (6.30)	−	
4bb	3,4,5-$(OMe)_3$	$(CH_2)_4CO$	17	166—168 (EtOH)	$C_{23}H_{37}N_3O_4\cdot$ 2MA·$0.5H_2O$	56.34 (56.35)	6.70 (6.87)	6.28 (6.36)	−	
4cc	2,3,4-$(OMe)_3$	CH_2CH_2	35	230—235 (dec.) (EtOH–Et_2O)	$C_{20}H_{33}N_3O_3\cdot$ 3HCl	50.80 (50.92)	7.67 (7.95)	8.89 (8.96)	−	

a, *b*) See footnotes *a*) and *b*) in Table I, respectively.

TABLE V. Comparative Pharmacological Effects of **4h**, **p**, **w**, **x**, and Reference Compounds

Compound	Antiulcer activity ED_{50} (mg/kg, *p.o.*)			Antisecretory activity ED_{50} (mg/kg, *p.o.*)	
	Indomethacin	Ethanol	Stress	Acid	Pepsin
4h	30	25	125	>300	>300
4p	31	34	89	>300	>300
4w	27	22	125	>300	>300
4x	29	30	113	>300	>300
Cetraxate hydrochloride	361	112	>1000	>300	>300
Sucralfate	216	150	NT	>300	NT
Teprenone	90	55	140	>300	NT

NT: not tested.

Typical Procedures of Method C——1-[2-(Pyrrolidinocarbonyl)ethyl]-4-(2,3,4-trimethoxybenzyl)piperazine Dimaleate (**4y**): A mixture of 1-(3-chloropropionyl)pyrrolidine (3.2 g), 1-(2,3,4-trimethoxybenzyl)piperazine dihydrochloride (6.8 g), K_2CO_3 (11.1 g) and CH_3CN (80 ml) was stirred at 70 °C for 15 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was diluted with EtOH (40 ml), and maleic acid (4.6 g) in EtOH (40 ml) was added to the solution. The precipitated solid was collected and recrystallized from EtOH to give **4y** (4.0 g).

Compounds **4a**—**i** and **4w**—**bb** were obtained in the same manner as described for **4y**. The yield, melting point and elemental analysis data are given in Tables II and IV.

1-[2-(Pyrrolidino)ethyl]-4-(2,3,4-trimethoxybenzyl)piperazine Trihydrochloride (4cc)——A mixture of chloroethylpyrrolidine[10] (4.08 g), 2.3.4-trimethoxybenzylpiperazine dihydrochloride (6.78 g), K_2CO_3 (13.8 g) and DMF (80 ml) was stirred at 80 °C for 3 h. The reaction mixture was poured into water and extracted with $CHCl_3$ (120 ml). The organic layer was washed with water and dried over $MgSO_4$. After removal of the solvent, HCl–EtOH was added. The precipitated solid was collected and recrystallized from EtOH–Et_2O to give **4cc** (3.3 g).

Antiulcer Activity——Indomethacin-Induced Gastic Ulcer[15]: Male Sprague–Dawley (SD) rats (weighing 180—

220 g, 8 weeks age, 16 rats per group) were fasted for 24 h, and the test compound (200 mg/kg, dissolved in distilled water or suspended in 1% aqueous gum arabic) or the vehicle was administered orally. After 15 min, indomethacin (30 mg/kg, dissolved in 3% aqueous Na_2CO_3 solution, s.c.) was administered. Five hours after the administration of indomethacin, the rats were sacrificed under ether anesthesia and the stomachs were removed. The stomachs were inflated with 1% formalin (12 ml), and placed in 1% formalin for 15 min to fix the outer layer of the stomach. After opening of the stomach along the greater curvature, the length (mm) of each ulcer found was measured for each rat. The sum of the length of ulcers in each rat was used as the ulcer index. The statistical significance of the difference between the mean ulcer index of the drug-treated group and that of the control group was calculated by using Student's *t* test. The ED_{50} value was calculated from the dose response curve which was drawn based on the ratio of the ulcer index of the drug-treated group to the mean ulcer index of the control group.

Ethanol-Induced Gastric Ulcer——Male SD rats (weighing 180—220 g, 8 weeks of age, 16 rats per group) were fasted for 24 h, and the test compound in the form of a solution in distilled water or a suspension in 1% aqueous gum arabic was administered orally. After 30 min, ethanol (99.5%, 1 ml) was orally administered to the rats in the same manner as described by Robert *et al.*[16] One hour after the administration of ethanol, the rats were sacrificed and the ulcer index as well as ED_{50} value were determined as described in the case of indomethacin-induced gastric ulcer.

Water-Immersion Stress-Induced Gastric Ulcer——Male SD rats (weighing 180—220 g, 8 weeks of age, 16 rats per group) were fasted for 24 h, and the test compound (dissolved in distilled water or suspended in 1% aqueous gum arabic) was administered orally. After 15 min, the rats were immersed vertically to the height of the xiphoid process in a water bath at 23 °C within a stress cage in the same manner as described by Takagi and Okabe.[17] After 17 h, the rats were sacrificed and the ulcer index as well as ED_{50} value were determined in the same manner as described in the case of indomethacin-induced gastric ulcer.

Gastric Antisecretory Activity——Male SD rats (weighing 180—200 g, 8 weeks of age, 8 rats per group) were fasted for 48 h. The small midline incision was performed, and the pylorus was ligated under ether anesthesia.[18] The test compound (dissolved in distilled water or suspended in 1% aqueous gum arabic) was administered intraduodenally to the rats immediately after the ligation. Four hours later, the abdomen was closed, the stomach was removed, and the volume of accumulated gastric juice therein was measured. The acid concentration was measured by titration against 0.05 N NaOH solution.

The peptic activity was determined according to Anson and Mirsky.[19]

Acute Toxicity——Male ddY mice (weighing 18—22 g, 4 weeks of age. 5 mice per group) fasted overnight were orally given the test compound in the form of a solution in distilled water or a suspension in 1% aqueous gum arabic. Thereafter, the death of mice was observed for 7 d. From the number of dead mice, the LD_{50} value was calculated according to Weil's method.[20]

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