Syntheses and Inhibitory Effects on Gastric Lesions of 4-Guanidinomethylbenzoic Acid Arylamides

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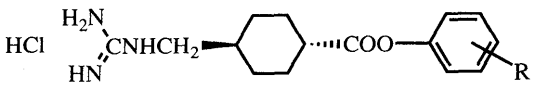
A novel series of 4-guanidinomethylbenzoic acid (GMBA) arylamides was synthesized. Several showed more potent inhibitory effects on stress-induced gastric lesion in rats than cetraxate. We selected 4-guanidinomethylbenzoic acid (2′-ethoxycarbonyl)phenylamide 3 for further pharmacological assessments because it had low toxicity. Compound 3 showed significant inhibitory effects on stress-, HCl-ethanol- and indomethacin-induced gastric lesions and gastric secretion, the ED_{50} values being 34.4, 45.0 and 23.0 mg/kg (*p.o.*) and 240 mg/kg (i.d.), respectively. Furthermore, this compound restored the reduction of gastric mucus caused by the stress-loading and inhibited compound 48/80-induced ulcer.

Keywords GMBA arylamide; GMBA (2′-ethoxycarbonyl)phenylamide; anti-ulcer activity; toxicological test; mucosal protection

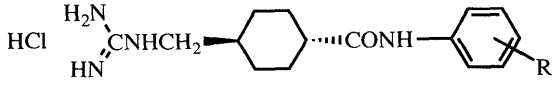
We have already synthesized a series of *trans*-4-guanidinomethylcyclohexanecarboxylic acid (*trans*-GMCHA) arylesters (Chart 1, type I) and arylamides (Chart 1, type II), and tested them for inhibitory effects on several kinds of serine proteases and for anti-ulcerous activities.[1,2] The *trans*-GMCHA (2′-benzyloxycarbonyl)phenylester and *trans*-GMCHA (2′-ethoxycarbonyl)phenylamide were found to be efficient anti-ulcer agents among type I and type II, respectively. Each compound showed a more potent inhibitory effect on HCl–ethanol-induced gastric lesion than cetraxate, and the anti-ulcer activity was not related to anti-serine protease activities.

Another group reported[3] that 4-guanidinobenzoic acid esters and amides have specific anti-trypsin activity. However, there has been no report on the pharmacological activity of 4-guanidinomethylbenzoic acid (GMBA) arylamides (Chart 1, type III).

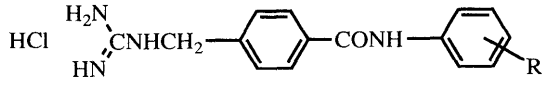
In this report, we describe syntheses of GMBA arylamides and the effects of these compounds on stress-induced gastric lesion, as well as toxicological assessments, and we discuss the related pharmacological effects of 4-guanidinomethylbenzoic acid (2′-ethoxycarbonyl)phenylamide **3**.



type I compounds



type II compounds



type III compounds

Chart 1

Materials and Methods

General All melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were measured with a Shimadzu IR-27G. The mass spectra (MS) were recorded on a Shimadzu MS LKB 9000B. All elemental analyses were found to be within 0.4% of the calculated values. Test compounds were given to animals as a suspension in 0.5% sodium carboxymethyl cellulose solution.

Materials Cetraxate HCl was a gift from Daiichi Seiyaku Co., Tokyo. Cimetidine was purchased from Sigma Chemical Co., St. Louis. Indomethacin, alcian blue 8GX and aniline compounds were purchased from Wako Pure Chemical Co., Osaka. Male Sprague–Dawley (SD) rats (200—220 g), Wistar rats (120—140 g) and ICR mice (20—30 g) were purchased from Charles River, Japan.

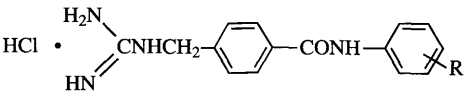
Synthesis of 4-Guanidinomethylbenzoic Acid Hydrochloride (GMBA–HCl) A solution of 2 M NaOH (200 ml) was added to a solution of *S*-methylisothiourea sulfate (17.7 g, 127 mmol) in H_2O (200 ml) with cooling in ice and stirring, then *p*-aminomethylbenzoic acid (10.0 g, 66 mmol) in boiling H_2O (50 ml) was added dropwise. The mixture was left to stand overnight at room temperature and then cooled for 1 h in an ice bath. The precipitated white crystals were filtered off and washed with cold H_2O. The yield of GMBA was 9.9 g (77.7%). GMBA (9.9 g, 54.7 mmol) was dissolved in warm 1 N HCl (99 ml) and insoluble material was removed by filtration. The GMBA–HCl precipitated from the filtrate on cooling and was recrystallized from H_2O–MeOH (8.4 g, 71.3%, mp 227—230 °C). *Anal.* Calcd for $C_9H_{11}N_3O_2 \cdot HCl$: C, 47.07; H, 5.27; N, 18.30. Found: C, 46.98; H, 5.15; N, 18.37.

Synthesis of 4-GMBA Arylamides Method A: GMBA–HCl (1.00 g, 4.4 mmol), the appropriate aniline (4.3—4.6 mmol) and *N,N′*-dicyclohexylcarbodiimide (1.18 g, 5.7 mmol) were dissolved in dry pyridine (20 ml), and the solution was stirred for 40 h at room temperature. Water (10 ml) was added, and the mixture was stirred for 30 min. The precipitate was removed by filtration, the filtrate was evaporated and the residue was dried *in vacuo* to give a crystalline residue. The residue was recrystallized from H_2O, providing compounds **1** and **2** (Table I).

Method B: One gram (9.0—13.0 mmol) of the appropriate aniline in dry pyridine (20 ml) was cooled to 0 °C with stirring. A solution of PCl_3 (5.0—5.9 mmol) in 10 ml of dry pyridine was added dropwise thereto. After 15 min, GMBA–HCl (10.0—11.0 mmol) was added at room temperature, and the mixture was stirred for 3 h. The volatile portion of the mixture was evaporated, followed by addition of cold H_2O to give a crystalline residue. The residue was recrystallized from H_2O, providing compounds **3**—**9** (Table I).

Toxicity Test The test compounds (400 mg/kg) were administered orally once daily for 90 d. Each test group consisted of 6 male Wistar rats, 5 weeks old at the start of dosing. At the end of the 90-d test period, all animals were killed and necropsied for clinical laboratory testing and histomorphological testing. Biochemical findings on sera were obtained with an Olympus AU550. For acute toxicity testing, male ICR mice (each group $n=5$), 5 weeks old, were used in the usual way.

TABLE I. Chemical and Physical Data for GMBA Arylamides



No.	R	Yield (%)	mp (°C)	IR ν_{max}^{KBr} cm^{-1}			MS m/z ($M^+ - HCl$)	Formula	*Anal.*[a]
				H_2N HN >CNH–	–CONH–	–COOR–			
1	H	38[b]	178—180	3350	1660		268	$C_{15}H_{16}N_4O \cdot HCl$	C, H, N
2	4′-Cl	16[b]	199—202	3370	1655		302, 304[d]	$C_{15}H_{15}ClN_4O \cdot HCl$	C, H, N
3	2′-COOEt	40[c]	211—213	3345	1600	1670	340	$C_{18}H_{20}N_4O_3 \cdot HCl$	C, H, N
4	4′-Me	53[c]	124—125	3355	1610		282	$C_{16}H_{18}N_4O \cdot HCl$	C, H, N
5	4′-*tert*-Bu	74[c]	254—256	3380	1605		324	$C_{19}H_{24}N_4O \cdot HCl$	C, H, N
6	4′-OMe	83[c]	260—263	3300	1630		298	$C_{16}H_{18}N_4O_2 \cdot HCl$	C, H, N
7	3′-COOEt	62[c]	217—220	3395	1660	1700	340	$C_{18}H_{20}N_4O_3 \cdot HCl$	C, H, N
8	4′-COOEt	32[c]	120—120.5	3335	1655	1720	340	$C_{18}H_{20}N_4O_3 \cdot HCl$	C, H, N
9	4′-NO_2	35[c]	260—262	3445	1690		313	$C_{15}H_{15}N_5O_3 \cdot HCl$	C, H, N

a) All elemental analyses were found to be within 0.4% of calculated values. *b*) Method A, see experimental section. *c*) Method B, see experimental section. *d*) $M^+ + 2$.

Assay for Anti-ulcer Activity Stress-Induced Gastric Lesion: Gastric lesion induced by stress in rats was induced by using the method described by Takagi and Okabe.[4] Namely, after 24 h starvation, male SD rats were immersed in a water bath (24±1 °C) vertically to the level of the xiphoid process. The animals were killed with an overdose of ether after 16 h stress load and the stomachs were removed. Test compounds were given orally 10 min before stress load.

HCl–Ethanol-Induced Gastric Lesion: HCl–ethanol-induced gastric lesion was caused by the method of Mizui and Doteuchi.[5] Male SD rats were starved for 24 h before the experiments, but allowed free access to water. A solution of ethanol (60%) in 150 mM HCl was given orally in 1 ml injections, and then the animals were killed with an overdose of ether 1 h later for removal of the stomach. Test compounds were given orally 10 min before the treatment with HCl–ethanol.

Indomethacin-Induced Gastric Lesion: Indomethacin-induced gastric lesion was produced by the method of Kasuya *et al.*[6] Male SD rats were starved for 24 h before the experiments, but allowed free access to water. Indomethacin was given subcutaneously (s.c.) at a dose of 20 mg/kg, and then the animals were killed with an overdose of ether 7 h later for removal of the stomach. Test compounds were given orally 10 min before the treatment with indomethacin.

Gastric Secretion: Gastric secretion was caused by the method of Shay *et al.*[7] Male SD rats were starved for 24 h before the experiments. The pylorus was ligated under ether anesthesia. The animals were killed with an overdose of ether 4 h later for removal of to remove the stomach. The stomach contents were collected and their volume was measured. Test compounds were given i.d. immediately after this ligation.

Measurement of Gastric Mucus in Stress-Induced-Treated Rat: Gastric mucus was measured by the method of Corne *et al.*[8] Male SD rats were starved for 24 h before the experiments, but allowed free access to water, then immersed in a water bath (24±1 °C) vertically to the level of the xiphoid process for 16 h, and killed with an overdose of ether. The stomach was removed immediately and the quantification of gastric mucus *in situ* was done by using the alcian blue method, (the dye combines with acidic mucopolysaccharides). Test compounds were given orally 30 min before the stress load.

Acetic Acid-Induced Gastric Ulcer: Acetic acid-induced gastric ulcer was produced by the method of Takagi *et al.*[9] Male SD rats were fasted for 24 h before the experiments, but allowed free access to water. After exteriorization of the stomach, the surface of the anterior wall of the glandular stomach was brought into contact with 1 ml of glacial acetic acid using a cylinder (10 mm in diameter) for 1 min. After closure of the incised abdomen, the animals were given feed and water *ad libitum*. They were killed by means of a blow on the head 14 d after the operation to remove the stomach. Test compounds were given orally once daily for 13 d from the day after the operation.

Compound 48/80-Induced Gastric Lesion: Compound 48/80-induced gastric lesion was produced by the method of Takeuchi *et al.*[10] Male SD rats were starved for 24 h before the experiments, but allowed free access to water. Compound 48/80 was given intraperitoneally at 1.0 mg/kg once daily for 6 d, and then the animals were killed with an overdose of ether 24 h later. Test compounds were given orally for 6 d (twice daily; 30 min before and 9 h after administration of compound 48/80).

Measurement of Gastric Lesion in Rat Stomachs: The stomachs were immediately removed, inflated by pouring in 10 ml of 1% formalin and left for 10 min to fix the gastric wall. Subsequently, 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. Lesion severity was expressed as the total length or area of lesions per stomach.

Results and Discussion

Chemistry We synthesized a novel series of 4-guanidinomethylbenzoic acid arylamide hydrochlorides by the following methods (compounds **1**—**9**, Table I). Compounds **1** and **2** were synthesized by condensation of GMBA–HCl and the corresponding aniline in pyridine with DCC. Compounds **3**—**9** were synthesized as follows. The corresponding aniline and PCl_3 were condensed in pyridine to give dianilide phosphine, and then GMBA–HCl was added. Compounds **3**—**9** were obtained by simple purification. The structures of these derivatives were determined by IR, MS and elemental analyses.

Pharmacology The effects of compounds **1**—**9** on stress-induced gastric lesions in rats are shown in Table II. Compounds **1** to **4** showed potent inhibitory effects, and compounds **5**, **6** and **9** showed moderate inhibitory effects. The GMBA–HCl (100 mg/kg, *p.o.*), a starting material for GMBA arylamides, exerted no effect on gastric lesions in rats (data not shown).

The anti-ulcer activity of lead compound **1** was lost when strongly electron-donating or strongly electron-accepting groups or large substituents were present. Compound **8** bearing a 4′-ethoxycarbonyl group, rather exacerbated gastric lesions, and compound **7** bearing a 3′-ethoxycarbonyl group exhibited a similar effect. However, compound **3** bearing a 2′-ethoxycarbonyl group showed a potent inhibitory effect.

Toxicity Test Compounds **1**—**4** did not cause moribundity or death at 400 mg/kg *p.o.* during the test period. Compounds **1** and **2** caused increases in GOT and GPT (Table III). All rats orally given compound **3** or **4** showed no toxicological sign. In acute toxicity studies, compound **4** showed a lethal dose of 1000 mg/kg (s.c.). However, the

TABLE II. Inhibitory Effect of GMBA Arylamides on Stress-Induced Gastric Lesions in Rats

Compound	Inhibition (%)	
	30 mg/kg	100 mg/kg[a]
1	71[b]	96[c]
2	69[b]	95[c]
3	63[b]	84[c]
4	67[b]	92[c]
5	32	58
6	47	71[b]
7	—[a]	−71[b,d]
8	—[a]	−31[d]
9	—[a]	51
Cetraxate	46	44

Each test $n=5$, significantly different from vehicle. *a*) Not tested. *b*) $p<0.05$. *c*) $p<0.001$. *d*) Exacerbation % of gastric lesions to vehicle.

TABLE III. GOT and GPT in Sera of Male Rats Orally Given GMBA Arylamides for 6 Weeks

Compound	GOT (1 U/l)	GPT (1 U/l)
Vehicle	94±15	32± 4
1	230±58[a]	122±78
2	96±28	77±50
3	94±17	36± 4
4	80±19	28± 4

Each test $n=6$, significantly different from vehicle. *a*) $p<0.05$.

TABLE IV. ED_{50} of Compound **3** on Experimental Gastric Lesions in Rats

Experimental gastric lesion	Route	ED_{50} (mg/kg)[a]
Stress	*p.o.*	34.4 (28.3— 41.9)
Indomethacin	*p.o.*	23.0 (15.5— 34.2)
HCl–ethanol	*p.o.*	45.7 (39.4— 52.9)
Gastric secretion	i.d.	240.2 (170.5—340.0)

a) The 95% confidence limits. Anti-ulcerous activity of compound **3** was investigated at 3 or 4 doses. Each dose was tested on at least 5 animals.

minimum lethal dose of compound **3** was over 2000 mg/kg (s.c.). So, compound **3** was selected for further pharmacological examinations.

Effects of Compound 3 on the Gastric Lesions The effects of compound **3** on gastric lesions induced by water-immersion stress, indomethacin, HCl–ethanol and gastric secretion were assessed. Compound **3** showed significant, dose-dependent inhibitory effects on these gastric lesions. The ED_{50} values are shown in Table IV. Compound **3** is a mucosa-protective anti-ulcer agent because its effective dose against gastric secretion was higher than that against HCl–ethanol-induced gastric lesion.

Cetraxate and benexate, typical mucosa-protective anti-ulcer agents, were proposed to inhibit the depression of prostaglandins and to increase gastric mucous secretion on gastric lesion.[11,12] Compound **3** exhibited more potent activity against indomethacin-induced gastric lesion than cetraxate (data not shown). In addition, compound **3** significantly increased gastric mucous secretion, which was reduced by stress loading (Table V). Azuumi *et al.*[13] reported that mucopolysaccharide plays a very important role in protection against gastric injury. From these results, anti-ulcer mechanisms of compound **3** might involve increase in the levels of prostaglandins and gastric mucous secretion.

TABLE V. Effect of Compound **3** and Cetraxate on Gastric Mucus

Treatment	Dose (mg/kg *p.o.*)	Mucus OD_{620} (mean±S.E.)
Normal		0.97±0.04
Stress		0.85±0.03
Stress+compound **3**	30	1.08±0.05[a]
	100	1.23±0.06[a]
Stress+cetraxate	100	1.22±0.06[a]

Each test $n=7$, significantly different from normal. *a*) $p<0.05$.

TABLE VI. Inhibitory Effect of Compound **3** on Gastric Ulcers Induced by Acetic Acid in Rats

Compound	Dose (mg/kg *p.o.*)	Ulcer index (mm^2) (mean±S.E.)	Inhibition (%)
Vehicle		48.3± 8.0	
Compound **3**	20	39.2±15.9	19
	50	36.5± 7.4	31
	150	32.9±11.8	42
Cetraxate	20	39.5±11.0	18
	50	32.1± 8.5	34
	150	46.4±14.6	4

Each test $n=7$.

TABLE VII. Inhibitory Effect of Compound **3**, Cetraxate and Cimetidine on Compound 48/80-Induced Gastric Lesions in Rats

Compound	Dose (mg/kg *p.o.*)	Ulcer index (cm^2) (mean±S.E.)	Inhibition (%)
Vehicle		5.6±0.5	
Compound **3**	15	3.5±0.4	37
	30	2.1±0.4[a]	63
	100	2.0±0.3[a]	64
Cetraxate	100	3.9±0.3	30
Cimetidine	100	2.6±0.6[b]	53

Each test $n=5$, significantly different from vehicle. *a*) $p<0.001$, *b*) $p<0.05$.

The curative effect of compound **3** on acetic acid-induced gastric ulcers was examined. Compound **3** dose-dependently reduced gastric ulcers induced by acetic acid, although its effect was not statistically significant (Table VI). Further, the effect of compound **3** was examined on compound 48/80-induced gastric lesions.[13] Compound **3** and cimetidine demonstrated more potent inhibitory effects than cetraxate (Table VII).

In conclusion, compound **3**, which was selected from among compounds **1**—**9**, has mucosal protective anti-ulcer activity like ω-guanidinoalkylcarboxylic acid derivatives.[1,2] Further, compound **3** has a mucosal protective effect and a slight anti-gastric secretory activity, exhibiting more potent anti-ulcer activity than cetraxate.

References

1) T. Satoh, M. Muramatsu, Y. Ooi, H. Miyataka, T. Nakajima, M. Umeyama, *Chem. Pharm. Bull.*, **33**, 647 (1985).
2) H. Ueda, Y. Momoi, T. Ikeda, H. Kakegawa, H. Miyataka, H.

Matsumoto, T. Satoh, *Chem. Pharm. Bull.*, **41**, 522 (1993).
3) F. Markwardt, P. Walsmann, J. Sturzebecher, H. Landmann, G. Wagner, *Pharmazie*, **28**, 326 (1973).
4) K. Takagi, S. Okabe, *Jpn. J. Pharmacol.*, **18**, 9 (1968).
5) T. Mizui, M. Doteuchi, *Jpn. J. Pharmacol.*, **33**, 939 (1983).
6) Y. Kasuya, T. Murata, S. Okabe, *Jpn. J. Pharmacol.*, **28**, 297 (1978).
7) H. G. Shay, D. C. H. Sun, M. Gruenstein, *Gastroenterology*, **26**, 906 (1954).
8) S. J. Corne, S. M. Morrissey, R. J. Woods, *J. Physiol.*, **242**, 116 (1974).
9) K. Takagi, S. Okabe, R. Saziki, *Jpn. J. Pharmacol.*, **19**, 716 (1969).
10) K. Takeuchi, O. Furukawa, H. Nishiwaki, "Jikken Kaiyou," Igakutosyoshuppan Co., Tokyo, 1987, pp. 91—104.
11) H. Kawai, *Jpn. J. Gastroenterol.*, **70**, 208 (1973).
12) Y. Azuumi, H. Okabe, H. Kuwata, K. Ishihara, K. Hotta, *Prog. Med.*, **6** (suppl. 1), 2092 (1986).
13) Y. Azuumi, S. Ohara, K. Ishihara, H. Okabe, K. Hotta, *Gut.*, **21**, 533 (1980).