Synthesis and Protective Effect of 1,3,5-Triazine Derivatives, Leukotriene C_4 Antagonist, on HCl·Ethanol-Induced Gastric Lesions in Rats

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2,4-Diamino-[*E*]-6-[2-(3-pyridyl)ethenyl]-1,3,5-triazine (3a), leukotriene C_4 (LTC_4) antagonist, was found to possess a protective effect on HCl·ethanol-induced gastric lesions. Analogues of 3a were synthesized and evaluated for the effect as well as antagonistic activity against LTC_4-induced contraction. Seven compounds (3a—d, f—h) exhibited a potent protective effect on gastric lesions which was considered to be based on the antagonistic activity against LTC_4. The structure–activity relationships of the derivatives (3a—k) are discussed.

Keywords 1,3,5-triazine; leukotriene C_4 antagonist; HCl·ethanol-induced gastric lesions; structure–activity relationship

Antiinflammatory activity has been reported for 1,3,5-triazine derivatives.[1] On the other hand, antiallergic activity has been reported for substituted ethenylpyridine derivatives.[2] And so, 2,4-diamino-[*E*]-6-[2-(3-pyridyl)-ethenyl]-1,3,5-triazine (**3a**, Chart 1), which was originally designed as an antiallergic agent, was recently prepared. In the biological screening, compound **3a** had low activity *in vivo*.[3] However, compound **3a** potently antagonized the contraction of leukotriene (LT) C_4 (LTC_4) on isolated rat gastric fundus strips.

Recently, Whittle and co-workers[4] have reported that the potent vasoconstrictor action of gastric submucosal LTC_4 that was observed in ethanol and HCl induced gastric mucosal damage model was identified as potential endogenous proulcerogenic agent. We therefore investigated the protective effect of compound **3a** against HCl·ethanol-induced gastric lesions in rats and found it to possess the potent protective effect. These facts prompted us to synthesize and test novel analogues of **3a**. This paper describes the synthesis of substituted ethenyl-1,3,5-triazine derivatives and the determination of their structure–activity relationships for antagonistic activity against LTC_4-induced contraction and for protective effect against HCl·ethanol-induced gastric lesions.

Chemistry All compounds were prepared by two general methods according to Chart 2.

In method A, **1** was condensed with substituted benzaldehyde derivatives (**2b—j**) by the use of methanesulfonic acid or formic acid without a solvent. Pyridine derivatives (**3a, l**) were also prepared in the same manner as described above. In method B, **1** was condensed with **2k** in the presence of potassium hydroxide in 2-methoxyethanol. All compounds prepared were purified by recrystallization.

Biological Results and Discussion Compounds **3a** and **3l** with a hydroxy substituent in place of the amino group in the 2 position of **3a** were listed in Table I. They were tested for antagonistic activity against LTC_4- and LTD_4-induced contraction. Compound **3a** inhibited the LTC_4-induced contraction of gastric fundus strips at 1×10^{-6} M, thereby indicating antagonistic activity against LTC_4. This anti-LTC_4 activity of **3a** in the gastric preparation was about 100 times more potent than its anti-LTD_4 activity in the ileal preparation. However, the anti-LTC_4 and -LTD_4 activity of compound **3l** were rather feeble compared with that of **3a**.

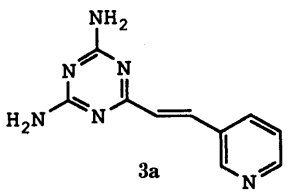
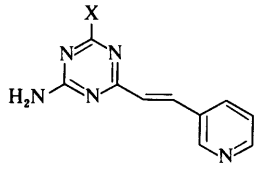


Chart 1

The listed compounds were tested for the protective effect at a dose of 5 mg/kg against HCl·ethanol-induced gastric lesions, using cimetidine as a standard. In the case of this test compound **3a** also showed substantial effect (>40% inhibition), but compound **3l** was not effective (<40% inhibition). These results suggested that the diamino substituents of **3a** were requisite for both the antagonistic activity and the protective effect.

Compounds **3b—k** with a phenyl group in place of the pyridyl group of **3a** were listed in Table II, and the effects of the substituent on the benzene ring were studied. These compounds showed remarkable variations in the activity as the substituent on the benzene ring was changed. The compounds with 2,4-dichloro (**3c**), 3,5-dichloro (**3d**), 2-trifluoromethyl (**3f**), 3-trifluoromethyl (**3g**), or 4-cyano

TABLE I. Biological Activity of 2-Amino-4-substituted-[*E*]-6-[2-(3-pyridyl)ethenyl]-1,3,5-triazine (**3a, l**)



Compd. No.	X	Antagonistic activity % inhibition against LTC_4-induced contraction at 1×10^{-5} M	1×10^{-6} M	LTD_4-induced contraction at 1×10^{-4} M	HCl·ethanol-induced gastric lesions % inhibition[a] at 5 mg/kg *p.o.*
3a	NH_2	60	43	36	75
3l	OH	21		5	31
Cimetidine		NT[b]		NT[b]	68[c]

a) Treatments in which inhibition values were more than 40% were evaluated as significantly effective. *b*) NT = not tested. *c*) Dose: 200 mg/kg.

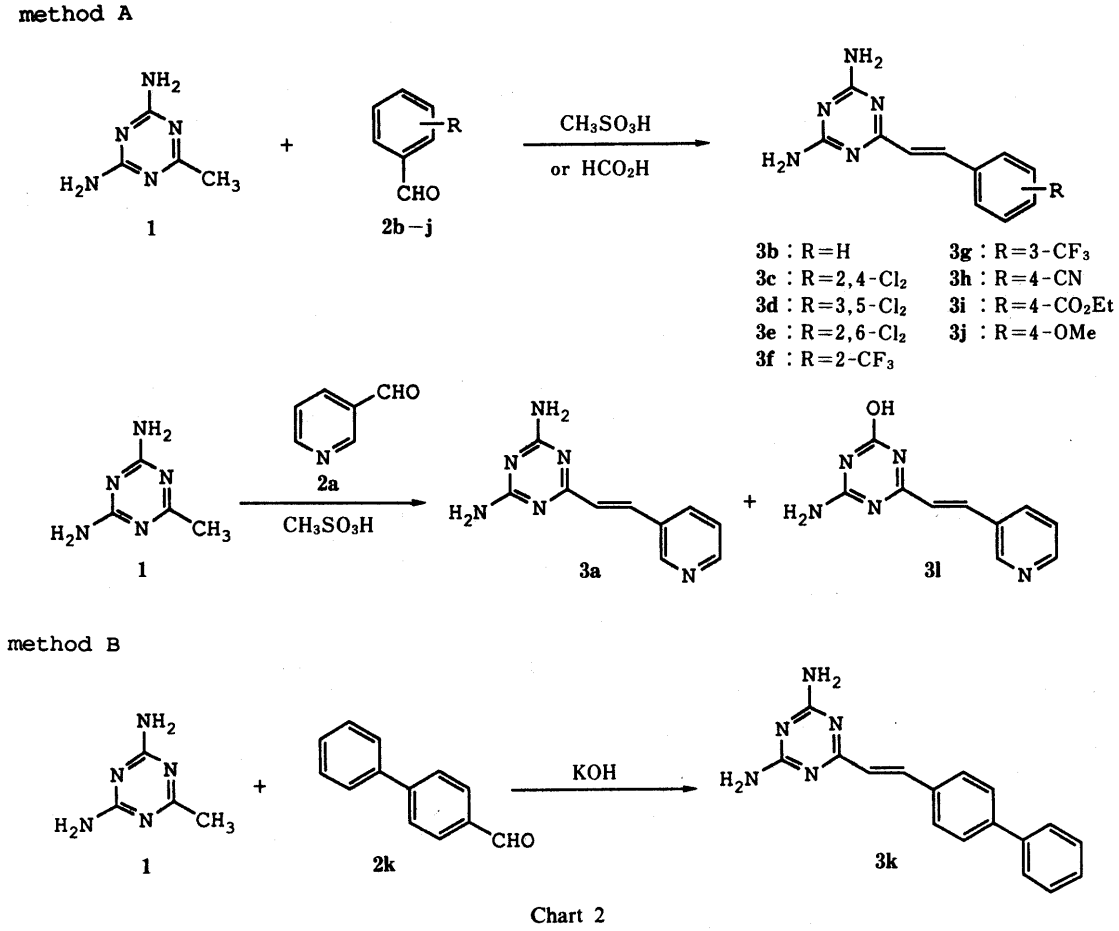
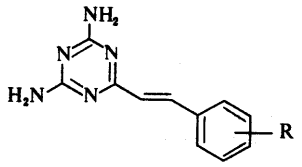


Chart 2

TABLE II. Biological Activity of 2,4-Diamino-[*E*]-6-[2-(substitutedphenyl)ethenyl]-1,3,5-triazine (**3b—k**)



Compd. No.	R	Antagonistic activity % inhibition against LTC_4-induced contraction at 1×10^{-5} M	HCl·ethanol-induced gastric lesions % inhibition[a] at 5 mg/kg *p.o.*
3b	H	53	44
3c	2,4-Cl_2	76	49
3d	3,5-Cl_2	41	67
3e	2,6-Cl_2	43	1
3f	2-CF_3	69	64
3g	3-CF_3	76	58
3h	4-CN	49	42
3i	4-CO_2Et	25	29
3j	4-OMe	16	8
3k	4-Ph	3	1

a) See footnotes *a*) in Table I.

(**3h**) group and the compound without substituents on the benzene ring (**3b**) showed both the antagonistic activity against LTC_4 and the protective effect on gastric lesions. Meanwhile, in these assays compounds with 4-ethoxycarbonyl (**3i**), 4-methoxy (**3j**) or 4-phenyl (**3k**) group were inactive. However, the compound with a 2,6-dichloro (**3e**) group showed only antagonistic activity against LTC_4.

These results suggested electron withdrawing substituents on the benzene ring except the 4-ethoxycarbonyl compound (**3i**) seemed to be preferable for both the anti-LTC_4 activity and the protective effect on gastric lesions. In the case of the 2,6-dichloro compound (**3e**), the results suggested that steric properties on the benzene ring might modify the protective effect on gastric lesions.

The results in Tables I and II suggested that the selectively potent antagonistic activity against a LTC_4-induced contraction of type 3 compounds, except compound **3e**, was closely correlated to the protective effect against HCl·ethanol-induced gastric lesions.

In conclusion, the present results demonstrate that **3a** and **3f** are suitable as lead compounds for the development of a new type of antiulcer drug.

Experimental

Melting points were determined on a Mettler FP62 apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined in dimethylsulfoxide (DMSO)-d_6 on a JEOL FX-200 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were measured with a JEOL JMS-DX 300 instrument. Infrared (IR) spectra were recorded on a Hitachi 270-30 spectrometer.

Typical Procedures of Method A 2,4-Diamino-[*E*]-6-styryl-1,3,5-triazine (**3b**): Benzaldehyde (**2b**, 4.4 ml) was added to a solution of **1** (5.25 g) in methanesulfonic acid (28 ml). The mixture was stirred for 2.5 h at 110 °C. It was cooled to room temperature and made basic with NaOH aqueous solution, with ice cooling. The precipitated solid was collected by filtration and recrystallized from 2-methoxyethanol to give **3b** (3.38 g, 38%), mp 273—274 °C. NMR δ: 6.65 (4H, br s), 6.74 (1H, d, J=15.9 Hz), 7.30—7.70 (5H, m), 7.80 (1H, d, J=15.9 Hz). IR ν (KBr):

3468, 3308, 3136, 1634, 1536, 1452 cm^{-1}. MS *m/z* (%): 213 (M^+, 54), 212 (M^+-1, 100), 170 (42), 144 (17), 128 (26). High resolution mass spectrum (HRMS) *m/z* Calcd for $C_{11}H_{11}N_5$ (M^+): 213.1013. Found: 213.1002. *Anal.* Calcd for $C_{11}H_{11}N_5$: C, 61.95; H, 5.20; N, 32.85. Found: C, 61.83; H, 5.43; N, 32.74.

Compounds **3a**, **3c**—**g** and **3j** were obtained in the same manner as described for **3b**.

2,4-Diamino-[*E*]-6-[2-(3-pyridyl)ethenyl]-1,3,5-triazine (**3a**): Yield, 51%. mp 287—289 °C (dec.) (2-methoxyethanol). NMR δ: 6.71 (4H, br s), 6.88 (1H, d, J=16.1 Hz), 7.43 (1H, dd, J=8.1, 4.6 Hz), 7.79 (1H, d, J=16.1 Hz), 8.12 (1H, br d, J=8.1 Hz), 8.55 (1H, d, J=4.6 Hz), 8.79 (1H, br s). IR ν (KBr): 3324, 3136, 1650, 1530 cm^{-1}. MS *m/z* (%): 215 (M^++1, 87), 214 (M^+, 100), 172 (50), 131 (32), 129 (23), 104 (17). HRMS *m/z* Calcd for $C_{10}H_{10}N_6$ (M^+): 214.0966. Found: 214.0966. *Anal.* Calcd for $C_{10}H_{10}N_6$: C, 56.06; H, 4.71; N, 39.23. Found: C, 56.39; H, 4.71; N, 38.90.

2,4-Diamino-[*E*]-6-[2-(2,4-dichlorophenyl)ethenyl]-1,3,5-triazine (**3c**): Yield, 66%. mp 264—265 °C (dec.) (2-methoxyethanol–MeOH). NMR δ: 6.75 (4H, br s), 6.82 (1H, d, J=15.9 Hz), 7.46 (1H, dd, J=8.6, 2.2 Hz), 7.70 (1H, d, J=2.2 Hz), 7.96 (1H, d, J=8.6 Hz), 8.08 (1H, d, J=15.9 Hz). IR ν (KBr): 3492, 3340, 3172, 1630, 1536, 976, 818 cm^{-1}. MS *m/z* (%): 285 (M^+), 283 (M^+, 19), 281 (M^+, 29), 248 (34), 246 (100), 206 (28), 204 (87), 162 (35). HRMS *m/z* Calcd for $C_{11}H_9Cl^*ClN_5$ (M^+): 283.0205. Found: 283.0197; Calcd for $C_{11}H_9{}^*Cl_2N_5$ (M^+): 285.0176. Found: 285.0176. *Anal.* Calcd for $C_{11}H_9Cl_2N_5$: C, 46.83; H, 3.22; N, 24.82. Found: C, 46.85; H, 3.37; N, 24.48.

2,4-Diamino-[*E*]-6-[2-(3,5-dichlorophenyl)ethenyl]-1,3,5-triazine (**3d**): Yield, 59%. mp 284—287 °C (dec.) (2-methoxyethanol–MeOH). NMR δ: 6.70 (4H, br s), 6.89 (1H, d, J=15.9 Hz), 7.60 (1H, d, J=15.9 Hz), 7.70—7.80 (3H, m). IR ν (KBr): 3432, 3344, 3148, 1644, 1532, 850 cm^{-1}. MS *m/z* (%): 285 (M^+), 283 (M^+, 66), 281 (M^+, 100), 240 (26), 238 (38), 212 (29), 162 (21). HRMS *m/z* Calcd for $C_{11}H_9Cl^*ClN_5$ (M^+): 283.0205. Found: 283.0210; Calcd for $C_{11}H_9{}^*Cl_2N_5$ (M^+): 285.0176. Found: 285.0179.

2,4-Diamino-[*E*]-6-[2-(2,6-dichlorophenyl)ethenyl]-1,3,5-triazine (**3e**): Yield, 48%. mp 251—252 °C (2-methoxyethanol–MeOH). NMR δ: 6.72 (1H, d, J=16.4 Hz), 6.76 (4H, br s), 7.37 (1H, dd, J=9.0, 7.1 Hz), 7.56 (1H, d, J=7.1 Hz), 7.56 (1H, d, J=9.0 Hz), 7.83 (1H, d, J=16.4 Hz). IR ν (KBr): 3492, 3472, 3316, 3112, 1666, 1648, 1548, 1430, 968, 808, 774 cm^{-1}. MS *m/z* (%): 285 (M^+), 283 (M^+, 33), 281 (M^+, 50), 248 (25), 246 (76), 206 (33), 204 (100), 162 (47). HRMS *m/z* Calcd for $C_{11}H_9Cl^*ClN_5$ (M^+): 283.0205. Found: 283.0196; Calcd for $C_{11}H_9{}^*Cl_2N_5$ (M^+): 285.0176. Found: 285.0168.

2,4-Diamino-[*E*]-6-[2-(2-trifluoromethylphenyl)ethenyl]-1,3,5-triazine (**3f**): Yield, 54%. mp 250—251 °C (EtOH). NMR δ: 6.72 (4H, br s), 6.78 (1H, d, J=15.9 Hz), 7.50—8.10 (4H, m), 8.11 (1H, br d, J=15.9 Hz). IR ν (KBr): 3496, 3344, 3168, 3072, 1672, 1640, 1532, 1312, 1164 cm^{-1}. MS *m/z* (%): 281 (M^+, 100), 212 (76), 170 (53), 111 (32). HRMS *m/z* Calcd for $C_{12}H_{10}F_3N_5$ (M^+): 281.0887. Found: 281.0886. *Anal.* Calcd for $C_{12}H_{10}F_3N_5$: C, 51.25; H, 3.58; N, 24.90. Found: C, 51.34; H, 3.81; N, 24.70.

2,4-Diamino-[*E*]-6-[2-(3-trifluoromethylphenyl)ethenyl]-1,3,5-triazine (**3g**): Yield, 39%. mp 171—173 °C (EtOH). NMR δ: 6.67 (4H, br s), 6.89 (1H, d, J=15.9 Hz), 7.60—7.80 (2H, m), 7.83 (1H, d, J=15.9 Hz), 7.90—8.00 (2H, m). IR ν (KBr): 3504, 3408, 3200, 1632, 1546, 1404, 1332, 1124 cm^{-1}. MS *m/z* (%): 282 (M^++1, 95), 281 (M^+, 100), 239 (50), 213 (34), 112 (40). HRMS *m/z* Calcd for $C_{12}H_{10}F_3N_5$ (M^+): 281.0888. Found: 281.0884.

2,4-Diamino-[*E*]-6-[2-(4-methoxyphenyl)ethenyl]-1,3,5-triazine (**3j**): Yield, 27%. mp 244 °C (dec.) (MeOH). NMR δ: 3.79 (3H, s), 6.59 (4H, br s), 6.59 (1H, d, J=15.9 Hz), 6.96 (2H, d, J=8.8 Hz), 7.56 (2H, d, J=8.8 Hz), 7.75 (1H, d, J=15.9 Hz). IR ν (KBr): 3324, 3168, 1636, 1546, 1510 cm^{-1}. MS *m/z* (%): 243 (M^+, 88), 242 (M^+-1, 100), 200 (32), 158 (19). HRMS *m/z* Calcd for $C_{12}H_{13}N_5O$ (M^+): 243.1019. Found: 243.1106.

2,4-Diamino-[*E*]-6-[2-(4-cyanophenyl)ethenyl]-1,3,5-triazine (**3h**): 4-Cyanobenzaldehyde (**2h**, 0.53 g) was added to a solution of **1** (0.5 g) in formic acid (10 ml). The mixture was refluxed for 57 h, and formic acid was evaporated off *in vacuo*. The residue was made basic with $NaHCO_3$ aqueous solution, with ice cooling. The precipitated solid was collected by filtration and recrystallized from 2-methoxyethanol to give **3h** (0.17 g, 18%), mp > 300 °C. NMR δ: 6.70 (4H, br s), 6.90 (1H, d, J=15.9 Hz), 7.80 (1H, d, J=15.9 Hz), 7.83 (4H, s). IR ν (KBr): 3504, 3440, 3328, 3176, 2220, 1640 cm^{-1}. MS *m/z* (%): 238 (M^+, 75), 237 (M^+-1, 100), 195 (41), 169 (25). HRMS *m/z* Calcd for $C_{12}H_{10}N_6$ (M^+): 238.0967. Found: 238.0974.

Compound **3i** was obtained in the same manner as described for **3h**.

2,4-Diamino-[*E*]-6-[2-(4-ethoxycarbonylphenyl)ethenyl]-1,3,5-triazine (**3i**): Yield, 15%. mp 230—231 °C (EtOH). NMR δ: 1.34 (3H, t, J=7.1 Hz), 4.33 (2H, q, J=7.1 Hz), 6.68 (4H, br s), 6.87 (1H, d, J=16.1 Hz), 7.76 (2H, d, J=8.3 Hz), 7.82 (1H, d, J=16.1 Hz), 7.97 (2H, d, J=8.3 Hz). IR ν (KBr): 3492, 3332, 3136, 1700, 1640, 1536, 1396, 1280 cm^{-1}. MS *m/z* (%): 285 (M^+, 74), 284 (M^+-1, 100), 256 (25), 242 (22). HRMS *m/z* Calcd for $C_{14}H_{15}N_5O_2$ (M^+): 285.1225. Found: 285.1245.

2-Amino-4-hydroxy-[*E*]-6-[2-(3-pyridyl)ethenyl]-1,3,5-triazine (**3l**): 3-Pyridinecarboxaldehyde (**2a**, 20.28 g) was added to a solution of **1** (19.75 g) in methanesulfonic acid (90 ml). The mixture was stirred for 2 h at 110 °C. The mixture was cooled to room temperature and made neutral with NaOH aqueous solution, with ice cooling. The precipitated solid was collected by filtration and recrystallized from DMSO–EtOH–MeOH to give **3l** (3.94 g, 12%), mp > 300 °C. NMR (D_2O–K_2CO_3) δ: 6.74 (1H, d, J=16.1 Hz), 7.43 (1H, dd, J=8.3, 4.9 Hz), 7.64 (1H, d, J=16.1 Hz), 8.05 (1H, br d, J=8.3 Hz), 8.44 (1H, dd, J=4.9, 1.5 Hz), 8.65 (1H, d, J=2.0 Hz). IR ν (KBr): 3368, 3128, 1690, 1588 cm^{-1}. MS *m/z* (%): 216 (M^++1, 85), 215 (M^+, 100), 172 (32), 147 (41), 137 (36), 132 (31), 130 (29), 104 (23). HRMS *m/z* Calcd for $C_{10}H_9N_5O$ (M^+): 215.0806. Found: 215.0796. *Anal.* Calcd for $C_{10}H_9N_5O$: C, 55.81; H, 4.22; N, 32.54. Found: C, 55.45; H, 4.16; N, 32.15.

Typical Procedure of Method B 2,4-Diamino-[*E*]-6-[2-(4-biphenylyl)-ethenyl]-1,3,5-triazine (**3k**): A mixture of **1** (1.25 g), KOH (0.66 g), 4-biphenylcarboxaldehyde (**2k**, 2.91 g) and 2-methoxyethanol (30 ml) was stirred for 16 h at 80 °C. The mixture was cooled to room temperature and the precipitated solid was collected by filtration and recrystallized from 2-methoxyethanol–MeOH to give **3k** (0.6 g, 21%), mp 276 °C (dec.). NMR δ: 6.66 (4H, br s), 6.79 (1H, d, J=15.9 Hz), 7.35—7.75 (5H, m), 7.72 (4H, s), 7.84 (1H, d, J=15.9 Hz). IR ν (KBr): 3324, 3112, 1632, 1538, 1530 cm^{-1}. MS *m/z* (%): 289 (M^+, 82), 288 (M^+-1, 100), 246 (34), 220 (13), 205 (16), 204 (31). HRMS *m/z* Calcd for $C_{17}H_{15}N_5$ (M^+): 289.1327. Found: 289.1306.

HCl·Ethanol-Induced Gastric Lesions[5] Male Wistar rats weighing 160—200 g (Charles-River Japan), 8 rats per group, were fasted but allowed free access to water for 24 h. One ml/200 g body weight of 60% ethanol (v/v) in 150 mM HCl (HCl·ethanol) was administered *p.o.* and the rats were sacrificed 1 h later. The sum of the length of each lesion (mm) was used as a lesion index. Percent inhibition was calculated as follows: [(lesion index of control-lesion index of test compound)/lesion index of control] × 100. 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 the Students' *t* test. Drugs were given *p.o.* 30 min before the HCl·ethanol administration suspended in 0.5% sodium carboxymethyl cellulose (CMC).

Bioassay of LTC_4 and LTD_4 LTs were performed on the isolated male Hartley guinea pigs (BW: *ca.* 300 g, Kiwa Lab. animals Co., Ltd.) ileum or male Sprague Dawley rats (BW: *ca.* 250 g, Charles-River Japan) fundus strips which were mounted at a load of 1.0 g in a 20 ml organ bath filled with the Tyrodes' solution and bubbled with 5% CO_2 in O_2. The contractile activities of 10^{-8} M LTC_4 and LTD_4 were assayed in the presence or absence of drugs. Drugs were given 10 min before the addition of LTs. To know the responsiveness of the tissue, histamine or acetylcoline (10^{-5} M) were added to the bath. Following the histamine or acetylcoline challenge, the tissues were washed and allowed 20 min to restabilize to base-line tension before LTs. Each data represents a mean of 4—5 animals.

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