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Ginger and Its Bioactive Component Inhibit Enterotoxigenic *Escherichia coli* Heat-Labile Enterotoxin-Induced Diarrhea in Mice

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Ginger is one of the most commonly used fresh herbs and spices. Enterotoxigenic *Escherichia coli* heat-labile enterotoxin (LT)-induced diarrhea is the leading cause of infant death in developing countries. In this study, we demonstrated that ginger significantly blocked the binding of LT to cell-surface receptor G_{M1}, resulting in the inhibition of fluid accumulation in the closed ileal loops of mice. Biological-activity-guided searching for active components showed that zingerone (vanillylacetone) was the likely active constituent responsible for the antidiarrheal efficacy of ginger. Further analysis of chemically synthesized zingerone derivatives revealed that compound 31 (2-[(4-methoxybenzyl)oxy]benzoic acid) significantly suppressed LT-induced diarrhea in mice via an excellent surface complementarity with the B subunits of LT. In conclusion, our findings provide evidence that ginger and its derivatives may be effective herbal supplements for the clinical treatment of enterotoxigenic *Escherichia coli* diarrhea.

KEYWORDS: Ginger; zingerone; enterotoxigenic *Escherichia coli*; heat-labile enterotoxin; diarrhea

INTRODUCTION

Ginger, the rhizome of *Zingiber officinale*, is a common condiment for various foods and beverages. Ginger has a long history of medicinal use in China and India for the treatment of headaches, nausea, rheumatism, and colds (*1*). Clinical studies indicate that ginger can be used as a "broad-spectrum antiemetic". It prevents nausea and/or emesis resulting from pregnancy, postoperation, motion sickness, and other causes (acetonemia or cytostatics) (*2*, *3*). Pharmacological studies indicate that ginger exerts anti-inflammatory, antipyretic, antimicrobial, antischistosomal, antitumorigenic, antioxidative, hypoglycemic hepatoprotective, diuretic, and hypocholesterolemic effects *in vitro* (*4*). Furthermore, ginger exhibits wide effects on the gastrointestinal tract by increasing bile secretion; preventing the occurrence of gastric ulcers; and enhancing pancreatic lipase, intestinal lipase, disaccharidases, sucrase, and maltase activities in animal models (*4*).

Diarrhea caused by intestinal pathogens is the leading cause of infant death in developing countries (*5*). Enterotoxigenic *Escherichia coli* (ETEC) is the leading bacterial cause of pediatric diarrhea, accounting for 210 million diarrhea episodes and approximately 380000 deaths annually. (*5*, *6*) Heat-labile enterotoxin (LT) is the major virulence factor of ETEC (*7*). LT consists of one A subunit for catalytic activity and five B subunits for receptor binding (*8*, *9*). When the B subunit (LTB) binds to the ganglioside G_{M1} on the intestinal cell surface, it induces a conformational change in the toxin molecule, followed by the translocation of the A subunit (LTA) into the cells. Inside the intestinal cells, LTA catalyzes the ADP-ribosylation of the stimulatory GTP-binding protein, resulting in the increased intracellular levels of cyclic AMP. The elevated levels of cyclic AMP in the cells cause massive losses of fluid and ions from the cells, leading to the symptom of diarrhea (*10*). Because the binding of LTB to G_{M1} is the first step of LT-induced diarrhea, it is an attractive target for developing drugs for the treatment and prophylaxis of LT-induced diarrhea (*9*).

Many medical plants and their constituents have been used for the treatment of diarrhea. For examples, *Croton lechleri*, *Galla chinensis*, rhubarb, and tea suppress enterotoxin-induced diarrhea through various mechanisms (*11*–*14*). In this study, we evaluated the antidiarrheal potential of ginger on the basis of its inhibitory effect on the LTB and G_{M1} interaction. Our data show that ginger was effective in the inhibition of LT-induced diarrhea via blocking the LTB and G_{M1} interaction. Zingerone was the likely active constituent of ginger responsible for antidiarrheal activity. Analysis of chemically synthesized derivatives of ginger constituents further suggested that com-

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pound 31 (2-[(4-methoxybenzyl)oxy]benzoic acid) might be the lead for further optimization.

MATERIALS AND METHODS

Extraction and Fractionation of Ginger. The dried ginger was a gift from Sun Ten Pharmaceutical Corporation (Taipei, Taiwan). The plant sample was ground with a homogenizer to a fine powder and extracted by mixing 100 g of the herb powder with methanol at 4 °C overnight. The supernatant was then collected and stored at –30 °C in small aliquots. The ginger extract was further evaporated under a vacuum to dryness, resuspended in deionized water, and partitioned with four different solvents (*n*-hexane, chloroform, ethyl acetate, and *n*-butanol) to yield *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous fractions, respectively. Each fraction was concentrated under reduced pressure at a temperature less than 40 °C, and the solid mass was then dissolved in methanol, divided into small aliquots, and kept at –30 °C until use.

Preparation of Zingerone Derivatives. Zingerone, 6-gingerol, and geraniol were purchased from Sigma (St. Louis, MO). 1-Phenyl-3,5-dodecenediones and 3-phenyl-acrylaldehydes were synthesized as described previously (*15, 16*). Briefly, 3,4-substituted cinnamic acids were used as starting materials to synthesize gingerdiones and dehydrogingerdione derivatives (1-phenyl-3,5-dodecenediones). Cinnamic aldehyde was reacted with a variety of substituted benzyl chlorides to synthesize 3-phenyl-acrylaldehydes. The benzyloxybenzenes were synthesized as follows. The starting substituted phenols were subjected to *O*-benzylation by reacting with substituted benzyl chloride in the presence of K_2CO_3 and KI to yield the corresponding substituted benzyloxybenzene derivatives (compounds **1–25**). Substituted benzyloxybenzoates were allowed to react with sodium hydroxide to afford corresponding substituted benzyloxybenzoic acids (compounds **26–34**).

Expression and Purification of *Escherichia coli* LT and LTB. Recombinant LT and LTB were expressed in the *E. coli* BL21(DE3)pLysS strain and purified by affinity chromatography as described previously (*12*). Briefly, cells induced by isopropyl-β-D-thiogalactopyranoside were collected 3 h after induction. The cell pellet was resuspended in TEAN buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 200 mM NaCl, and 3 mM Na_3N), lysed by sonication, and centrifuged at 15000*g* for 20 min at 4 °C. The supernatant was collected and mixed with D-galactose resin (Pierce, Rockford, IL), and the recombinant LT and LTB were then eluted by 1× TEAN buffer containing 1 M galactose. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and quantified with a Bradford assay (Bio-Rad, Hercules, CA).

Biotinylation of LTB. LTB was biotinylated as described previously (*19*). Briefly, LTB was mixed with Sulfo-NHS-LS-biotin (Pierce, Rockford, IL) in a ratio of 1:10. After a 2 h incubation on ice, the unincorporated biotin was removed by centricon-10 (Millipore, Bedford, MA), and the biotinylated LTB was stored at 4 °C until further analysis.

G_{M1}-Enzyme-Linked Immunosorbent Assay (ELISA). The interaction of LTB with ganglioside G_{M1} was evaluated by a G_{M1}-ELISA as described previously (*12*). Briefly, biotinylated LTB (16 ng) was mixed with various amounts of the compound and incubated at 4 °C with shaking. After a 3 h incubation, the mixture was added to wells, which were coated with 200 ng of G_{M1}, and incubated at 37 °C for 1 h. Following three washes, peroxidase-conjugated avidin and a chromatic substrate, 2,2′-azinobis(3-ethylbenzithiazoline-sulfonic acid), were sequentially added. The absorbance was read at 405 nm in an ELISA plate reader (Anthos Labtec Instruments, Austria). The percentage of inhibition was calculated by [1 –(OD value of mixture containing LTB and compound/OD value of mixture containing LTB only)] × 100.

Fluid Accumulation Assay. Female BALB/c mice (8 weeks old, 20 ± 1 g weight) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). Mouse experiments were conducted under ethics approval from the China Medical University Animal Ethics Committee (Ethics Approval Number 2005-3). Fluid accumulation induced by LT was evaluated with mouse ileal loops as described previously (*13*). Briefly, mice were fasted for 24 h with water available ad libitum. Mice were then anesthetized with ketamine and xylazine, and the intestines were exteriorized through a midline incision. One intestinal segment (about 4 cm) was ligated, and the LT (1 μg) and/or compounds in a total volume of 200 μL were simultaneously injected into the loop. Twenty-four hours later, the mice were sacrificed and the loops were excised. The fluid accumulation (g/cm) was calculated by dividing the weight of the fluid-containing loop by the length of the loop.

Patent Mouse Gut Assay. LT-induced diarrhea was evaluated by patent mouse gut assay as described previously (*12*). Briefly, six mice per group were fasted for 16 h with water available ad libitum. Each mouse was inoculated intragastrically with 0.5 mL of 10 μg LT and/or compounds. Six hours later, the mice were sacrificed. The entire intestine from duodenum to rectum was carefully removed to retain any accumulated fluid, and the residual mesentery was removed prior to weighing. The carcass was weighed separately. LT-induced diarrheal ability was presented as the gut/carcass weight ratio by dividing the weight of gut by the weight of the carcass.

Docking Analysis. Gasteiger–Huckel charges were deployed to compounds in the Maybridge database using the SYBYL 7.0.1 program. The position and conformation of each compound were optimized by the anchor fragment orientation and then torsion minimization methods implemented in the DOCK4.0.2 program or DOCK5.3.0 (*18*). There were 50 configurations and 100 maximum anchor orientations for each compound used in the anchor-first docking algorithm. All the docked configurations were energy-minimized by 100 maximum iterations and one minimization cycle. The X-Score program was used to correct the docking score (*19*). The LIGPLOT 4.22 program was used to identify specific contacts between ligands and LTB (*20*).

Statistical Analysis. Data were presented as mean ± standard errors. Student's *t* test was used for comparisons between two experiments. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effects of Ginger on the Binding of LTB to G_{M1}. The inhibitory abilities of ginger on the binding of LTB to G_{M1} were evaluated by G_{M1}-ELISA. As shown in **Figure 1A**, ginger exhibited a significant effect on the abolishment of the LTB–G_{M1} interaction. The inhibitory effect of the ginger extract was dose-dependent, with an IC_{50} value of 2.0 μg/mL. These data suggest that ginger might have antidiarrheal activities by blocking the binding of LTB to G_{M1}.

Effects of Ginger on LT-Induced Fluid Accumulation in Mice. The antidiarrheal efficacy of ginger extract was tested *in vivo* using the toxin-induced fluid accumulation assay in the short circuit of small intestines in mice. In this assay, the closed loops of small intestines were created *in vivo*, and the lumens of loops were injected with small volumes of saline, LT, or LT-containing ginger extracts. Luminal fluid accumulation was determined after 24 h. As seen in **Figure 1B**, there was marked fluid accumulation and distention in LT-treated loops, whereas the normal (saline) loop remained empty. The methanolic extract of ginger significantly suppressed fluid accumulation in the toxin-treated intestinal loops in a dose-dependent manner. Methanol alone did not affect the fluid accumulation induced by LT. These findings indicate that the ginger extract blocks the binding of LTB to G_{M1}, resulting in the suppression of LT-induced diarrhea.

Effects of Zingerone, a Ginger Constituent, on LT-Induced Fluid Accumulation and LTB-G_{M1} Interaction. Biological-activity-guided searching for active components was performed by screening various solvent fractions of ginger using G_{M1}-ELISA. As shown in **Figure 2**, aqueous, *n*-butanol soluble, and ethyl acetate soluble fractions of ginger did not inhibit the interaction between LTB and G_{M1}. However, the fractions from nonpolar solvents (*n*-hexane and chloroform) blocked the

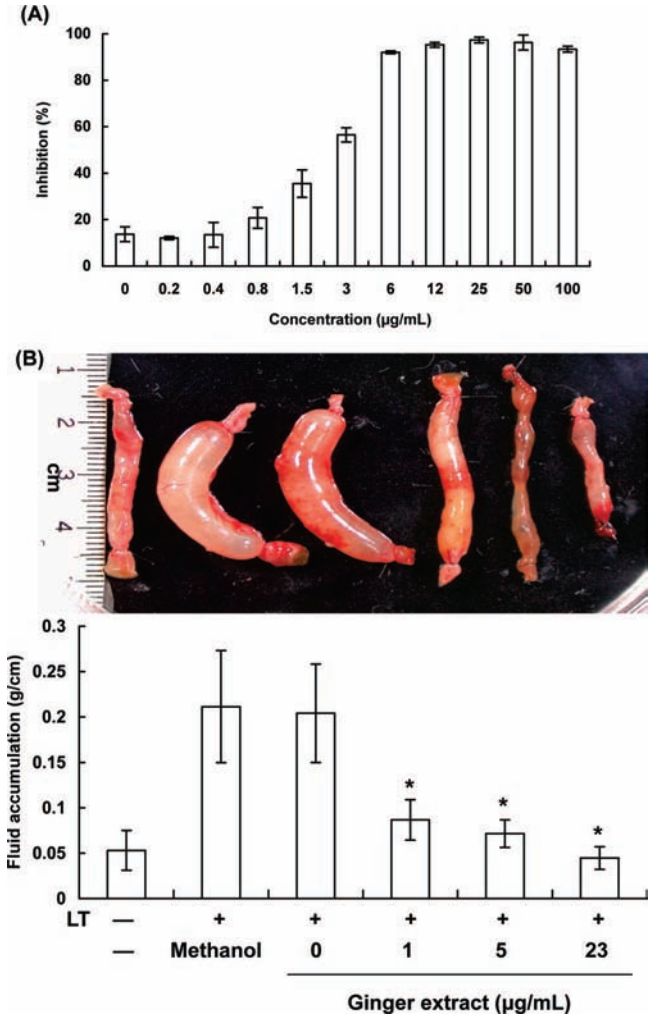

Figure 1. Antidiarrheal effect of ginger. (**A**) G_{M1}-ELISA. Various amounts of ginger extract were incubated with 16 ng of biotinylated LTB, and G_{M1}-ELISA was performed as described in the Materials and Methods. Results are expressed as inhibition (%). Values are mean ± standard errors of six independent assays. (**B**) Fluid accumulation assay. LT (1 μg) and/or various amounts of ginger extract were simultaneously injected into the ileal loops, and mice were sacrificed 24 h later. Cross appearance of the ileal loops is shown at the top. The dissected ileal loops from a normal mouse treated with saline and a solvent control mouse treated with LT and methanol are shown in the first and the second columns, respectively. Fluid accumulation (g/cm) is shown at the bottom. Values are mean ± standard errors of three independent assays. *$p < 0.05$, compared with LT treatment.

binding of LTB to G_{M1} in a dose-dependent manner. These data indicate that the active constituents of ginger might be lipophilic. Zingerone (vanillylacetone), 6-gingerol, and geraniol, which are present in the ginger oil (*21–23*), were further tested for their inhibitory effects. As shown in **Figure 3B**, zingerone and 6-gingerol significantly abolished the binding of LTB to G_{M1}, while geraniol did not affect the LTB–G_{M1} interaction. Antidiarrheal efficacies of zingerone and 6-gingerol were further evaluated by a fluid accumulation assay. Zingerone significantly inhibited LT-induced fluid accumulation in the closed loops (**Figure 3C**), while 6-gingerol did not affect it (data not shown). The IC_{50} of zingerone was 2.5 mM. These findings indicate that zingerone was the likely active constituent responsible for the antidiarrheal activity of ginger.

Effect of Compound 31, a Zingerone Derivative, on the Binding of LTB to G_{M1}. Comparing the structures of zingerone

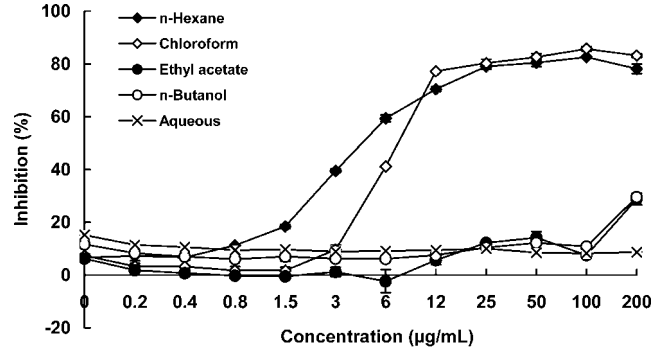

Figure 2. Inhibitory effects of ginger fractions on the interaction of LTB with G_{M1}. Various amounts of ginger fractions were incubated with 16 ng of biotinylated LTB, and G_{M1}-ELISA was performed as described in the Materials and Methods. Results are expressed as inhibition (%). Values are mean ± standard errors of two independent assays.

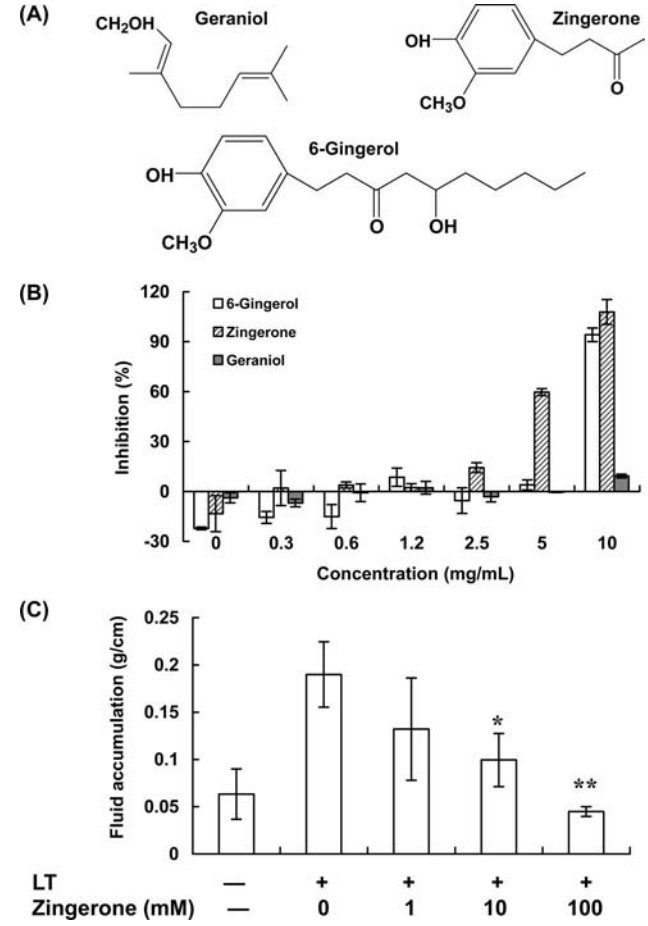

Figure 3. Inhibitory effects of zingerone, 6-gingerol, and geraniol on the LTB–G_{M1} interaction and LT-induced fluid accumulation. (**A**) Structures of zingerone, 6-gingerol, and geraniol. (**B**) G_{M1}-ELISA. Various amounts of zingerone, 6-gingerol, and geraniol were incubated with 16 ng of biotinylated LTB. Results are expressed as inhibition (%). Values are mean ± standard errors of two independent assays. (**C**) Fluid accumulation assay. LT (1 μg) and/or various amounts of zingerone were simultaneously injected into the ileal loops, and mice were sacrificed 24 h later. Results are expressed as fluid accumulation (g/cm). Values are mean ± standard errors of three independent assays. *$p < 0.05$, **$p < 0.01$, compared with LT treatment.

and 6-gingerol, we found that they shared a common skeleton with a benzene ring and a hydrocarbon chain. We wondered whether the number of carbon atoms or phenolic groups was

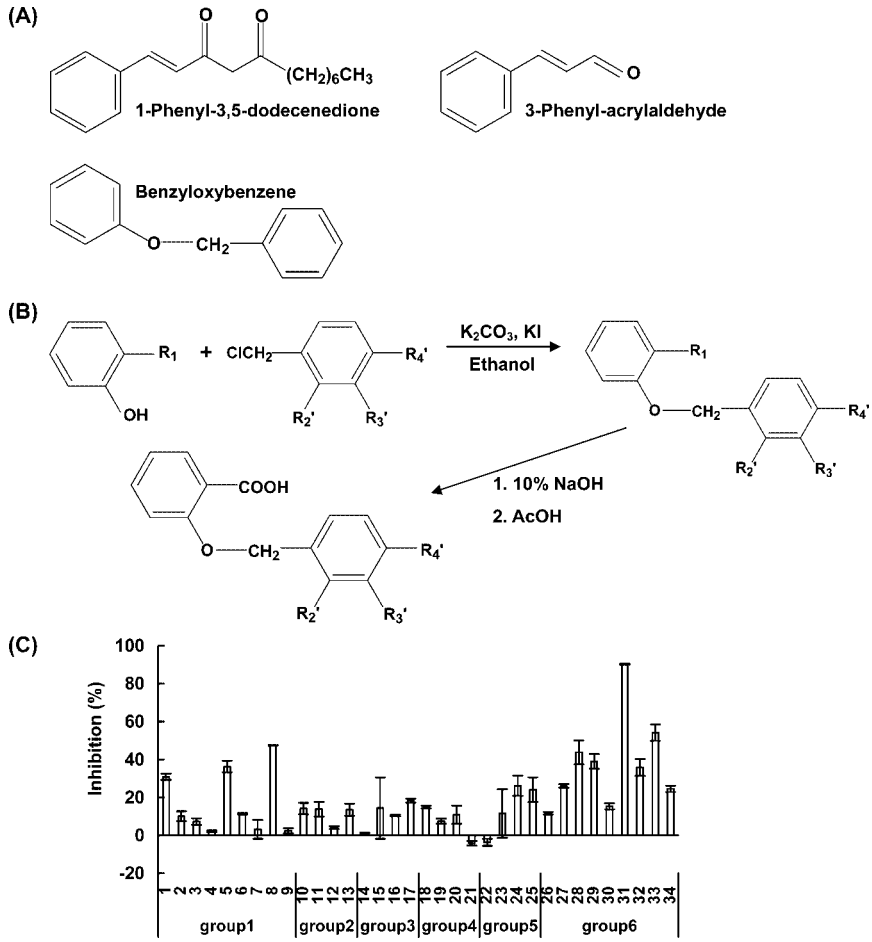

Figure 4. Inhibitory effects of zingerone derivatives on the LTB and G_{M1} interaction. (**A**) Structures of 1-phenyl-3,5-dodecenedione, 1-phenyl-acrylaldehyde, and benzyloxybenzene. (**B**) Solution-phase synthesis of benzyloxybenzoic acid analogs. (**C**) G_{M1}-ELISA. Biotinylated LTB (16 ng) was mixed with 10 mM benzyloxybenzoic acid analogs. Results are expressed as inhibition (%). Values are mean ± standard errors of two independent assays.

significant for the interaction with LTB; various derivatives were synthesized and their inhibitory effects on the LTB-G_{M1} interaction were evaluated by G_{M1}-ELISA (**Figure 4**). We first elongated and shortened the carbon chain to synthesize 1-phenyl-3,5-dodecenediones and 3-phenyl-acrylaldehydes, respectively (**Figure 4A**). Unfortunately, these compounds showed no effect on the LTB and G_{M1} interaction (data not shown). We further combined two phenolic groups to synthesize benzyloxybenzene derivatives (**Figure 4B** and **Table 1**). Data showed that benzyloxybenzene derivatives displayed various inhibitory efficacies on the binding of LTB to G_{M1} (**Figure 4C**). Compounds **8** and **33** exhibited approximately 50% inhibition, while compound 31 (2-[(4-methoxybenzyl)oxy]benzoic acid) displayed a 90% inhibition on the LTB–G_{M1} interaction. In comparison with *m*-nitrophenyl-α-D-galactopyranoside (MNPG), the receptor-binding antagonist derived from the simple sugar galactose (*24*), we found that compound **31** displayed a more effective inhibition (**Figure 5**). At 10 mM, compound **31** exhibited a 90% inhibition, while MNPG displayed an approximately 20% inhibition on the LTB and G_{M1} interaction.

Effect of Compound 31 on LT-Induced Diarrhea in Mice. Fluid accumulation and patent mouse gut assay were performed to evaluate the antidiarrheal effect of compound **31**. As shown in **Figure 6A**, LT induced massive fluid accumulation in the closed ileal loops. Solvent (dimethyl sulfoxide, DMSO) alone did not decrease the volume of LT-induced fluid in the intestinal loops. However, compound **31** suppressed LT-induced intestinal fluid secretion in a dose-dependent manner, with the IC_{50} of 0.1 mM. The patent mouse gut assay also showed that compound **31** significantly inhibited LT-induced fluid accumulation in the gut, with a concentration-dependent decrease in the gut–carcass ratio (**Figure 6B**). These findings indicate that compound **31**, a zingerone derivative, exhibited antidiarrheal effects by blocking the LTB–G_{M1} interaction. Moreover, it was more effective than zingerone in the prevention of LT-induced diarrhea.

Compound 31 Interacted with LTB. DOCK and X-Score programs were performed to analyze the interaction between compound **31** and LTB (see the Supporting Information for results). The docking data of MNPG were consistent with the X-ray diffraction data, in which there are hydrogen-bond interactions with the side chains of Gly-33, Gln-61, and Asn-90, and hydrophobic contacts with the side chains of His-57 and Trp-88 of LTB (**Figure 7A**) (*25*). Compound **31** docked into LTB with complementarity (**Figure 7A**). Critical hydrogen-bond interactions with the side chains of Arg-13 and Asn-90 and hydrophobic contacts with the side chains of Tyr-12, His-57, and Trp-88 of LTB were found in the LTB and compound 31 interactions. Comparing the docking results of MNPG and compound 31, we found that they shared a hydrogen-bond interaction with Asn-90 and two hydrophobic interactions with His-57 and Trp-88 of LTB (**Figure 7B**). Analysis of the binding affinity of LTB–compound interaction showed that the docking score of compound **31** was higher than that of MNPG. These findings suggest that compound **31** may be more effective than MNPG in binding to LTB.

Table 1. Functional Groups of 34 Benzyloxybenzene Derivatives

group	compound	R1	R2′	R3′	R4′
1	**1**	CN	H	H	H
	2	CN	Cl	H	H
	3	CN	H	Cl	H
	4	CN	H	H	Cl
	5	CN	F	H	H
	6	CN	H	F	H
	7	CN	H	H	F
	8	CN	H	OCH_3	H
	9	CN	H	H	OCH_3
2	**10**	CH_3	H	H	H
	11	CH_3	Cl	H	H
	12	CH_3	H	Cl	H
	13	CH_3	H	H	Cl
3	**14**	CH_2OH	H	H	H
	15	CH_2OH	Cl	H	H
	16	CH_2OH	H	Cl	H
	17	CH_2OH	H	H	Cl
4	**18**	$COOCH_3$	H	H	H
	19	$COOCH_3$	Cl	H	H
	20	$COOCH_3$	H	Cl	H
	21	$COOCH_3$	H	H	Cl
5	**22**	$COOC_2H_5$	H	H	H
	23	$COOC_2H_5$	Cl	H	H
	24	$COOC_2H_5$	H	Cl	H
	25	$COOC_2H_5$	H	H	Cl
6	**26**	COOH	H	H	H
	27	COOH	Cl	H	H
	28	COOH	H	Cl	H
	29	COOH	H	H	Cl
	30	COOH	H	OCH_3	H
	31	COOH	H	H	OCH_3
	32	COOH	F	H	H
	33	COOH	H	F	H
	34	COOH	H	H	F

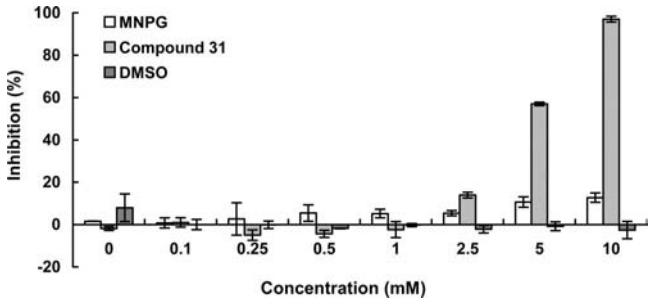

Figure 5. Inhibitory effects of MNPG and compound **31** on the binding of LTB to G_{M1}. Various amounts of MNPG, compound **31**, or a solvent (DMSO) were mixed with 16 ng of biotinylated LTB. Results are expressed as inhibition (%). Values are mean ± standard errors of two independent assays.

DISCUSSION

Today, the most common principle of management for diarrhea is fluid replacement combined with pharmacologic therapy (*26*). Fluid and electrolyte replacement is used to replace fluid losses by the administration of sugar–electrolyte solutions; however, it does not facilitate the reabsorption of secreted fluid and therefore does not lessen diarrhea. Antibiotics and antimotility agents are used to control symptoms. Antibiotics kill bacteria but cannot inhibit the toxicity of the bacterial toxin. Moreover, antibiotic therapy is not a viable solution because of the rapid increase in antibiotic resistance (*27*). Antimotility agents, such as loperamide and diphenoxylate, can lessen stool frequency and volume in mild diarrheas. However, such agents are not suitable in severe diarrhea because they may cause pooling of large fluid volumes in paralyzed bowel loops.

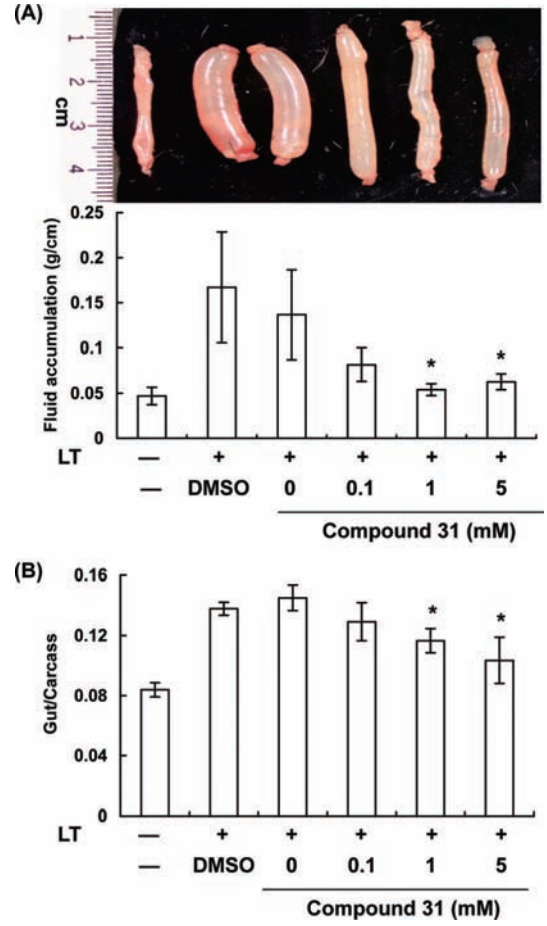

Figure 6. Antidiarrheal effect of compound **31** on the LT-induced diarrhea in mice. (**A**) Fluid accumulation assay. LT (1 μg) and/or various amounts of compound **31** were simultaneously injected into the ileal loops, and mice were sacrificed 24 h later. Cross appearance of the ileal loops is shown at the top. The dissected ileal loops from a normal mouse treated with saline and a solvent control mouse treated with LT and DMSO are shown in the first and the second columns, respectively. Fluid accumulation (g/cm) is shown at the bottom. (**B**) Patent mouse gut assay. Six mice per group were orally administered saline, 10 μg of LT, 10 μg of LT mixed with DMSO, or 10 μg of LT mixed with various amounts of compound 31. After a 6 h incubation, the mice were sacrificed. The gut/carcass weight ratio was calculated for each group. Values are mean ± standard errors of triplicate assays. *$p < 0.05$, compared with LT treatment.

Ginger is one of the most commonly used fresh herbs and spices. Ginger is among the 20 top-selling herbal supplements in the United States (*28*). Ginger also ranks second in the top-selling fresh herbs and spices in Australia, with an estimated annual consumption of over 550 tons (*24*). Today, pharmacopoeias from different countries list that ginger extract is used for various digestive diseases (*29, 30*). In this study, we demonstrated for the first time that, in addition to an antiemetic effect, ginger extracts exhibit antidiarrheal activities by blocking the binding of LTB to G_{M1}. Because LT and cholera toxin share an 83% amino acid sequence homology, consisting of an A subunit for enzymatic activity and five B subunits for receptor recognition, and the fact that LT causes choleralike diarrhea (*10*), our findings suggest that ginger extract could be used as an herbal supplement to prevent choleralike diarrhea in developing countries.

The LT holotoxin structure points to three potential target areas for inhibitor design: blocking the enzyme-active site located in the A1 domain, inhibiting holotoxin assembly by

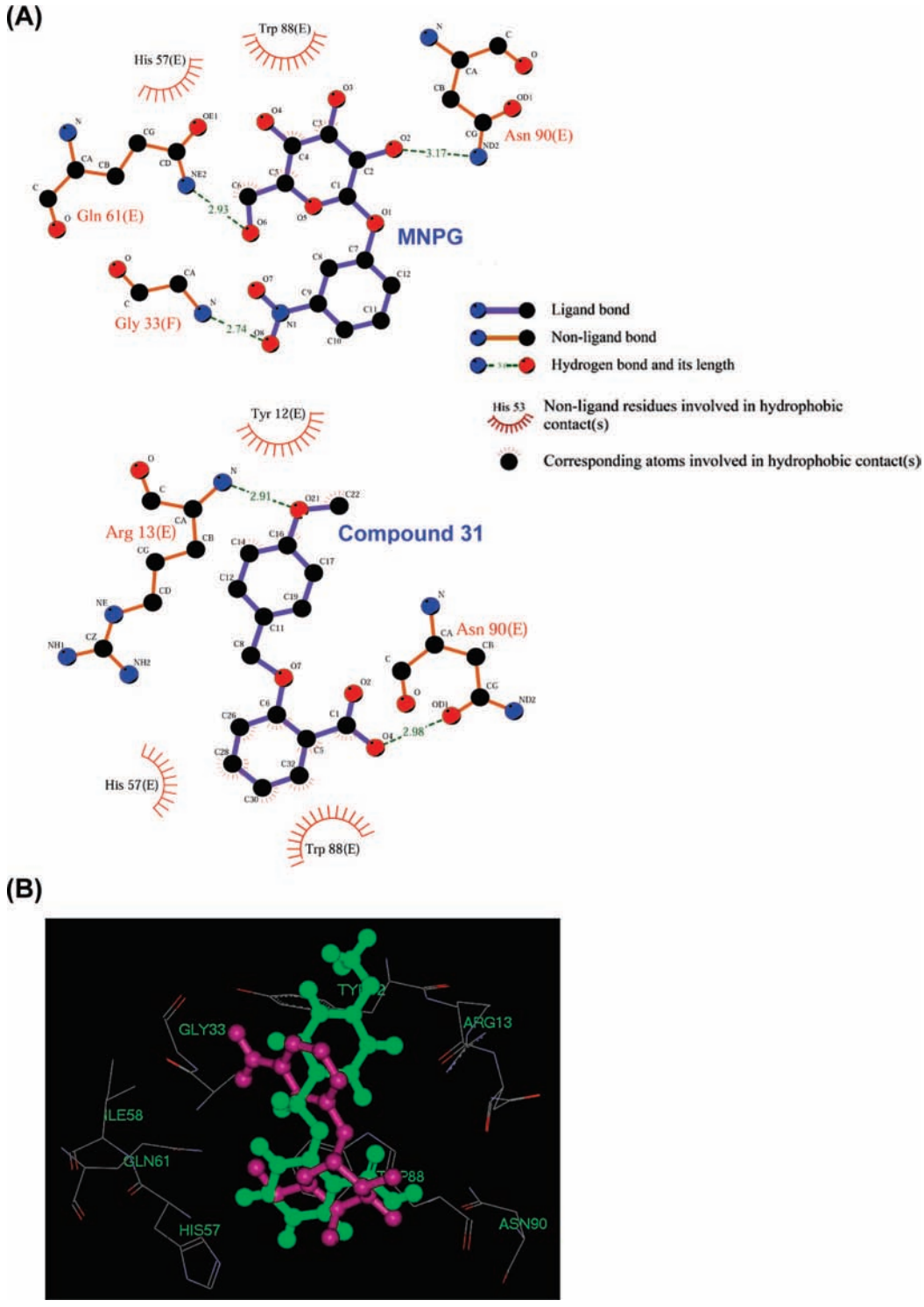

Figure 7. Interaction of compound **31** with the receptor-binding site of LTB. (**A**) Schematic illustrations of the interactions of MNPG (top panel) and compound **31** (bottom panel) with residues around the receptor-binding site of LTB. (**B**) Superimposed structure of MNPG (purple) and compound **31** (green) bound to LTB.

interrupting A2-B pentamer interactions, and blocking the toxin and cellular receptor interaction (*8*, *9*). We screened the antidiarrheal potential of herbs on the basis of the LTB–G_{M1} interaction. Their antidiarrheal abilities were further evaluated by a fluid accumulation assay. Results showed that there were correlations between the inhibition of the LTB–G_{M1} interaction and the suppression of LT-induced diarrhea in most compounds (data not shown). One exception was 6-gingerol, which displayed an inhibitory effect on the LTB–G_{M1} interaction, while it did not inhibit LT-induced diarrhea in mice. Nakazawa and Ohsawa (*31*) indicated that 6-gingerol is metabolized by gut flora to various metabolites, such as vanillin acid, ferulic acid, and 6-gingerol-4-O-β-glucuronide. The metabolic fate of 6-gingerol by normal flora in the gut might explain why it failed in the fluid accumulation assay.

Zingerone was the likely active constituent responsible for the antidiarrheal activity of ginger. Three series of zingerone derivatives were synthesized, and data showed that compound **31** (2-[(4-methoxybenzyl)oxy]benzoic acid) was more active than zingerone both *in vitro* and *in vivo*. Additionally, the *in*

vitro assay indicated that compound **31** was more effective than MNPG in the inhibition of the LTB–G_{M1} interaction. Like MNPG, compound **31** interacted with the surface of LTB via hydrogen bonds and hydrophobic contacts. The phenolic substituents of compound **31** and MNPG increased the area of hydrophobic contact with the toxin. In contrast, MNPG buried roughly half of the binding-site surface covered by the full receptor G_{M1} pentasaccharide (*32*), while compound **31** buried the binding-site surface and extruded into the surface. These findings suggested that compound 31 might form a stereobarrier to exclude the entry of G_{M1}. Because compound **31** significantly suppressed the LT-induced diarrhea in mice and no detectable tissue damage was observed (data not shown), compound **31** might be the lead candidate for further optimization.

In conclusion, pharmacologic therapy specifically targeting the bacterial toxin is an ideal management for the treatment or prevention of diarrhea. However, today, the most common principle of management for diarrhea is either the replacement of fluid losses (fluid replacement therapy) or the killing of bacteria (antibiotics therapy). In this study, we demonstrated that ginger, the commonly used herb and spice, was effective in inhibiting choleralike diarrhea in mice via the abolishment of toxin–receptor interaction. By biological-activity-guided searching for active components of ginger, zingerone was identified as the likely active constituent responsible for the observed antidiarrheal activity of ginger. Further analysis of the chemically synthesized zingerone derivatives revealed that compound **31** was more effective than zingerone in both the blockade of toxin-receptor interaction and the suppression of diarrhea in mice. The U.S. Food and Drug Administration classifies ginger as "Generally Recognized as Safe," and the German Commission E Monographs report that ginger has no known side effects and no known drug/herb interactions (*30*). Therefore, our observations suggest that ginger may be an effective candidate for the clinical treatment of ETEC diarrhea. Furthermore, our findings demonstrate for the first time that LT receptor-binding antagonists are effective in the suppression of LT-induced diarrhea *in vivo*.

ABBREVIATIONS USED

ETEC, enterotoxigenic *Escherichia coli*; LT, heat-labile enterotoxin; LTB, B subunit of LT; LTA, A subunit of LT; *E. coli*, *Escherichia coli*; ELISA, enzyme-linked immunosorbent assay; MNPG, *m*-nitrophenyl-α-D-galactopyranoside; DMSO, dimethyl sulfoxide.

Supporting Information Available: Solid-phase synthesis of 1-phenyl-3,5-dodecenediones and 3-phenyl-acrylaldehydes, the superimposition of cocrystal and docking structures of MNPG around the receptor-binding domain of LTB, X-Score results of MNPG and compound 31, and spectral characteristics of benzyloxybenzene compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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