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Notes

Synthesis and 5-Lipoxygenase Inhibitory Activities of Some Novel 2-Substituted 5-Benzofuran Hydroxamic Acids

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A series of 2-substituted benzofuran hydroxyamic acids were synthesized as rigid analogs of simple (benzyloxy)phenyl hydroxamates, evaluated for their *in vitro* and *in vivo* 5-lipoxygenase activity and found to be potent inhibitors of the enzyme. Substituents which enhanced lipophilicity near the 2-position of the benzofuran nucleus increased inhibitor potency but reduced oral activity. Incorporation of small polar substituents such as methoxymethylene, hydroxymethylene, and amino (urea) on the acyl group led to more consistent oral activity. The most potent inhibitors of this series *in vitro* were N-hydroxy-N-[1-(2-phenyl-5-benzofuranyl)-ethyl]furancarboxamide (**12**) and methyl 5-[N-hydroxy-N-[1-(2-(3,4,5-trimethoxyphenyl)-5-benzofuranyl]ethyl]-5-oxopentanoate (**17**), both with IC₅₀ values of 40 nM, and *in vivo* the most potent compound was N-hydroxy-N-[1-(2-phenyl-5-benzofuranyl)ethyl]urea, **20**, with an ED₅₀ = 10.3 mg/kg.

Introduction

Leukotrienes (LTs) are biological mediators derived from arachidonic acid through the action of the enzyme 5-lipoxygenase $(5-LO)^1$ and are implicated in several inflammatory and allergic reactions.^{2,3} The non-peptidic leukotriene LTB_4 is a potent chemotactic agent to a number of pro-inflammatory leukocytes in vitro and promotes aggregation, chemokinesis, and superoxide release by these cells.^{4,5} In vivo LTB₄ causes leukocyte accumulation in both animals and humans.⁶⁻⁸ The peptidoleukotrienes LTC₄, LTD₄, and LTE₄ are known to induce contraction of human airway smooth muscle preparation and mucus formation in human airways.^{9,10} In addition, it is becoming very evident that LTs are involved in several human disease states such as asthma, allergic rhinitis, rheumatoid arthritis, gout, and inflammatory bowel disease.^{1,11-13} Thus, the control of leukotriene biosynthesis through the inhibition of 5-lipoxygenase represents a potential method for treating such diseases. Known inhibitors of the enzyme include a variety of molecules containing the hydroxamic acid functionality, such as compounds derived from the (phenylalkoxy)benzylamines 1-3.14,15

In the search for novel 5-lipoxygenase inhibitors, we synthesized a series of 2-substituted 5-benzofuran hydroxamic acids as rigid analogs of compounds 1-3, with the phenylalkoxy portion incorporated into a substituted furan ring and fused onto the phenyl ring. Several derivatives were synthesized to help establish the structure-activity relationships (SAR) of this series of compounds. The synthesis and biological activities of these compounds are reported herein.

Chemistry

The synthetic route to these compounds is shown in Scheme 1. The synthesis of **26a** has been reported by





 a (a) R₁ = phenyl; (b) R₁ = 3,4,5-trimethoxyphenyl; (c) R₁ = 6-methoxy-2-naphthyl; (d) R₁ = n-decyl; (e) R₁ = n-butyl.

Bisangni and Royer,¹⁶ involving cyclodehydration of 2-hydroxy-5-acetylbenzyl phenyl ketone (**30**). This route to the benzofurans nucleus was found unattractive due to the inaccessibility of **30** and its analogs, which were obtained in low yields from degradation of 1-(3-benzoyl-2-ethyl-5-benzofuranyl)ethanone (**29**) (Scheme 2).¹⁶ Compounds **26a**-**e** were therefore synthesized by reacting the appropriately substituted acetylenes **24a**-**e** with 3-iodo-4-hydroxyacetophenone (**25**) in the presence of either bis(triphenylphosphine)palladium(II) acetate and cuprous iodide in anhydrous dimethylformamide¹⁷ or cuprous oxide in pyridine.¹⁸ The ketones were then converted to the oximes **27a**-**e** with hydroxylamine

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Scheme 2¹⁶



Table 1. In Vitro 5-Lipoxygenase Inhibitory Activities of Compounds $1-3^{15}$

$(CH_2)_n \xrightarrow{R}_{O} \xrightarrow{CH_3}_{OH} CH_3$						
no.	n	R	<i>in vitro</i> 5-LO inhibn: IC ₅₀ (μM) (95% CL)			
1 2 3	0 0 1	H CH3 H	$\begin{array}{c} 0.37 \ (0.24 {-} 0.51)^{15} \\ 0.50 \ (0.50 {-} 0.64)^{15} \\ 0.41 \ (0.36 {-} 0.48)^{15} \end{array}$			

hydrochloride and sodium hydroxide (compounds 27c and 27e were reduced to the final products without full characterization), followed by reduction with sodium cyanoborohydride under acidic conditions¹⁹ to give hydroxylamines 28a-e. The hydroxamic acids 4-19 and 22 were prepared by acylating the hydroxylamines with the appropriate acid chlorides in the presence of triethylamine. The ester 14 obtained from 28a, and acetoxyacetyl chloride was selectively hydrolyzed with lithium hydroxide in 2-propanol and water²⁰ to give the hydroxyl derivative 23. The urea analogs 20 and 21 were prepared by reaction of 28a and 28b respectively with phosgene followed by ammonium hydroxide.²⁰

Biological Results and Discussions

Simple compounds such as 1 have been reported to possess reasonable in vitro and in vivo 5-lipoxygenase inhibitory activity with a rather short duration of action due to metabolic oxidation at the benzylic position (Table 1).^{14,15} Structural modifications to limit such in vivo metabolism either reduced or eliminated the in vitro and/or in vivo inhibitory activities associated with the resulting compounds.¹⁵ It was envisioned that metabolic inactivation of these compounds could be blocked by incorporation of the phenylalkoxy portion into a substituted furan ring, assuming the resulting compounds will be active. Table 2 contains the in vitro and in vivo activities of the test compounds, compared to the clinical candidate, zileuton.²¹ As shown in the table, compound 4 exhibited in vitro and in vivo activities, but was 10 times less potent in vivo than zileuton. Other derivatives were therefore synthesized to improve both the in vitro and/or in vivo potencies. Several factors regarding the enzyme and its inhibitors were considered in the design of derivatives of 4. In an earlier review Cashman has summarized the key structural elements of the 5-lipoxygenase active site as a nonheme ferric iron, a hydrophobic domain, and a carboxylate binding area.²² Other studies since then have supported some of the suggested elements in the active site. In a study involving QSAR of a large series of the so-called type A hydroxamic acids, it was concluded that a hydrophobic binding region within the active site of the enzyme was a major contributor to inhibitor potency.²³ Furthermore other workers have reported that structural changes which increase hydrophobicity are

accompanied by an increase in potency.²⁴ In addition, compounds containing groups capable of chelation of the ferric ion have been shown to be successful inhibitors of the enzyme.^{1,25} Due to the pivotal role of the hydroxamic acid group in this type of inhibitors, this portion was conserved in our series and modifications concentrated at the positions bearing R_1 and R_2 to change the properties of the molecules to modify their interaction with the two remaining portions of the active site.

(a) Substituents at Position 2 (\mathbf{R}_1). Four derivatives, $\mathbf{5-8}$, bearing substituents with different lipophilic and electronic properties at position 2 of the benzofuran ring were prepared. Replacement of the phenyl group with hydrophobic groups with lower electron density such as *n*-butyl **5** led to a 10-fold increase *in vitro* potency. Compound **6** with the larger *n*-decyl group was less potent than **5**, suggesting that though hydrophobic-ity may be important in this portion of the inhibitors, there is a limiting size contribution. Replacement of the phenyl group with 3,4,5-trimethoxyphenyl (**7**) and 6-methoxynaphthyl (**8**) did not affect the potency to any great extent, although compound **8** was slightly more potent than **4**.

(b) Acyl Substituents (R₂). Modifications made included replacing the methyl moiety with groups with different lipophilic and electronic properties such as phenyl, 9, substituted phenyl, 10 and 11, and heterocycles, 12 and 13. Replacement of the methyl with a more lipophilic group such as phenyl reduced the potency almost 10 times while the 3,4-dimethoxyphenyl analog, 10, improved the potency. Though the lipophilicity of 11 is the same as 10, the presence of the 2,6dimethoxy substitution may affect the conformation of the hydroxamic acid functionality, which in turn reduced the potency. The best replacement for the methyl group within this series is the furan analog, 12, which is more polar than either benzene, 9, or thiophene, 13. In general this portion of the active site appears to tolerate polar substituents better, which is in agreement to some of the reported hydroxamic acid inhibitors.²³ We previously reported substantial increases in in vitro potency by the introduction of various esters on the acyl group of other hydroxamate 5-lipoxygenase inhibitors.²⁰ A similar approach within the two phenyl series resulted in very potent compounds, with the best compounds, 15 and 16, possessing two and three methylene units between the carbonyls of the ester and the hydroxamate groups. The ester functionality could be involved in a hydrophilic type interaction with the carboxylate binding area. Maintaining the methyl butyrate functionality on the acyl unit and replacing the 2-position phenyl group with 3,4,5-trimethoxyphenyl (17), *n*-butyl (18), and *n*-decyl (19) did not affect the potency of the resulting compounds. However, compound **19** was the least active among the three, probably due to the excessive length of the molecule. This supports the importance of the distance between the hydrophobic and ionic binding sites.

Though several of the initial modifications of the lead compound gave very potent compounds, *in vitro*, most of the analogs had low oral activity, probably due to their higher overall CLogP values. In an attempt to reduce the lipophilicity and improve the oral activity, derivatives 20-23 containing small polar groups on the

Table 2. In Vitro and in Vivo 5-Lipoxygenase Inhibitory Activities of Compounds 4-23 Compared to Zileuton



no.	R ₁	R_2	in vitro 5-LO (5-HETE) inhibn. ^α IC ₅₀ (μM) (95% CL)	<i>in vivo</i> 5-LO (LTC ₄) inhibn ^b % inhibn at 30 mg/kg po ± SE [ED ₅₀ (mg/kg po) (95% CL)
4	phenyl	CH3	0.49 (0.37-0.65)	- [22.1 (15.2-42.0)]
5	C_4H_9	CH_3	0.05 (0.02-0.09)	41.4 ± 10.6
6	$C_{10}H_{21}$	CH_3	0.41 (0.15-0.77)	19.5 ± 12.0
7	3,4,5-trimethoxyphenyl	CH_3	0.31 (0.18-0.35)	42.1 ± 8.60
8	6-methoxy-2-naphthyl	CH_3	0.12 (0.08-0.16)	25.6 ± 7.40
9	phenyl	phenyl	4.83 (3.07-7.40)	46.2 ± 4.90
10	phenyl	3,4-dimethoxyphenyl	0.20(0.08 - 0.42)	NS
11	phenyl	2,6-dimethoxyphenyl	3.00 (2.06-5.55)	NS
12	phenyl	furanyl	0.04 (0.01-0.06)	32.9 ± 10.2
13	phenyl	thiophenyl	0.25 (0.19-0.35)	29.1 ± 12.4
14	phenyl	CH_2OAc	0.16 (0.08-0.27)	18.9 ± 13.0
15	phenyl	$CH_2CH_2CO_2Et$	0.05 (0.01-0.09)	NS
16	phenyl	$(CH_2)_3CO_2Me$	0.06 (0.02-0.11)	NS
17	3,4,5-trimethoxyphenyl	$(CH_2)_3CO_2Me$	0.04 (0.02-0.06)	42.1 ± 8.60
18	C_4H_9	$(CH_2)_3CO_2Me$	0.05 (0.04-0.07)	28.6 ± 12.4
19	$C_{10}H_{21}$	$(CH_2)_3CO_2Me$	0.15 (0.09-0.24)	37.3 ± 7.60
20	phenyl	$\rm NH_2$	0.41 (0.30-0.61)	- [10.3 (5.30-15.0)]
21	3,4,5-trimethoxyphenyl	$\rm NH_2$	0.05(0.02 - 0.11)	-[17.5(9.10-40.2)]
22	phenyl	CH_2OMe	0.16 (0.03-0.33)	-[18.61(9.80-27.6)]
23	phenyl	CH_2OH	0.70 (0.61-0.79)	- [27.5 (19.2 $-$ 59.8)]
	Zileuton		0.37(0.24 - 0.54)	- [2.71 (1.39-6.93)]

^a IC₅₀ with 95% confidence limits²⁷ in parentheses for the in vitro inhibition of 5-lipoxygenase (5-HETE) from 9000g supernatant of RBL broken cell assay (see Methods). ^b ED₅₀ with 95% confidence limits²⁷ in parentheses or mean percent inhibition values + SEM for inhibition of 5-lipoxygenase (LTC₄) in the mouse zymosan peritonitis assay (see methods). ^c NS = no significant activity at 30 mg/kg.

acyl unit were prepared. One substituent which has been an effective replacement for the acyl unit in inhibiting the enzyme is urea.²⁶ This was found to be effective within this series also. Conversion of compounds 4 and 7 to the urea analogs 20 and 21 improved the *in vivo* potencies for both compounds and also improved the *in vitro* potency for 4 while maintaining that of 7. Compound 20 was found to be the most orally potent inhibitor within the series. An important finding within this series is that the methoxymethylene and the hydroxylmethylene groups (22 and 23) also serve as efficient bioisosteric replacements for the acyl methyl group of the acetyl hydroxamic acids, while maintaining both the *in vitro* and *in vivo* potencies.

In summary, a novel series of 2-substituted benzofuran hydroxamic acids were shown to be potent inhibitors of the enzyme, 5-lipoxygenase. The more rigid benzofurans were equipotent to the simple (benzyloxy)phenyl derivatives, 1-3. In addition, we have further demonstrated the limiting but important hydrophobic binding region of the enzyme. In the region adjacent to the hydroxamate binding site, a hydrophilic area was found which can be used to alter the physicochemical parameters and thus the pharmacodynamics of the inhibitor molecules.

Experimental Section

Melting points were determined on a Meltemp II apparatus and are uncorrected. Elemental analyses (within 0.4% of the theoretical values unless otherwise indicated) and the mass spectral data (chemical ionization technique) were performed by the analytical group at the R. W. Johnson Pharmaceutical Research Institute. All ¹H NMR spectra were recorded on a GE-300 spectrometer, and values are reported in ppm from Me₄Si.

General Procedure for the Preparation of 1-(2-Substituted 5-benzofuranyl)ethanones 26a-e. The following procedures for the preparation of 1-(2-phenyl-5-benzofuranyl)ethanone (26a) are representative. Method A. To a stirred suspension of anhydrous sodium acetate (6.65 g, 81.1 mmol) in DMF (75 mL) was added phenylacetylene (6.63 g, 64.9 mmol), 4-hydroxy-3-iodoacetophenone (8.50 g, 32.4 mmol), bis(triphenylphosphine)palladium(II) acetate (487 mg, 0.65 mmol), and copper(I) iodide (246 mg, 1.30 mmol). The mixture was then heated at 65-70 °C for 4 h under nitrogen and cooled to 25 °C, and H₂O (600 mL) was added. The resulting tan solid was filtered, air-dried, packed on silica gel column, and eluted with EtOAc/hexane (1:5) to give 6.68 g (87%) of a tan solid, mp 156-159 (lit.¹⁶ mp 160 °C).

Method B. To a suspension of cuprous oxide (179 mg, 1.25 mmol) in pyridine (8 mL) was added phenylacetylene (213 mg, 2.09 mmol) and 4-hydroxy-3-iodoacetophenone (524 mg, 2.00 mmol), and the mixture was refluxed for 5 h under nitrogen. The mixture was then filtered, H_2O (50 mL) was added, and the resulting solid was filtered, air-dried, packed on silica gel column, and purified as before to give 0.37 g (78%) of the product.

General Procedure for the Preparation of 1-(2-Substituted 5-benzofuranyl)ethanone Oximes 27a-e. The following procedure for the preparation of 1-(2-phenyl-5-benzofuranyl)ethanone oxime (27a) is representative. To a mixture of 26a (3.93 g, 16.6 mmol) and hydroxylamine hydrochloride (4.05 g, 58.3 mmol) in EtOH (50 mL) was added powdered NaOH (5.90 g, 0.15 mol) in small portions. After addition, the mixture was stirred at 25 °C for 30 min, refluxed for 10 min, cooled to 25 °C, and poured into a mixture of 12 N HCl (25 mL) and H₂O (100 mL). The resulting precipitate was filtered and recrystallized from aqueous EtOH to give 3.00 g (72%) of a tan solid: mp 207-209 °C; MS (CI, CH₄) MH⁺ at 254; ¹H NMR (DMSO-d₆) δ 2.23 (s, 3H, CH₃), 7.47 (m, 4H, 3H, and 3', 4', and 5' phenyl Hs), 7.61 (m, 2H, 2' and 6' phenyl Hs), 7.92 (m, 3H, 4, 6, and 7 Hs) 5.85, 11.15 (s, 1H, OH). Anal. (C₁₆H₁₃N₁O₂·0.5H₂O) C, H, N.

General Procedure for the Preparation of N-Hydroxy-1-(2-substituted 5-benzofuranyl)ethanamines. The following procedure for the preparation of N-hydroxy-1-(2phenyl-5-benzofuranyl)ethanamine (28a) is representative. To a solution of 27a (1.76 g, 6.76 mmol), NaBH₃CN (0.88 g, 14.0 mmol) in MeOH (50 mL), and THF (50 mL) containing methyl orange (1 mg) was added dropwise 12 N HCl until the color remained pink. The mixture was stirred continuously for 4 h with the occasional addition of 12 N HCl to maintain the pink color of the reaction. The reaction mixture was then evaporated to dryness under reduced pressure, the solid was suspended in H_2O (50 mL), and enough sodium hydroxide was added to adjust the pH to 9 and extracted with CH_2Cl_2 (3 \times 250 mL). The combined organic extracts were dried with magnesium sulfate and concentrated to dryness to give an offwhite solid. The solid material was packed on a silica gel column and eluted with EtOAc/hexane (3:1) to give 1.70 g (99%) of a white product: mp 164–166 °C; MS (CI, CH_4) MH^+ at 253; ¹H NMR (DMSO-d₆) δ 1.3 (d, 3H, CH₃), 4.16 (q, 1H, CH), 7.18 (s, 1H, 3H), 5.85 (br s, 1H, OH), 7.28-7.5 (m, 6H, phenyl Hs and 7H), 7.9 (d, 2H, 4 and 7 Hs) (s, 1H, NH). Anal. $(C_{16}H_{15}N_1O_2)$ C, H, N.

General Procedure for the Preparation of N-hydroxy-N-[1-(2-substituted 5-benzofuranyl)ethyl]acetamides 4-19 and 22. The following procedure for the preparation of N-hydroxy-N-[1-(2-phenyl-5-benzofuranyl)ethyl]acetamide (4) is representative. To a solution of 28a (0.50 g, 1.97 mmol) and triethylamine (1 mL) in dry THF (50 mL) was added acetyl chloride (0.16 g, 1.97 mmol). The reaction mixture was stirred at 25 °C for 20 min, concentrated to dryness, and recrystallized from H₂O, THF, and a few drops of AcOH to give 0.39 g (66%) of an off-white solid: mp 181-183 °C; MS (CI, CH₄) MH⁺ at 296; ¹H NMR (DMSO-d₆) δ 1.51 (d, 3H, CH₃), 2.02 (s, 3H, CH₃C(O)), 5.88 (q, 1H, CH), 7.2- $7.61\ (m,\ 7H,\ phenyl\ Hs,\ 3\ and\ 4\ Hs),\ 7.95\ (d,\ 2H,\ 6\ and\ 7\ Hs),$ 9.58 (br s, 1H, OH). Anal. (C₁₈H₁₇N₁O₃) C, H, N.

General Procedure for the Preparation of N-Hydroxy-N-[1-(2-substituted 5-benzofuranyl)ethyl]ureas 20 and 21. The following procedure for the preparation of N-hydroxy-N-[1-(2-phenyl-5-benzofuranyl)ethyl]urea (20) is representative. Dry HCl gas was bubbled through a solution of 28a (0.20 g, 0.79 mmol) in THF (35 mL) for 5 min. The solution was added dropwise to a stirred 20% phosgene in toluene solution (4.09 mL, 7.90 mmol), stirred at 25 °C for 4 h, and poured into cold aqueous 38% NH4OH solution (50 mL). The resulting solution was extracted with EtOAc $(3 \times 125 \text{ mL})$. The combined organic extracts were washed with H_2O (100 mL), dried with magnesium sulfate, and concentrated to dryness. The solid obtained was packed on a silica gel column and eluted with EtOAc/hexane (1:4) to give 0.14 g (62%) of a white product: mp 164-166 °C; MS (\overline{CI} , CH_4) \overline{MH}^+ at 297; ¹H NMR (DMSO-*d*₆) δ 1.47 (d, 3H, CH₃), 5.4 (q, 1H, CH), 6.28 (s, 2H, NH_2), 7.3–7.6 (m, 7H, phenyl Hs, 3 and 4 Hs), 7.92 (d, 2H, 6 and 7 Hs), 9.07 (s, 1H, OH). Anal. (C₁₇H₁₆N₂O₃), C, H, N.

N,2-Dihydroxy-N-[1-(2-phenyl-5-benzofuranyl)ethyl]acetamide (23). To a solution of 14 (0.43 g, 1.21 mmol) in warm 2-propanol (50 mL) and THF (10 mL) was added H₂O (2 mL), and the mixture was cooled to 25 °C. Solid LiOH (0.46 g, 19.0 mmol) was added and the mixture stirred at 25 °C for 2 h. Enough 2 N HCl was added to bring the pH to about 2, followed by H_2O (50 mL). The resulting precipitate was filtered, and the aqueous layer was extracted with EtOAc (2 \times 50 mL), washed with H₂O (2 \times 75 mL), and dried with magnesium sulfate. The solvent was evaporated, and the resulting solid was added to the filtered solid, packed on a silica gel column, and eluted with EtOAc/hexane (2:3) to give 0.18 g (47%) of an off-white product: mp 187-190 °C; MS (CI, CH₄) MH⁺ at 312; ¹H NMR (DMSO- d_6) δ 1.5 (d, 3H, CH₃), 4.13 $(m,\,2H,\,CH_2),\,4.5\,(q,\,1H,\,CH),\,5.70\,(q,\,1H,\,CH),\,7.28-7.62\,(m,\,2H$ 7H, phenyl Hs, 3 and 4 Hs), 7.90 (d, 2H, 6 and 7 Hs), 9.50 (s, 1H, OH). Anal. (C₁₈H₁₇N₁O₄•0.1H₂O) C, H, N.

RBL-1 5-Lipoxygenase Inhibition. RBL-1 cells from the American Type Culture Collection (ATCC) were grown in suspension cultures and harvested by centrifugation at 2000g for 5 min. Washed cells at a concentration of 5×10^7 cells/ mL were suspended in $NaHPO_4/CaCl_2$ buffer, homogenized at 0 °C, and then centrifuged at 9000g for 50 min. The 5-lipoxygenase activity in the 9000g supernatant was determined radiometrically by measuring the conversion of arachidonic acid to 5-HETE. Increasing logarithmic doses of test compound were utilized in order to determine a dose-response curve for each drug. Doses were chosen such that the IC_{50} concentration of the drug fell within the linear portion of the sigmoidal dose response curve. A mixture of 5.5 μ L of test compound and 500 μ L of enzyme supernatant was preincubated for 5 min at 37 °C. Then, 10 μ L of 50 μ Ci/mL [¹⁴C]arachidonic acid was added to each sample followed by a 20 min incubation period at 37 °C. The reaction was stopped by the addition of 1.0 mL of 2 N formic acid per sample. The primary 5-LO product, 5-HETE, was isolated by chloroform extraction, followed by TLC on silica gel, and detection of radioactive emissions of product via a Bioscan imaging system plate scanner. The inhibition of 5-LO product formation is expressed as a percentage of the arachidonic acid converted to 5-HETE by the control group vs the drug treatment group. IC_{50} values with 95% confidence limits (CL) were determined by the method of Finney.²⁷

Mouse Zymosan Peritonitis Model. Male mice (CD-1), 18-25 g, were dosed orally with test compound suspended in polyethylene glycol 200. One hour later, the animals were injected (ip) with 3 mg of zymosan-A suspended in 0.5 mL of 0.9% sterile saline. Fifteen minutes after receiving zymosan, the mice were sacrificed by CO_2 inhalation. The abdomens were injected with 2 mL of a 10 μ M indomethacin solution. Subsequent to massaging the abdominal area, the skin was removed and the abdominal wall was opened. A 0.2 mL aliquot of peritoneal fluid was withdrawn and added to 1 mL of cold 95% ethanol. The solutions were incubated in an ice bath (minimum of 30 min) and then centrifuged at 28000g for 15 min at 4 °C. Supernatant fractions were decanted and evaporated under a stream of nitrogen at room temperature. The samples were capped and stored at -70 °C until assayed. Radioimmunoassays (RIAs) for LTC_4 were performed on a 1:20 dilution of original samples using [3H]RIA kits from Advanced Magnetics, Inc., according to kit instructions. ED_{50} values (that dose calculated to cause a 50% reduction in the immunoreactive LTC₄ with 95% confidence limits of (CL)) were calculated from the percentage of inhibition determined for each animal at the doses tested and then fitted to a straight line by a log-linear regression analysis according to the method of Finney.27

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