q, J = 7.0 Hz), 6.95 (2 H, d, J = 9.0 Hz), 7.38 (2 H, d, J = 9.0 Hz); MS, m/e (relative intensity) 279 (M^+, 7.2), 237 (10.7), 179 (5.0), 178 (5.6), 177 (11.5), 153 (39.0), 136 (16.8), 135 (73.5), 109 (10.0), 108 (13.8), 107 (26.3), 51 (100). Spectral properties are consistent with those previously reported for related compounds.[5c,7,9]

***N*-Acetyl-*p*-benzoquinone Imine (4).** This material was synthesized by the procedure of Dahlin and Nelson.[11]

Kinetic Measurements. All kinetics were performed in 5 vol % CH_3CN–H_2O at 0.50 M ionic strength (KCl). Procedures for purification of solvents, preparation of solutions, and general methods for following the progress of reactions by UV absorption spectroscopy have been described.[5a,b]

Kinetic measurements were performed at (25.0 ± 0.1) °C over the pH range 1.0–8.0 in HCl solutions and in acetate or phosphate buffers. All pH readings were taken at (25.0 ± 0.1) °C with an Orion Model 801 digital pH meter equipped with a Radiometer Model GK 2402C combination electrode. Measurements of pH on standardized HCl solutions in the pH range 1.0–3.0 established the following relationship between pH and the meter reading:

$$\text{pH} = \text{meter reading} - 0.05 \quad (6)$$

Equation 6 was used to correct all pH measurements.

Procedures used to monitor the hydrolysis kinetics of 4 have been published.[9] Identical procedures were used for **3c**. Methods for handling the kinetic data have also been described.[5a–c,9]

Product Analyses. Product studies were performed in solutions identical to those used in the kinetic studies except for higher concentrations of **3c** (ca. 1.0×10^{-4} M). The purity of **3c** was determined by NMR peak integration (ca. 90–95% pure). A correction based on this calculated purity was applied to all product yield data. Product yields were determined by HPLC methods that have been published.[9] All HPLC peak areas were calibrated with a known concentration of authentic samples, which were obtained commercially or prepared in previous studies.[5a]

Detection of 4 during the Hydrolysis of 3c by HPLC Methods. The hydrolysis of **3c** was initiated by the injection of 25 μL of a ca. 2.0×10^{-2} M solution of **3c** in CH_3CN into 5 mL of an appropriate buffer solution that had been incubating for sufficient time at (25.0 ± 0.1) °C. Aliquots of 10 μL were periodically removed and injected directly onto an HPLC column (μ-Bondapak C-18 column, 1:1 MeOH/H_2O eluent, 1 mL/min, UV absorbance monitored at 250 nm). HPLC peaks for **4** (rt = 4.3 min) and its labile hydrolysis product **8** (rt = 3.1 min) could be observed at appropriate times and pH values. These materials decomposed in a manner identical to that previously reported for authentic **4** and **8**.[9] No other intermediates were observed during the hydrolysis of **3c**.

Acknowledgment. This work was supported by a grant from the American Cancer Society (BC-348). The HPLC used in this study was purchased with funds provided by the Cotrell Research Grants Program of the Research Corp.

Registry No. 1, 62-44-2; **3a**, 69783-20-6; **3b**, 69783-19-3; **3c**, 102725-57-5; 4, 50700-49-7.

Synthesis of [[(Naphthalenylmethoxy)- and [[(Quinolinylmethoxy)phenyl]amino]oxoalkanoic Acid Esters. A Novel Series of Leukotriene D_4 Antagonists and 5-Lipoxygenase Inhibitors

John H. Musser,* Dennis M. Kubrak, Joseph Chang, and Alan J. Lewis

Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, Pennsylvania 19101. *Received October 18, 1985*

A series of novel [[(naphthalenylmethoxy)- and [[(quinolinylmethoxy)phenyl]amino]oxoalkanoic acid esters have been prepared. These compounds were tested as inhibitors of rat polymorphonuclear leukocyte (PMN) 5-lipoxygenase (LO) in vitro and as inhibitors of ovalbumin (OA) and leukotriene D_4 (LTD_4) induced bronchospasm in the guinea pig (GP) in vivo. Many naphthalenyl compounds were potent inhibitors of 5-LO, and several quinolinyl compounds were potent inhibitors of LTD_4-mediated bronochospasms in the GP. The most potent naphthalenyl compound, 4-[[3-(2-naphthalenylmethoxy)phenyl]hydroxyamino]-4-oxobutanoic acid, methyl ester (**6v**), had an IC_{50} of 0.6 μM in the 5-LO assay. The most potent compound in vivo, 4-[[3-(2-quinolinylmethoxy)phenyl]hydroxyamino]-4-oxobutanoic acid, methyl ester (**6e**), had ED_{50}'s of 3.3 mg/kg and 27.4 mg/kg (intraduodenally) against LTD_4- and OA-induced bronchospasm, respectively. When tested as an antagonist of LTD_4-induced contraction of isolated GP tracheal spiral strips, **6e** was shown to be a competitive inhibitor with a pK_B value of 5.33.

Leukotrienes (LT), products of the 5-lipoxygenase (LO) biosynthetic pathway, may play an important role in the pathophysiology of such disease states as asthma, psoriasis, ulcerative colitis, and rheumatoid arthritis.[1,2] The sulfidopeptide LTs, LTC_4, LTD_4, and LTE_4, previously referred to as slow-reacting substance of anaphylaxis (SRS-A), are known to contract lung smooth muscle and evoke bronchoconstriction in the guinea pig (GP) and man.[3] In humans, LTC_4 and LTD_4 also stimulate the release of mucus from airways in vitro,[4] are potent vasodilators in skin,[5] and produce a wheal and flare response when injected intradermally.[6] The nonpeptidic LT, LTB_4, is an extremely potent chemotactic, and aggregating agent for a variety of leukocytes in vitro; in vivo, it stimulates cell accumulation and increases vascular permeability.[7] The multiple actions and potency of LTs have prompted the development of agents that inhibit the formation or action of these substances.

FPL-55,712 (**1**) (Figure 1) was the first compound to be described as an antagonist of sulfidopeptide LTs.[8] As a pharmacological tool, it has been extensively used to define

(1) Samuelsson, B. *Science (Washington, D.C.)* **1983**, *220*, 568.
(2) Ford-Hutchinson, A. W. *Fed. Proc.* **1985**, *44*, 25.
(3) Piper, P. J. *Int. Arch. Allergy Appl. Immunol. Suppl. 1* **1985**, *76*, 43.
(4) Marom, Z.; Shelhamer, J. H.; Bach, M. K.; Morton, D. R.; Kaliner, M. *Am. Rev. Respir. Dis.* **1982**, *126*, 449.
(5) Bisgaard, H.; Kristensen, J.; Sondergaard, J., *Prostaglandins* **1982**, *23*, 797.
(6) Camp, R. D. R.; Coutts, A. A.; Greaves, M. W.; Kay, A. B.; Walport, M. J. *Br. J. Pharmacol.* **1982**, *80*, 497.
(7) Bray, M. A. *Br. Med. Bull.* **1983**, *39*, 249.
(8) Augstein, J.; Farmer, J. B.; Lee, T. B.; Sheard, P.; Tattersall, M. L., *Nature (London) New Biol.* **1973**, *245*, 215.

0022-2623/86/1829-1429$01.50/0

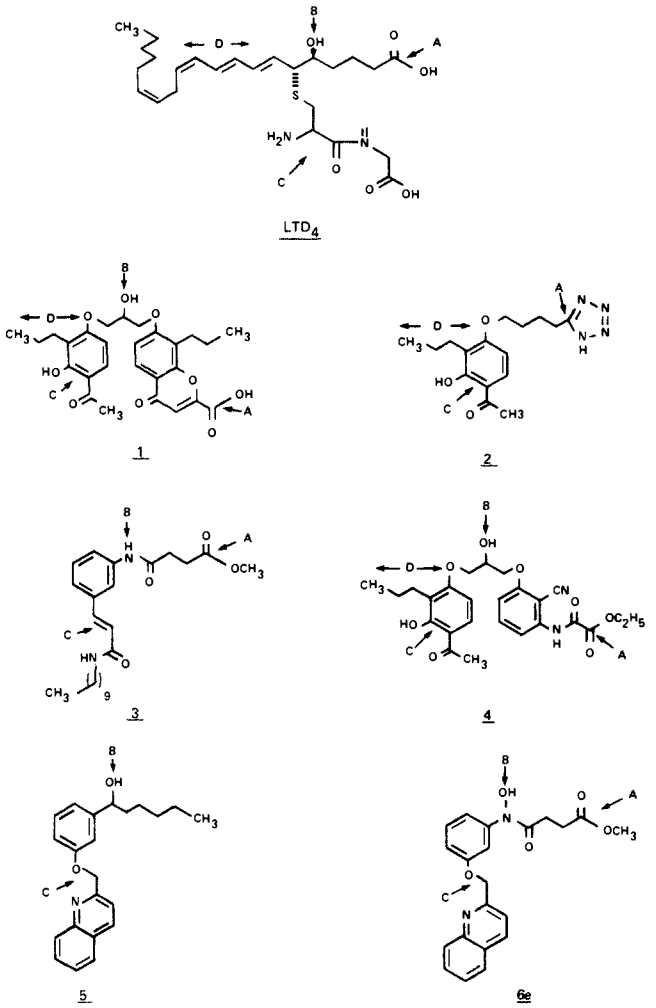
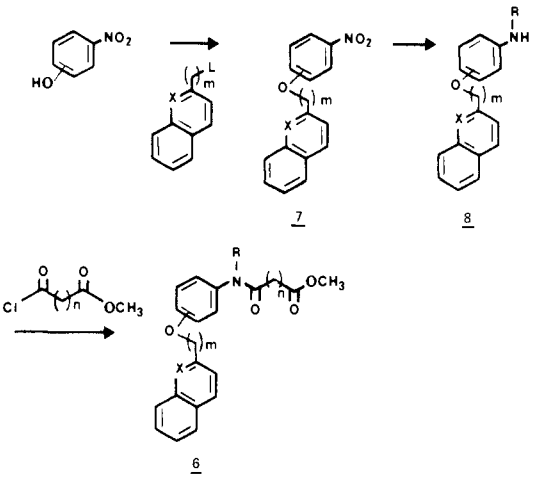


Figure 1. Structural relationships among LTD_4 and LTD_4 antagonists.

the role sulfidopeptide LTs play in immediate hypersensitivity reactions in man and animals.[9] Numerous research groups have obtained sulfidopeptide LT antagonists by substantial modification of the right-hand portion of FPL-55,712 while retaining the left-hand hydroxyacetophenone moiety constant.[10] An example that has received recent attention is LY-171,883 (2). It is orally active against both LTD_4- and antigen-induced increase in total pulmonary resistance in the GP and is a competitive antagonist of LTD_4 on GP ileum and parenchyma.[11]

Structurally unrelated to the above compounds, which contain a hydoxyacetophenone moiety, is a series of compounds represented by structure **3** which are claimed as antagonists of sulfidopeptide LTs.[12,13] Although no details of the biologic data were presented, we thought that the (phenylamino)-4-oxobutanoic acid group of **3** could approximate the 5-hydroxy-6-thio-6'-vinylhexanoic acid portion of LTD_4 (see Structural Relationship section for details). We have successfully employed the (phenylamino)-4-oxobutanoic acid group in the synthesis of sulfidopeptide LT antagonist of novel structure.[14]

Scheme I



We were also interested in examining our compounds as 5-LO inhibitors. Agents that antagonize sulfidopeptide LTs and inhibit 5-LO have precedent in that known sulfidopeptide LT antagonists FPL-55,712 (1) and Wy-44,329 (4) are also 5-LO inhibitors.[15,16]

Two years ago a series of aryleicosanoids, which were designed and synthesized by one of us,[17,18] were reported to be specific inhibitors of 5-LO.[19] Surprisingly aryleicosanoid **5** is also a modest sulfidopeptide LT antagonist.[20] We now think that the sulfidopeptide LT antagonist activity of **5** could be due to the 2-oxymethylquinoline portion of the molecule.

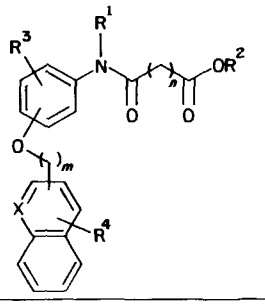
Although neither the (phenylamino)-4-oxobutanoic acid group nor the 2-oxymethylquinoline group was structurally novel,[21] we thought the juxtaposition of the two partial structures striking. Herein, we report the synthesis and LTD_4 antagonist activity of a novel series of [[(naphthalenylmethoxy)- and (quinolinylmethoxy)phenyl]amino]alkanoic acid esters.

Chemistry

The generalized synthetic pathway for the preparation of compounds **6** listed in Table I is shown in Scheme I. Reaction of 2-, 3-, or 4-nitrophenol with 2-(chloromethyl)naphthalene or 2-(chloromethyl)quinoline in acetone with cesium carbonate as a base[22] gave the bis-aryl system **7**. Reduction of **7** with sodium dithionite or powdered iron in ethanolic HCl gave the amino compounds **8** (R = H) and partial reduction of **7** with hydrazine and palladium on carbon gave hydroxyamino compounds, **8** (R

(9) Chand, N. *Agents Actions* **1979**, *9*, 133.
(10) Musser, J. H.; Kreft, A. F.; Lewis, A. J. *Agents Actions*, in press.
(11) Fleisch, J. H.; Rinkema, L. E.; Haisch, K. D.; Swanson-Bean, D.; Goodson, T.; Ho, P. P. K.; Marshall, W. S. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 148.
(12) Kadin, S. B. U.S. Patent 4 296 120 and 4 296 129, 1981.
(13) Kadin, S. B. U.S. Patent 4 331 683, 4 342 781, and 4 343 813, 1982.
(14) Musser, J. H.; Kees, K. L. U.S. Patent 4 528 392, 1985.
(15) Casey, F. B.; Appleby, B. S.; Buck, D. C. *Prostaglandins* **1983**, *25*, 1.
(16) Lewis, A. J.; Chang, J.; Hand, J.; Kreft, A., *Int. J. Immunopharmacol* **1985**, *7*, 384.
(17) Musser, J. H.; Chakraborty, U. *E.P. Publication No. 110405 (11.30.83.) Derwent Ref. No. 84-147669.*
(18) Musser, J. H.; Chakraborty, U.; Scortino, S.; Gordon, R. J.; Neiss, E. S.; Pruss, T. A.; VanIwegen, R.; Weinryb, I.; Wolf, P. S.; Coutts, S. M. *J. Med. Chem.*, in press.
(19) Coutts, S. M.; Khandwala, A.; Van Inwegan, R.; Bruens, J.; Jariwala, N.; Dally-Meade, V.; Ingram, R.; Chakraborty, J.; Musser, J.; Jones, H.; Pruss, T.; Neiss, E.; Weinryb, I. *IVth International Washington Spring Symposium on Prostaglandins Leukotrienes*, 1984, Abstr. No. 70.
(20) Gordon, R. J.; Trvis, J.; Pruss, T. P.; Neiss, E.; Musser, J.; Chakraborty, U.; Jones, H.; Liebowitz, M., *IVth International Washington Spring Meeting on Prostaglandins and Leukotrienes*, 1984, abstr. No. 266.
(21) Dewhirst, F. E. U.S. Patent 4 244 956, 1981.
(22) Zaugg, H. E. *J. Org. Chem.* **1976**, *41*, 3419.

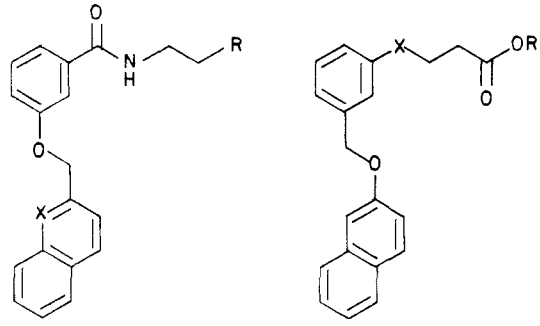
Table I. Inhibition of Rat PMN 5-LO and GP Bronochospasm



compd no.	pos Ar	X	*m*	pos Bz	R^1	*n*	R^2	R^3	R^4	mp, °C	formula[d]	synthetic method	in vitro PMN: IC_{50},[a] μM % yield	5-LO	CO	in vivo (50 mg/kg, id) LTD_{14}[b] % inh	*N*[c]	OA[b] % inh	*N*[c]
6a	2	N	1	3	H	2	CH_3	H	H	121–122	$C_{21}H_{20}N_2O_4$	**6b**	69	28	NA[e]	86*	3	43	6
6b	2	C	1	3	H	2	CH_3	H	H	132–133	$C_{22}H_{21}NO_4$	**6b**	44	3.2	NA	54[g]	2	10[g]	3
6c	2	N	1	3	H	2	H	H	H	180–182	$C_{20}H_{18}N_2O_4$	**6c**	52	NA		66*	4	−20	3
6d	2	N	1	2	H	2	CH_3	H	H	127–128	$C_{21}H_{20}N_2O_4$	**6b**	64	NA		−6	2	9[h]	2
6e	2	N	1	3	OH	2	CH_3	H	H	187–189	$C_{21}H_{20}N_2O\cdot HCl$	**6b**	53	1.4	40	97*	8	76*	13
6f	2	N	1	4	H	2	CH_3	H	H	140–142	$C_{21}H_{20}N_2O_4$	**6b**	95	2.6	NA	−7	2		
6g	2	N	1	3	H	0	CH_3	H	H	158–161	$C_{19}H_{16}N_2O_4\cdot {}^1/_4H_2O$	**6b**	91	5.3	NA	−2	2		
6h	2	N	1	3	H	3	CH_3	H	H	127–129	$C_{22}H_{22}N_2O_4\cdot {}^1/_4H_2O$	**6b**	30	10.4		−6	2		
6i	2	N	1	3	H	1	CH_2CH_3	H	H	66–71	$C_{21}H_{20}N_2O_4\cdot HCl$	**6b**	45	19.0	NA	84*	5	67	5
6j	2	C	1	3	H	0	CH_3	H	H	140–143	$C_{20}H_{17}NO_4\cdot {}^1/_4H_2O$	**6b**	90	6.9	NA	−1	2		
6k	2	C	1	3	H	3	CH_3	H	H	120–125	$C_{23}H_{23}NO_4\cdot {}^1/_{10}H_2O$	**6b**	88	12.6		5	2		
6l	2	N	1	3	H	2	CH_3	2-CN	H	167–170	$C_{22}H_{19}N_3O_4$	**6b**	06	NA	NA	−6	2		
6m	2	C	1	2	H	2	CH_3	H	H	91–92	$C_{22}H_{21}NO_4\cdot {}^1/_4H_2O$	**6b**	87	86.2	NA	−14	2		
6n	2	C	1	4	H	2	CH_3	H	H	191–192	$C_{22}H_{21}NO_4$	**6b**	66	NA		12	2		
6o	2	N	1	3	H	2	CH_2CH_3	H	H	97–100	$C_{22}H_{22}N_2O_4$	**6b**	80	12.6	NA	95*	2	6	6
6p	2	N	1	3	H	2	CH_3	2-CH_3	H	152–153	$C_{22}H_{22}N_2O_4\cdot {}^1/_{10}H_2O$	**6b**	88	15.5	NA	3	3		
6q	2	N	1	3	H	2	CH_3	6-CH_3	H	148–149	$C_{22}H_{22}N_2O_4$	**6b**	93	7.6	NA	23	2		
6r	2	N	1	3	H	1	CH_3	H	H	116–114	$C_{20}H_{18}N_2O_4\cdot {}^1/_4H_2O$	**6b**	71	24.6	NA	96*	2	57	6
6s	2	N	1	3	H	2	CH_3	*f*	H	179–183	$C_{26}H_{27}N_3O_7\cdot {}^1/_4H_2O$	**6b**	77	NA		18	2	34[h]	2
6t	2	C	1	3	H	1	CH_3	H	H	119–121	$C_{21}H_{19}NO_4\cdot {}^1/_{10}H_2O$	**6b**	43	9.5	NA	−16	2		
6u	2	C	1	3	OH	2	Na	H	H	132–133	$C_{21}H_{18}NO_5\cdot Na$	**6c**	34	15.7		−21	2	−35	2
6v	2	C	1	3	OH	2	CH_3	H	H	106–109	$C_{22}H_{21}NO_5$	**6b**	72	0.6	7.4	−32	3	18	2
6w	8	N	1	3	H	2	CH_3	H	H	147–149	$C_{21}H_{20}N_2O_4$	**6b**	55	NA		−18	2		
6x	2	N	0	3	H	2	CH_3	H	H	148–149	$C_{20}H_{18}N_2O_4\cdot {}^1/_{10}H_2O$	**6x**	95	26.0		29	3	−55	3
6y	2	N	1	3	H	2	CH_3	H	7-CH_3	178–180	$C_{22}H_{22}N_2O_4$	**6b**	65	NA	NA	−6.1	2		
6z	2	N	1	3	CH_3	2	CH_3	H	H	149–151	$C_{22}H_{22}N_2O_4\cdot HCl\cdot {}^1/_2H_2O$	**6b**	38	14.0	NA	48*	6	2	6
5	(RHC 5901A)													0.3	NA				

[a] All IC_{50}'s were calculated by nonlinear regression analysis and were significant at $p < 0.05$ level. [b] Starred results (*) are statistically significant using the two-tail Students *t* tests ($p < 0.05$). [c] *N* = number of animals. [d] All compounds had elemental analysis (C, H, N) within ±0.4 of theoretical values. [e] NA <50% inhibition at 100 μm. [f] 4-$NHCO(CH_2)_2CO_2CH_3$. [g] Drug given iv at 10 mg/kg. [h] Drug given id at 100 mg/kg.

Table II. Inhibition of Rat PMN, and GP Bronchospasm



9 10

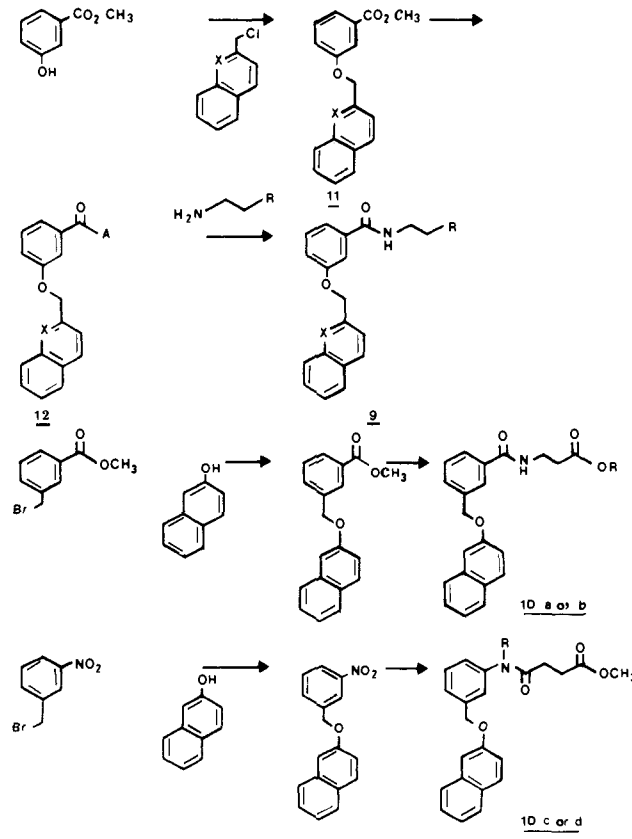
compd no.	X	R	mp, °C	formula[d]	synthetic method	% yield	in vitro PMN: I_{50},[a] μM 5-LO	in vitro PMN: I_{50},[a] μM CO	in vivo (50 mg/kg, id) LTD_4[b] % inh	in vivo (50 mg/kg, id) LTD_4[b] N[c]
9a	C	CN	137–138	$C_{21}H_{18}N_2O_2$	**9b**	91	NA[e]			
9b	C	CO_2CH_3	80–81	$C_{22}H_{21}NO_4$	**9b**	44	29.5	NA	−12	2
9c	C	CO_2H	173–176	$C_{21}H_{19}NO_4$	**6c**	69	NA		10	3
9d	N	CO_2CH_3	102–103	$C_{21}H_{20}N_2O_4$	**9b**	18	35.2		−3	2
10a	O ‖ CNH	CH_3	87–88	$C_{22}H_{21}NO_4$	**9b**	76	60.5		−6	3
10b	O ‖ CNH	H	127–128	$C_{21}H_{19}NO_4$	**6c**	55	NA		2	2
10c	H | NC ‖ O	CH_3	128–129	$C_{22}H_{21}NO_4$	**6b**	83	1.91	NA	1	2
10d	OH | N—C ‖ O	CH_3	95–97	$C_{22}H_{21}NO_5$	**6b**	28	0.86	6.1	5	2

[a] All I_{50}'s were calculated by nonlinear regression analysis and were significant at $p < 0.05$ level. [b] Starred results (*) are statistically significant using the two-tail Students t tests ($p < 0.05$). [c] N = number of animals. [d] All compounds had elemented analysis (C, H, N) within ±0.4 of theoretical values. [e] NA <50% inhibition at 100 μm.

= OH). Treatment of **8** with the appropriate chloroacylcarboxylate reagent gave target compounds **6a,b,d–k,m–o,r,t,v**. Compounds **6c,u** were prepared by hydrolyzing the parent esters **6a,v** with Claisen's alkali. Compounds **6l,p,q** were made by employing 2-cyano-,[23] 2-methyl-, or 6-methyl-substituted 3-nitrophenol, and compounds **6w,y** were made by using 8-(chloromethyl)-quinoline and 2-(chloromethyl)-7-methylquinoline instead of the corresponding starting materials of Scheme I. The synthesis of the bis(-amino-4-oxobutanoic acid methyl ester) **6s** started with 2-amino-5-nitrophenol. The preparation of ether **6x** started with condensing 2-chloroquinoline with 3-nitrophenol neat. Finally, **6z** was made by treating **8** (R = H) with ethyl formate followed by reduction with sodium borohydride and acetylation with 3-carbomethoxypropanoyl chloride.

The synthetic pathways for the preparation of compounds **9** and **10** listed in Table II are shown in Scheme II. Reaction of methyl 3-hydroxybenzoate with 2-(chloromethyl)quinoline or 2-(chloromethyl)naphthalene gave bis-aryl compounds **11**. Base hydrolysis followed by carbonyl activation and treatment with 3-aminopropionitrile or β-alanine methyl ester produced compounds **9a,b,d**. Condensation of methyl 2-(bromomethyl)benzoate with 2-naphthol followed by hydrolysis, carbonyl activation with thionyl chloride, and treatment with β-alanine methyl ester provided **10a**, and condensation of 2-(bromomethyl)-

Scheme II



(23) Kreft, A. F.; Klaubert, D. H.; Bell, S. C.; Pattison, T. W.; Yardley, J. P.; Hand, J. M.; Chang, J.; Carlson, R. P.; Lewis, A. J. *J. Med. Chem.*, in press.

Table III. Effect of Compound **6e** and LY-171,883 on LTC_4- and LTD_4-Induced Contraction of the GP Trachea

agonist/ antagonists[e]	% max.[a] contractility ± SE			−log ED_{50} ± SE[c]			
	control	treated	P^b	control	treated	P	pK_B ± SE[d]
LTC_4							
compd **6e**	77 ± 4	77 ± 3	NS	8.49 ± 0.08	7.50 ± 0.25	*	5.35 ± 0.39
LY-171,883	81 ± 1	73 ± 2	NS	8.72 ± 0.12	7.47 ± 0.10	*	5.74 ± 0.22
LTD_4							
compd **6e**	59 ± 2	63 ± 2	NS	8.71 ± 0.12	7.79 ± 0.12	*	5.33 ± 0.22
LY-171,883	73 ± 3	57 ± 4	*	8.69 ± 0.13	7.49 ± 0.19	*	5.63 ± 0.31

[a] Percentage of maximal response of tissue to carbachol (1 mM). [b] Significance of control vs. treated; unpaired Student's t test; an asterisk indicates $p < 0.05$ response. [c] −Log of agonist concentration (M) producing half-maximal agonist. [d] Estimate of pK_B determined as follows: $-\log K_B = -\log([B]/\text{dose ratio} - 1)$, where B = molar concentration of antagonist and dose ratio = ED_{50} treated ÷ ED_{50} control. [e] Antagonist concentration = 3×10^{-5} M.

nitrobenzene with 2-naphthol followed by full or partial reduction produced **10c** or **10d**. Base hydrolysis of **9b** and **10a** yielded **9c** and **10b**, respectively.

Biological Results and Discussion

The results obtained for compounds **6**, **9**, and **10** as inhibitors of LTD_4- and OA-induced bronchospasm in the GP and as inhibitors of rat PMN 5-LO are listed in Tables I and II. The naphthalenyl ring containing compounds tend to be more active in vitro (see pairs **6a6b, 6c6u, 6d6m, 6e6v, 6r6t**; exceptions are pairs **6h6k, 6g6j, 6n6f**), whereas all of the compounds significantly active in vivo contain the quinolinyl heterocycle. Compounds with a hydroxamic acid moiety produce the most potent analogues (**6e, 6v, 10d**) in vitro. Factors that contribute to maximal biologic activity include meta substitution of the benzene ring (see series **6a, 6d, 6f** and **6b, 6m, 6n**), terminal ester groups (compare **6a** with **6c** and **6u** with **6v**), and an oxobutanoate or oxopropanoate side chain (see series **6a, 6g, 6h, 6i** and **6b, 6j, 6k, 6t**). Structural modifications that reduce or abolish activity include shortening (**6x**) or reversing (**10c, 10d**) the ether linkage and alkylating (**6z**) or reversing (**9b, 9c, 9d**) the amide group.

Compounds in Tables I and II are selective inhibitors of 5-LO vs. CO. For example, 10 compounds (**6b,e–g,j,-q,t,v,10c,d**) have IC_{50}'s less than 10 μM in the 5-LO assay, whereas only **6v** and **10d** have IC_{50}'s less than 10 μM in the CO assay, and their 5-LO/CO inhibitory IC_{50} ratios are approximately 10 and 3, respectively.

Compound **6v** is the most potent 5-LO inhibitor in this series (IC_{50} = 0.6 μM); however, it is not active in vivo. This may be due to inadequate absorption or distribution. In general, no significant correlation between the inhibition of 5-LO in vitro and inhibition of OA- or LTD_4-induced bronchospasm in vivo is seen.

There are seven compounds in Table I that have significant activity against LTD_4-induced bronchospasm in the GP (**6a,c,e,i,o,r,z**). Only compound **6e** has significant activity against OA-induced bronchospasm.

Compound **6e** was studied in detail and its biological profile was compared with that of LY-171,883. Cumulative concentration–response curves to LTs C_4 and D_4 were obtained on the isolated GP trachea. The results are summarized in Table III. Compound **6e** and LY-171,883, under these experimental conditions,[24] appear to be equally active as antagonists of exogenously applied LTC_4 and LTD_4. Similar mean pK_B values (Table III) indicate nearly equivalent potency for both compounds against either LT. Compound **6e** and LY-171,883 were also examined against bronchoconstriction induced by iv administered LTD_4 and aerosolized administered OA. Compound **6e** and LY-171,883 administered intraduodenally were equipotent against LTD_4- [IC_{50} with 95% confidence interval: **6e** = 3.3 (1.8–7.2) mg/kg; LY-171,883 = 6.0 (3.3–8.8) mg/kg] and OA-induced [IC_{50} with 95% confidence interval: **6e** = 27.4 (5.1–37.0) mg/kg; LY-171,883 = 16.7 (8.1–31.8) mg/kg] bronchoconstriction. Finally, the IC_{50}'s for **6e** and LY-171,883 in the rat PMN 5-LO assay were determined to be 1.4 μM and 18.9 μM, respectively.

We have described the preparation of a novel series of 5-LO inhibitors and LTD_4 antagonists. Compound **6e** is a potent inhibitor of both LTD_4- and OA-induced bronchoconstriction in the GP. The fact that it competitively inhibits the binding of LTD_4 in isolated GP tracheal spiral strips indicates that it is acting at the receptor level. Although the data on compound **6e** is significant, it is not being further developed as a drug candidate because of its mutagenic activity in the Ames assay.[25]

Structural Relationships

The structural relationship among the above sulfidopeptide LT antagonists and LTD_4 deserves comment. All the compounds in Figure 1 (except **5**) contain three or more of the following structural features: (A) a terminal carboxylic acid (or equivalent), (B) a hydroxyl (or equivalent), (C) a potential metal chelating moiety, and (D) a lipophilic tail. The long lipophilic tail of LTD_4 may confer agonist properties because most antagonists either lack this portion or have it in truncated form. Both FPL-55,712 (**1**) and Wy-44,329 (**4**) have all four functional groups; however, the spatial relationships among the groups do not closely approximate the distances found in LTD_4. LY-171,883 (**2**) contains a tetrazolyl moiety, which has a similar pK_a and electronic configuration as a carboxylic acid, in addition to functional groups C and D. The amide group of compound **3** may act as a substitute for the hydroxyl (B) because the pK_a's are similar for both groups. Compound **5** has only functional groups B and C. It would be interesting to examine a compound similar to **5** with a carboxylic acid substituted for the hexyl chain methyl to determine whether sulfidopeptide LT antagonism would increase significantly.

With respect to the potential metal chelating moiety (C), there is evidence that Ca^{2+}, Mg^{2+}, and Mn^{2+} enhance binding of LTD_4 at receptor sites.[25] Compound **6e** contains an especially good potential metal chelating group (C) in the 2-oxymethylquinoline. Furthermore, groups A–C in **6e** closely approximate the distances found in LTD_4.

(24) Buckner, C. K.; Hand, J. M.; Wong, S. K. Int. *J. Immunopharmacol.* **1979**, *1*, 173.
(25) Dizio, S., internal correspondence, Wyeth Laboratories, Inc.
(26) Pong, S. S.; DeHaven, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7415.
(27) Furchgott, R. F. In *Handbook of Experimental Pharmacology*; Eichler, O., Farah, A., Herlen, H., Welch, A. D., Eds.; Springer-Verlag: New York, 1972; pp 283–335.

Therefore, it is intriguing to speculate that cationic metal chelation at the receptor is involved with the above antagonists because they all contain a potential metal cation binding functional group.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. NMR spectra were recorded on a Varian XL-300 at 300 MHz, a Varian XL-100 at 100 MHz, or a Varian FT-80A at 80 MHz. Mass spectra were recorded on a Kratos MS-25. IR spectra were recorded with a Perkin-Elmer 299 infrared spectrophotometer. Elemental analysis were recorded with a Perkin-Elmer 240C elemental analyzer, and all compounds were within 0.4% of theoretical value.

Typical Procedures for Scheme I. 3-(2-Naphthalenylmethoxy)nitrobenzene (7). A mixture of 2-(chloromethyl)naphthalene (25 g, 0.141 mol), 3-nitrophenol (19.6 g, 0.141 mol), cesium carbonate (11.5 g, 0.035 mol), sodium carbonate (15 g, 0.141 mol), acetone (600 mL), and potassium iodide (500 mg, 0.3 mmol) was heated at reflux for 3 days. The reaction was filtered through Celite and silica gel. The product was crystallized from acetone, giving 24.8 g (65% yield) of solid: mp 115–117 °C.

3-(2-Naphthalenylmethoxy)aniline (8, R = H). Method A. To a mixture of 3-(2-naphthalenylmethoxy)nitrobenzene (24.8 g, 88.9 mmol), methanol (300 mL), tetrahydrofuran (300 mL), H_2O (300 mL), and sodium hydroxide (14.2 g, 0.356 mol) was added sodium dithionite (61.9 g, 0.356 mol). The reaction was stirred overnight. The mixture was filtered and the filtrate was concentrated. The residue was refluxed in acetone. The acetone extract was dried ($MgSO_4$) and concentrated to a solid, giving 5.3 g (24% yield) of product: mp 110–114 °C.

8 (R = H). Method B. To a suspension of 3-(2-naphthalenylmethoxy)nitrobenzene (24.8 g, 88.9 mmol) in ethanolic hydrochloric acid was added excess powdered iron. The reaction was stirred overnight at room temperature. The reaction was filtered to remove excess iron. After neutralization with saturated aqueous sodium bicarbonate, the mixture was extracted with methylene chloride (3×). The extract was dried ($MgSO_4$) and concentrated to a solid, giving 15.9 g (72% yield) of product: mp 108–113 °C.

[3-(2-Naphthalenylmethoxy)phenyl]hydroxylamine (8, R = OH). To a solution of 3-(2-naphthalenylmethoxy)nitrobenzene (5.0 g, 17.9 mmol) in tetrahydrofuran with 10% palladium on carbon (50 mg) was slowly added hydrazine hydrate (1.0 mL, 19.7 mmol). Fresh catalyst was added twice (100 mg) over 4 h. The mixture was filtered through Celite and the solvent was removed in vacuo. The remaining solid was crystallized from methanol, giving 2.5 g (53% yield) of product: mp 142–144 °C.

4-[[3-(2-Naphthalenylmethoxy)phenyl]amino]-4-oxobutanoic Acid, Methyl Ester (6b). To a solution of 3-(2-naphthalenylmethoxy)aniline (3.9 g, 15.7 mmol) and triethylamine (2.4 mL, 17.2 mmol) in tetrahydrofuran (200 mL) at 0 °C was slowly added a solution of 3-carbomethoxypropanoyl chloride (2.1 mL, 17.2 mmol) in tetrahydrofuran (50 mL). The reaction was allowed to warm to room temperature and was stirred for an additional hour. The mixture was filtered and the solvent was removed in vacuo. The remaining oil was dissolved in methylene chloride, washed with 1 N aqueous HCl (2×), dried ($MgSO_4$), treated with charcoal, filtered through Celite and silica gel, and concentrated to an oil. The oil was crystallized in diisopropyl ether, giving 2.5 g (44% yield) of solid: mp 132–133 °C.

In a like manner as above using the appropriate combination of 2-(chloromethyl)naphthalene or 2-(chloromethyl)quinoline with 2-nitrophenol, 3-nitrophenol, or 4-nitrophenol the following compounds were prepared: **6a, 6d–k, 6m–o, 6r, 6t,** and **6v**. (See Table I for yield and melting points.)

Compounds **6l,p,q** were made by the same procedures employed in the synthesis of **6b**, except 2-cyano-,[23] 2-methyl-, or 6-methyl-3-nitrophenol was used instead of 3-nitrophenol.

Compounds **6w,y** were also made by the same procedures employed in the synthesis of **6b**, except 8-(chloromethyl)quinoline and 2-(chloromethyl)-7-methylquinoline were used instead of 2-(chloromethyl)quinoline.

The synthesis of compound **6s** started with 2-amino-5-nitrophenol, and the only modifications to the **6b** procedures was to use 2 equiv of 3-carbomethoxypropanoyl chloride.

4-[[3-(2-Quinolinylmethoxy)phenyl]amino]-4-oxobutanoic Acid (6c). Compound **6a** (3.0 g, 8.24 mmol) was dissolved in Claisen's alkali (200 mL) and stirred overnight at room temperature. The reaction was washed with ether. The aqueous layer was neutralized with dilute aqueous HCl and extracted with methylene chloride. The methylene chloride extract was dried ($MgSO_4$) and concentrated to give 1.5 g (52% yield) of solid: mp 180–182 °C.

In the same manner compound **6u** was prepared.

4-[[3-(2-Quinolinyloxy)phenyl]amino]-4-oxobutanoic Acid, Methyl Ester (6x). A mixture of 2-chloroquinoline (8.2 g) and 3-nitrophenol (7.0 g) was heated at 160 °C for 2 h. After cooling, the mixture was dissolved in methylene chloride, washed with saturated aqueous sodium bicarbonate (4×), dried (Na_2SO_4), and concentrated to a solid. The solid was crystallized from acetone/hexanes, giving 12.7 g (95% yield) of product: mp 112–114 °C.

The 3-(2-quinolinyloxy) nitrobenzene was reduced and acetylated in the same manner as in the synthesis of **6b** to give **6x**: mp 148–149 °C.

Typical Procedures for Scheme II. Methyl 3-[(2-Naphthalenylmethyl)oxy]benzoate (11). This compound was prepared by using the procedure described for the preparation of **7** but employing methyl-3-hydroxybenzoate instead of 3-nitrophenol.

3-[(2-Naphthalenylmethyl)oxy]benzoic Acid. A solution of methyl 3-[(2-naphthalenylmethyl)oxy]benzoate (12.62 g, 43.1 mmol), methanol (400 mL), and 1 N aqueous sodium hydroxide (375 mL) was heated at 90 °C for 2 h. The mixture was concentrated, acidified with 1 N aqueous HCl, and extracted with methylene chloride. The extract was dried ($MgSO_4$) and concentrated to give 11.5 g (97% yield) of solid: mp 184–187 °C.

3-[(2-Naphthalenylmethyl)oxy]benzoyl Chloride (12). A mixture of 3-[(2-naphthalenylmethyl)oxy]benzoic acid (5.3 g, 19.0 mmol), oxalyl chloride (2.66 g, 20.9 mmol), toluene (300 mL), and dimethylformamide (0.25 mL) was heated at 120 °C for 1.5 h. The reaction was cooled, washed with dilute aqueous $NaHCO_3$, and dried ($MgSO_4$), and the solvent was removed in vacuo giving 5.5 g (98% yield) of solid: mp 89–91 °C.

***N*-[3-[(2-Naphthalenylmethyl)oxy]benzoyl]-β-alanine, Methyl Ester (9b).** To a solution of 3-[(2-naphthalenylmethyl)oxy]benzoyl chloride (5.6 g, 19.0 mmol) and triethylamine (2.1 g, 20.9 mol) in methylene chloride (250 mL) was added a solution of β-alanine methyl ester (2.06 g, 19.9 mmol) in methylene chloride (50 mL). The reaction was stirred for 30 min. The mixture was washed with dilute aqueous HCl, dried ($MgSO_4$), and concentrated to an oil. The oil was crystallized from diisopropyl ether, giving 3.0 g (44% yield) of product: mp 80–81 °C.

Compound **9a** was prepared as above except that 3-aminopropionitrile was used instead of β-alanine methyl ester, and compound **9d** was prepared as above except that 2-(chloromethyl)quinoline was used instead of 2-(chloromethyl)-naphthalene. (See Table II for yield and melting points.)

***N*-[3-[(2-Naphthalenyloxy)methyl]benzoyl]-β-alanine, Methyl Ester (10a).** This compound was prepared by the procedures used in the synthesis of **9b** but using methyl 3-(bromomethyl)toluate and 2-naphthol.

Compounds **10c,d** were prepared with 3-(bromomethyl)nitrobenzene and 2-naphthol as the starting material and by use of the procedures described for the preparation of **7, 8,** and **6b**. Compound **10b** was prepared from **10a** by hydrolysis in the same manner as **6c**.

Rat PMN 5-LO. Peritoneal PMNs were obtained from female Wistar rats (150–250 g) that received an intraperitoneal (ip) injection of 6% glycogen (10 mL). After 24 h, rats were killed by CO_2 asphyxiation and peritoneal cells were harvested by peritoneal lavage using Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution (HBSS). The peritoneal exudate was centrifuged at 400*g* for 10 min. The lavaged fluid was removed, and the cell pellet was resuspended in HBSS containing Ca^{2+} and Mg^{2+} and 10 mM L-cysteine at a concentration of 2×10^7 cells/mL. To 1-mL portions of cell suspension, test drugs or vehicle was added and incubated at 37° C for 10 min. Following this preincubation, the calcium ionophore A23187 (10 μM) was added together with 0.5 μCi [^{14}C]arachidonic acid and further incubated for 10 min. The

reaction was stopped by the adding ice-cold water (3 mL) and acidifying to pH 3.5. LO and CO products were then extracted twice into diethyl ether. The pooled ether extracts were evaporated to dryness under nitrogen, and the residue was redissolved in a small volume of methanol and spotted on aluminum-backed, precoated thin-layer chromatographic plates. The samples were then cochromatographed with authentic reference 5-HETE, 12-HETE, and LTB_4 in the solvent system hexane/ether/acetic acid (50:50:3). For assessing CO activity, the methanolic extracts were chromatographed in the solvent system ether acetate/formic acid (80:1) with authenic PGE_2 and TxB_2. After chromatography, the areas associated with authentic 5-HETE, LTB_4, or PGE_2 and TxB_2 were identified by autoradiography, cut out, and quantitated by liquid scintillation. IC_{50} values for each metabolite and test compound were calculated from the line generated by nonlinear regression analysis of raw dose–response data. The results are summarized in Tables I and II.

LTD_4-Induced Bronchospasm in the GP. Male Martley strain GPs (350–600 g) were anesthetized with pentobarbital sodium (50 mg/kg, ip). The jugular vein was cannulated for injection of drugs and the carotid artery for monitoring blood pressure. The trachea was cannulated for artificial ventilation by a miniature Starling pump and for indirect measurement of respiratory volume changes as described infra. Additional pentobarbital sodium (15 mg/kg) was administered intravenously to arrest spontaneous respiration. A CO inhibitor, indomethacin (10 mg/kg in Tris buffer, iv), was administered 9 min before LTD_4 challenge to shunt arachidonate metabolism to the LO pathways. Submaximal bronchoconstrictor responses were established in control animals by varying the dose levels of LT. Intravenous (iv) dose level range for LTD_4 was from 0.3 to 1 μg/kg. Test drugs were administered by the intraduodenal (id) route (except where noted) 10 min before induction of bronchospasm by administration of LTD_4 at the predetermined dose levels. Control animals receive saline in place of drug. Respiratory volume changes were determined by a calibrated piston whose travel was recorded, via a linear transducer, on a Beckman dynograph recorder. Maximal bronchoconstrictor volume was determined by clamping off the trachea at the end of the experiment. Overflow volumes at 1, 3, and 5 min were obtained from the recorded charts. The overflow volume at 1, 3, and 5 min was expressed as a percentage of maximal bronchoconstriction. Combined group values were used from each of these time intervals to determine the inhibitory effect of drugs:

$$\% \text{ inhibition} = [(\% \text{ bronchoconstriction (bc) in control group} - \% \text{ bc in drug-treated groups})/\% \text{ bc in control group}] \times 100$$

A Student's t test for unpaired data was used to determine statistical significance. Dose–response curves were generated, and ED_{50} doses were interpolated from the regression lines.

Results for compounds are summarized in Tables I and II.

OA-Induced Bronchospasm in the GP. Male Hartley strain GP weighing 350–600 g were sensitized to OA (5 mg ip and 5 mg sc) on day 0. Three to five weeks later, the animals were anesthetized with pentobarbital sodium (50 mg/kg, ip). The jugular vein was cannulated for injection of drugs and the carotid artery for monitoring blood pressure. The trachea was cannulated for artificial ventilation by a miniature Starling pump and for indirect measurement of respiratory volume changes as described infra. Additional pentobarbital sodium (15 mg/kg, iv) was administered to arrest spontaneous respiration. A CO inhibitor, indomethacin (10 mg/kg in Tris buffer, iv at 9 min prior to OA challenge), was administered to shunt arachidonic metabolism to the LO pathways. One minute later, chlorpheniramine (1.0 mg/kg in saline, iv) was given to attenuate the histaminic component of anaphylactic bronchoconstriction. Test drugs were administered id (except where noted) at 10 min before antigen challenge. Anaphylactic bronchoconstriction was induced by administration of aerosolized OA (1%). Control animals receive saline in place of drug. Respiratory volume changes and determinations of the inhibitory effects of drugs were determmined as described in the preceding section.

Acknowledgment. We thank J. M. Hand, S. Schwalm, T. Smith, and A. Blumenthal for their contributions and P. Stevens for preparing the manuscript.

Registry No. **6a**, 102649-84-3; **6b**, 102649-85-4; **6c**, 102649-86-5; **6d**, 102649-87-6; **6e**, 102649-88-7; **6f**, 102649-89-8; **6g**, 102649-90-1; **6h**, 102649-91-2; **6i**, 102649-92-3; **6j**, 102649-93-4; **6k**, 102649-94-5; **6l**, 102649-95-6; **6m**, 102649-96-7; **6n**, 102649-97-8; **6o**, 102649-98-9; **6p**, 102649-99-0; **6q**, 102650-00-0; **6r**, 102650-01-1; **6s**, 102650-02-2; **6t**, 102650-03-3; **6u**, 102650-04-4; **6v**, 102650-05-5; **6w**, 102650-06-6; **6x**, 102650-07-7; **6y**, 102650-08-8; **6z**, 102650-09-9; **7**, 79807-83-3; **8** (R = H), 79807-85-5; **8** (R = OH), 102649-83-2; **9a**, 102650-10-2; **9b**, 102650-11-3; **9c**, 102650-12-4; **9d**, 102650-13-5; **10a**, 102650-14-6; **10b**, 102650-15-7; **10c**, 102650-16-8; **10d**, 102650-17-9; **11** (X = C), 102650-18-0; **11** (X = C, acid), 102650-19-1; **12** (X = C, A = Cl), 102650-20-4; LO, 80619-02-9; LTD_4, 73836-78-9; $ClCO(CH_2)_2CO_2CH_3$, 1490-25-1; $ClCOCO_2CH_3$, 5781-53-3; $ClCO(CH_2)_3CO_2CH_3$, 1501-26-4; $ClCOCH_2CO_2CH_2CH_3$, 36239-09-5; $ClCO(CH_2)_2CO_2CH_2CH_3$, 14794-31-1; $ClCOCH_2CO_2CH_3$, 37517-81-0; $2\text{-}O_2NC_6H_4OH$, 88-75-5; $3\text{-}O_2NC_6H_4OH$, 554-84-7; $4\text{-}O_2NC_6H_4OH$, 100-02-7; $3\text{-}HOC_6H_4CO_2CH_3$, 19438-10-9; $H_2N(CH_2)_2CO_2CH_3$, 4138-35-6; $H_2N(CH_2)_2CN$, 151-18-8; $3\text{-}BrCH_2C_6H_4CO_2CH_3$, 1129-28-8; 2-(chloromethyl)naphthalene, 2506-41-4; 2-(chloromethyl)quinoline, 4377-41-7; 2-cyano-3-nitrophenol, 72106-43-5; 2-methyl-3-nitrophenol, 5460-31-1; 6-methyl-3-nitrophenol, 5428-54-6; 8-(chloromethyl)quinoline, 94127-04-5; 2-(chloromethyl)-7-methylquinoline, 102649-82-1; 2-amino-5-nitrophenol, 121-88-0; 2-chloroquinoline, 612-62-4; 3-(2-quinolinyloxy)nitrobenzene, 51248-09-0; 2-naphthol, 135-19-3; 3-(bromomethyl)-nitrobenzene, 3958-57-4.