Leukotriene D_4 Antagonists and 5-Lipoxygenase Inhibitors. Synthesis of Benzoheterocyclic [(Methoxyphenyl)amino]oxoalkanoic Acid Esters

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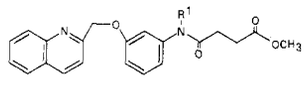
A series of novel benzoheterocyclic [(methoxyphenyl)amino]oxoalkanoic acid esters has been prepared. These compounds were tested as inhibitors of rat polymorphonuclear leukocyte 5-lipoxgenase (LO) in vitro and as inhibitors of leukotriene D_4 (LTD_4) and ovalbumin (OA) induced bronchospasm in the guinea pig (GP) in vivo. In general, inhibitory activity against 5-LO, LTD_4, and OA was broadest for benzthiazole-containing analogues (benzthiazole > benzimidazole >> benzoxazole, benzofuran). The most potent 5-LO inhibitor, 4-[[3-(2-benzthiazolylmethoxy)-phenyl]hydroxyamino]-4-oxobutanoic acid methyl ester (**7**), had an IC_{50} of 0.36 μM. Compound **7**, however, was inactive vs. OA. The most potent compound in vivo, 4-[[3-[(1-methyl-2-benzimidazolyl)methoxy]phenyl]-amino]-4-oxobutanoic acid methyl ester **4**, inhibited both LTD_4- and OA-induced bronchospasm by 83% and 60%, respectively, at 50 mg/kg intraduodenally. Compound **4** was studied in the Ames assay employing five strains of bacteria (TA1535, TA1537, TA1538, TA98, and TA100) with and without S-9 rat liver enzyme metabolic activation, and there was no significant number of reversions noted.

We have recently shown that 4-[[3-(2-quinolinylmethoxy)phenyl]hydroxyamino]-4-oxobutanoic acid methyl ester (**1a**) is a potent inhibitor of rat polymorphonuclear leukocyte (PMN) 5-lipoxygenase (5-LO) in vitro and is a potent inhibitor of both leukotriene D_4 (LTD_4) and ovalbumin (OA) induced bronchospasm in the guinea pig (GP).[1,2] This paper covers a portion of our continuing investigation of this novel structural class and is concerned with the synthesis and biological evaluation of heterocyclic groups other than quinoline and/or phenyl side chains other than aminooxoalkanoic acid esters.



1a R^1 = OH
1b R^1 = H

As pointed out by us, compound **1a** is not being further developed as a drug candidate because of its mutagenic activity in the Ames[3] assay. Certain aryl hydroxamic acids are known to form sulfate or acetate esters in vivo, which then heterolytically cleave to give arylacylnitrenium ions as the metabolic ultimate electrophilic mutagens.[4] Since **1a** contains an aryl hydroxamic acid, we thought this portion of the molecule was suspect. Alternatively, the (quinolinylmethoxy)phenyl system of **1a** may be acting as an intercalating agent.[5]

Therefore, our goals were to discover new nonmutagenic analogues of compound **1** that retain potent LTD_4 antagonist activity in vivo. Past experience in this series has shown that a 2-oxymethyl nitrogen heterocycle, meta substitution, and a terminal alkyl carboxylate are elements needed for LTD_4 antagonist activity. We chose to substitute a benzthiazole because it is a bioisostere of quinoline.[6] Benzimidazole and benzoxazole were employed because they extend the 2-oxymethyl nitrogen heterocyclic series and benzofuran would examine the effect of a 2-oxymethyl oxygen heterocycle. With respect to phenyl substitution, we prepared some 2-oxopyrrolidine-4-carboxylates because this moiety is found in previously reported LT antagonists.[7]

Chemistry

The synthetic pathways for the preparation of compounds **2–14** listed in Table I are shown in Scheme I. Reaction of 2-aminophenol, *N*-methyl-2-phenylenediamine, or 2-aminophenol with methyl chloroacetimidate[8] gave 2-chloromethyl heterocycles **16**. Treatment of **16** with 3-nitrophenol in acetone with cesium carbonate[9] at reflux provided the bis-aryl system **17**. Reduction of **17** with iron and ethanolic HCl followed by treatment with 3-carbomethoxypropionyl chloride gave compounds **2–4**. The reagents used in the preparation of the remaining target compounds **5–14** are noted in Scheme I and details of the reactions are provided in the Experimental Section.

The preparation of target compounds **18–24** listed in Table II started with 3-aminophenol (Scheme II). Fusion of 3-aminophenol with itaconic acid followed by acid-catalyzed esterification provided intermediate **25**. Alkylation of **25** with the appropriate chloromethyl benzoheterocycle **16** gave compounds **18–21**. Condensation of 3-aminophenol with 3-carbomethoxypropionyl chloride provided intermediate **26**. Reaction of **26** with the appropriate benzoheterocycle **16** in acetone with cesium carbonate at reflux effected both alkylation and cyclization to give target compounds **22–24**.

Physical data for key intermediates in Schemes I and II are summarized in Table IV.

Biological Results and Discussion

The results obtained for compounds **2–14** and **18–24** as inhibitors of rat PMN 5-LO and LTD_4- and OA-induced bronchospasm in the GP are listed in Tables I and II. As expected on the basis of bioisosteric equivalence, benzthiazole **2** was equiactive both in vitro and in vivo with quinoline **1b**. Unexpectedly, benzimidazole **4** was

(1) Musser, J. H.; Kubrak, D. M.; Chang, J.; Lewis, A. J. *J. Med. Chem.* **1986**, *29*, 1429.
(2) Compound **1a** was recently retested as an antagonist of LTD_4-induced contractions of isolated GP tracheal spiral strips and was shown to be a competitive inhibitor with a pk_B value of 6.60, which is different than the pk_B value of 5.33 reported in ref 1. The reason for the difference is that L-cysteine was added to inhibit conversion of LTD_4 to LTE_4. Furthermore, **1a** does not inhibit LTC_4 in the presence of glutathione and it does not inhibit histamine dose–response curves.
(3) Ames, B. N.; McCann, J.; Yamasaki, E. *Mutat. Res.* **1975**, *31*, 347.
(4) Gassman, P. G.; Granrud, J. E. *J. Am. Chem. Soc.* **1984**, *106*, 1498 and references cited therein.
(5) Epler, J. L.; Winton, W.; Ho, T.; Larimer, F. W.; Rao, T. K.; Hardigree, A. A. *Mutat. Res.* **1977**, *39*, 285.
(6) Thornber, C. W. *Chem. Soc. Rev.* **1979**, *18*, 564.
(7) Kadin, S. B. U. S. Patent 4 296 120.
(8) Roger, R.; Neilson, A. G. *Chem. Rev.* **1961**, *61*, 179.
(9) Zaugg, H. E. *J. Org. Chem.* **1976**, *41*, 3419.

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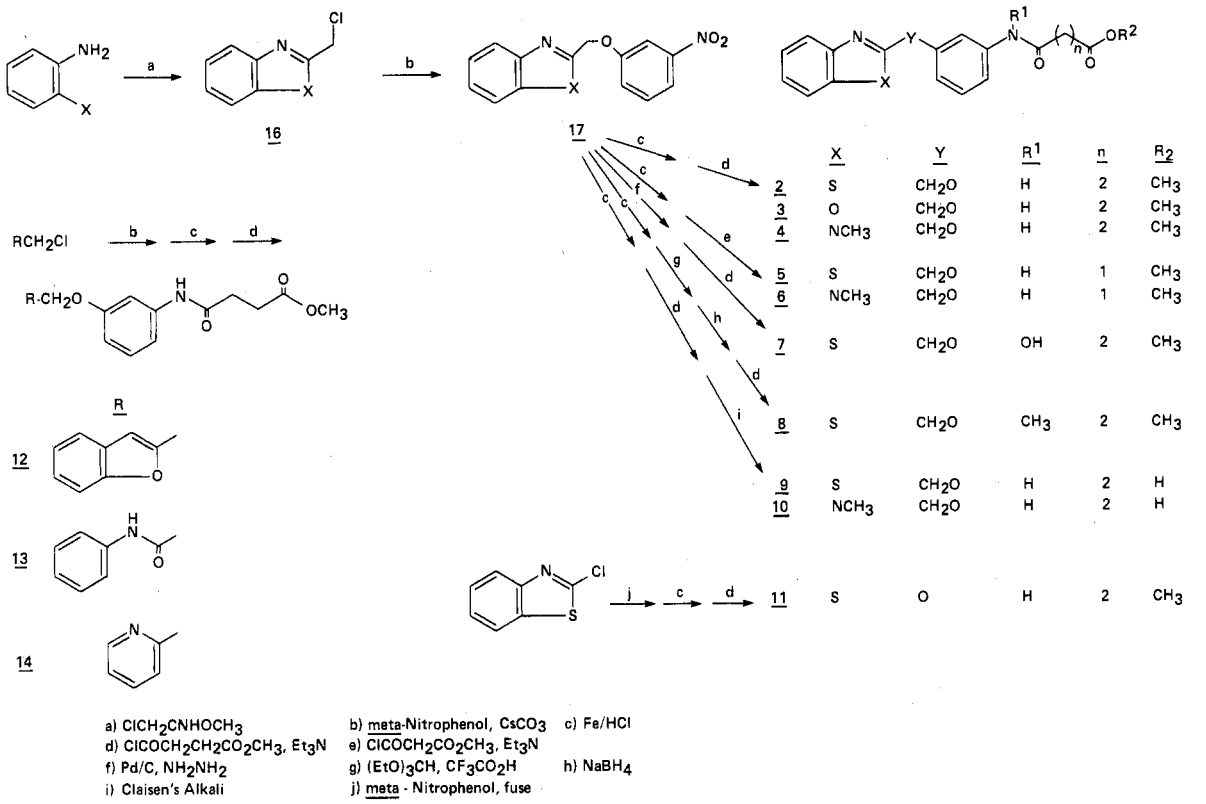
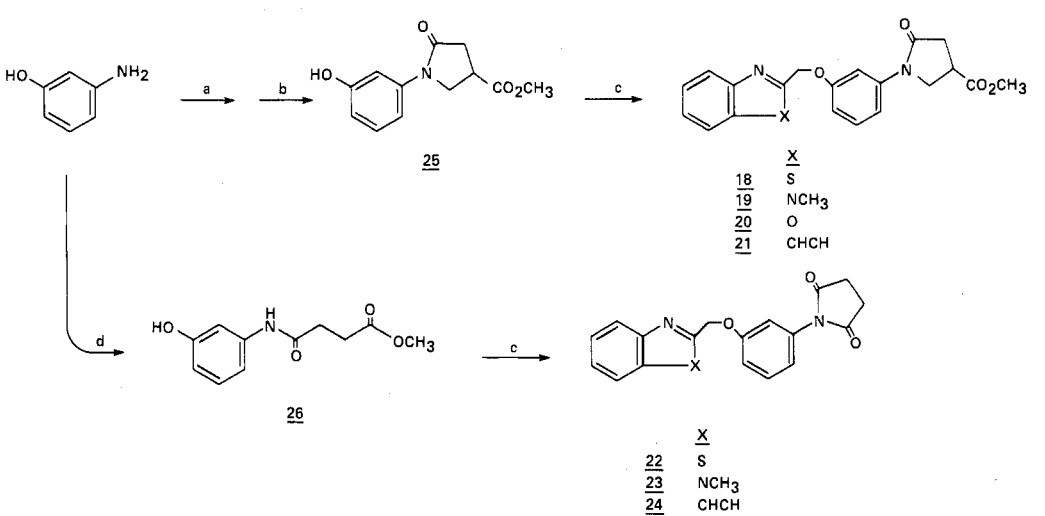
Scheme I



	X	Y	R^1	n	R_2
2	S	CH_2O	H	2	CH_3
3	O	CH_2O	H	2	CH_3
4	NCH_3	CH_2O	H	2	CH_3
5	S	CH_2O	H	1	CH_3
6	NCH_3	CH_2O	H	1	CH_3
7	S	CH_2O	OH	2	CH_3
8	S	CH_2O	CH_3	2	CH_3
9	S	CH_2O	H	2	H
10	NCH_3	CH_2O	H	2	H
11	S	O	H	2	CH_3

Scheme II



	X
18	S
19	NCH_3
20	O
21	CHCH

	X
22	S
23	NCH_3
24	CHCH

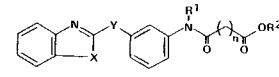
equiactive with **1b**, whereas benzoxazole **3** was essentially inactive in all three assays. Furthermore, the benzthiazole malonate **5** was equiactive with **1b** and **2**, whereas the benzimidazole malonate **6** was only active vs. LTD_4-induced bronchospasm.

In general, further modification of the benzoheterocyclic system lowered potency or eliminated activity. The pyrido analogue of **1b** was prepared to determine the importance of the fused benzyl ring; pyrido analogue **14** was completely inactive. Results obtained for benzofuran **12** and acetanilide **13** indicated that a 2-oxymethyl oxygen heterocycle and an oxyacetanilide, respectively, could not be substituted for the 2-oxymethylquinoline and retain activity.

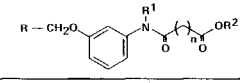
Several modifications made in the quinoline series[1] were also made in this study for comparison purposes. In most cases the results were consistent with our earlier findings. For example, carboxylic acids (**9**, **10**) were less potent than the corresponding esters (**2**, **4**) and removing the ether link (**11**) or methylating the amide (**8**) abolished activity in vivo. However, benzthiazole hydroxamic acid **7** relative to quinoline hydroxamic acid **1a** was more potent in vitro, less potent vs. LTD_4-induced bronchospasm, and inactive vs. OA-induced bronchospasm.

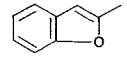
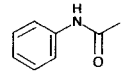
Certain compounds with a pyrrolidinone ring instead of the succinate group on the phenyl retained activity. Like the above series, methyl 2-oxopyrrolidine-4-carboxylates containing benzthiazole (**18**) and quinoline (**21**) were active as inhibitors of 5-LO and LTD_4-induced bronchospasm and benzoxazole (**20**) was inactive. In contrast, the benzimidazole 2-oxopyrrolidine-4-carboxylate **19** was not significantly active. It is interesting that compound **8** was inactive. Possibly the methyl group presents some steric

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



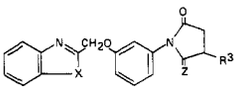
no.	X	Y	R^1	n	R^2	mp, °C	formula[d]	synth meth[c]	% yield	in vitro PMN: IC_{50},[a] μM 5-LO	in vitro PMN: IC_{50},[a] μM CO	in vivo (50 mg/kg id) LTD_4[b] % inh	in vivo (50 mg/kg id) LTD_4[b] N[c]	in vivo (50 mg/kg id) OA % inh	in vivo (50 mg/kg id) OA N
1a	CHCH	CH_2O	OH	2	CH_3	187–189	$C_{21}H_{20}N_2O_5$	*f*	69	1.4	40	97*	8	76*	13
1b	CHCH	CH_2O	H	2	CH_3	121–122	$C_{20}H_{20}N_2O_4$	*f*	58	28	NA[g]	86*	3	43	6
2	S	CH_2O	H	2	CH_3	129–130	$C_{19}H_{18}N_2O_4S$	2	83	15.7	NA	41*	6	63*	6
3	O	CH_2O	H	2	CH_3	104–105	$C_{19}H_{18}N_2O_5$	2	66	69.7	NA	3	2	−65	2
4	NCH_3	CH_2O	H	2	CH_3	136–138	$C_{20}H_{21}N_3O_4$	2	69	7.2	NA	83*	6	60*	6
5	S	CH_2O	H	1	CH_3	109–112	$C_{18}H_{16}N_2O_4S$	2	64	17.0	NA	77*	6	58*	6
6	NCH_3	CH_2O	H	1	CH_3	167–169	$C_{19}H_{19}N_3O_4 \cdot {}^1/_{10}H_2O$	2	81	NA		60*	6	5	6
7	S	CH_2O	OH	2	CH_3	123–125	$C_{19}H_{18}N_2O_5S$	7	82	0.36	NA	79*	6	6	6
8	S	CH_2O	CH_3	2	CH_3	107–110	$C_{20}H_{20}N_2O_4S \cdot 1.4HCl$	8	63	21	NA	21	3	19	3
9	S	CH_2O	H	2	H	191–193	$C_{18}H_{16}N_2O_4S \cdot {}^1/_2H_2O$	9	99	NA		57*	3		
10	NCH_3	CH_2O	H	2	H	213–214	$C_{19}H_{19}N_3O_4$	9	86	NA		30	3		
11	S	O	H	2	CH_3	78–82	$C_{18}H_{16}N_2O_4S$	11	97	27.1	NA	−2	3	7	2



no.	R	R^1	n	R^2	mp, °C	formula[d]	synth meth[e]	% yield	in vitro PMN: IC_{50},[a] μM 5-LO	in vitro PMN: IC_{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]	in vivo (50 mg/kg id) OA % inh	in vivo (50 mg/kg id) OA N
12		H	2	CH_3	112–113	$C_{20}H_{19}NO_5$	12	27	17.9	NA	−8	2	12	2
13		H	2	CH_3	164–165	$C_{19}H_{20}N_2O_5$	2	73	NA		25	2	7	2
14		H	2	CH_3	97–98	$C_{17}H_{18}N_2O_9$	2	86	NA		−1	2		
(REV 5901)[12]									0.3	NA	44.2	3	60.2*	6
(LY-171,883)[13]									18.9	NA	92.6*	6	79.7*	6

[a] All IC_{50} values were calculated by nonlinear regression analysis and were significant at the $p < 0.05$ level. [b] Starred results (*) are statistically significant with the two-tail Student's *t* tests ($p < 0.05$). [c] N = number of animals. [d] All compounds had elemental analysis (C, H, N) within ±0.4 of theoretical value. [e] Experimental Section compound number. [f] See ref 1. [g] NA <50% inhibition at 100 μM.

Table II. Inhibition of Rat PMN 5-LO and GP Bronchospasm



no.	X	Z	R^3	mp, °C	formula[d]	synth meth[e]	% yield	in vitro PMN: IC_{50},[a] μM 5-LO	in vitro PMN: IC_{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]	in vivo (50 mg/kg id) OA[b] % inh	in vivo (50 mg/kg id) OA[b] N
18	S	H_2	CO_2CH_3	99–102	$C_{20}H_{18}N_2O_4S$	18	62	14.0	NA	67*	3		
19	NCH_3	H_2	CO_2CH_3	131–133	$C_{21}H_{21}N_3O_4$	18	53	NA[f]		26	2		
20	O	H_2	CO_2CH_3	107–109	$C_{20}H_{18}N_2O_5$	18	61	30.0	NA	15	2		
21	CHCH	H_2	CO_2CH_3	101–104	$C_{22}H_{20}N_2O_4 \cdot {}^1/_{10}H_2O$	18	39	19.1	NA	89*	6	29	6
22	S	O	H	175–176	$C_{18}H_{14}N_2O_3S$	22	56	NA		68*	6	18	6
23	NCH_3	O	H	150–152	$C_{19}H_{17}N_3O_3$	22	49	NA		79*	3		
24	CHCH	O	H	159–160	$C_{20}H_{16}N_2O_3$	22	32	NA		88*	6	26	6

[a] All IC_{50} values were calculated by nonlinear regression analysis and were significant at the $p < 0.05$ level. [b] Starred results (*) are statistically significant with the two-tail Student's t test ($p < 0.05$). [c] N = number of treated animals. [d] All compounds had elemental analysis (C, H, N) within ±0.4 of theoretical value. [e] Experimental Section compound number. [f] NA <50% inhibition at 100 μM.

Table III. Ames Test Results for Compounds **1a**, **1b**, **21**, and **24**[a]

test article	amount per plate	mean no. of revertants[b] without activation	mean no. of revertants[b] with activation[c]
vehicle control[d]	0.2 mL	14	13
positive control[e]	0.1 mg	2710	738
1a	0.005 mg	15	13
	0.015	9	10
	0.05	13	16
	0.15	31	12
	0.5	943	716
1b	0.05 mg	24	15
	0.15	25	15
	0.5	696	566
	1.5	2930	2767
	5.0	2258	2879
vehicle control	0.2 mL	7	12
positive control	0.1 mg	1376	634
21	0.02 mg	10	12
	0.06	13	10
	0.2	16	10
	0.6	421	15
	2.0	475	84
vehicle control	0.2 mL	8	5
positive control	0.1 mg	1350	1103
24	0.005 mg	6	5
	0.015	9	7
	0.05	12	7
	0.15	43	8
	0.5	155	45

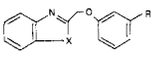
[a] None of the test compounds produced a significant amount of revertants in strains TA98, TA100, TA1535, and TA1538, either in the presence or absence of the metabolic activation system. [b] Three plates per treatment; two plates in cases of toxic response or contamination. [c] Activation provided by S-9 rat liver homogenate. [d] Me_2SO-d_6. [e] Without activation, 9-aminoacridine; with activation, 2-aminoanthracene.

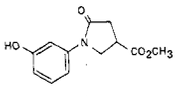
interaction that is alleviated in the constrained pyrrolidinone ring.

Pyrrolidine-2,5-dione **24** was determined to be a metabolite of **1b**.[10] Apparently, compound **1b** is hydrolyzed to the corresponding acid, which is interconvertible with the cyclic imide **24**. Compound **24** was prepared and was found to be an inhibitor of LTD_4-induced bronchospasm. As expected, both benzthiazole **22** and benzimidazole **23** were also LTD_4 antagonists in vivo. None of the pyrrolidinedione-containing compounds were active as 5-LO inhibitors.

(10) Tio, C. Internal Correspondence, Wyeth Laboratories, Inc.

Table IV. Physical Data for Key Intermediates[a]



X	R	mp, °C	formula[b]
S	NO_2	156–158	$C_{14}H_{10}N_2O_3S$
S	NH_2	118–120	$C_{14}H_{12}N_2OS$
NCH_3	NO_2	183–185	$C_{15}H_{13}N_3O_3$
NCH_3	NH_2	149–153	$C_{15}H_{15}N_3O \cdot {}^1/_4H_2O$
		188–189	$C_{12}H_{13}NO_4$

25

[a] (Chloromethyl)benzimidazole and (chloromethyl)benzthiazole are known compounds; see ref 14 and 15, respectively. [b] All compounds had elemental analysis (C, H, N) within ±0.4 of theoretical value.

The most potent 5-LO inhibitor compound **7** (IC_{50} of 0.36 μM in vitro) was not active in vivo against OA challenge. Indeed, no significant correlation between inhibition of 5-LO and inhibition of OA-or LTD_4-induced bronchospasm in vivo was seen, which is consistent with our earlier study.

In Tables I and II there are 11 compounds that had significant inhibitory activity against LTD_4-induced bronchospasm (**2**, **4–7**, **9**, **18**, and **21–24**). Only three compounds had significant inhibitory activity against OA-induced bronchospasm (**2**, **4**, and **5**). This may be due to the differences in the absorption, distribution, or metabolism of the compounds or the difference with exogenously applied LTD_4 vs. endogenously generated LTD_4.

Compounds **2** and **4** were chosen as biological leads and studied further in the Ames assay. Results obtained for compounds **1a** and **1b** are reported herein for the first time and are included to furnish some insight into their mechanism of action as mutagens. Compound **21** was examined in the Ames assay because it is a potent LTD_4 antagonist that could not be readily metabolized to a hydroxamic acid, and compound **24** was examined because it is a metabolite of **1b** that may have been responsible for the observed mutagenic activity.

The assay used is essentially the one Ames recommends for general mutagenicity testing.[3] Three standard tester strains of *Salmonella typhunium* bacteria, TA1535, TA1537, and TA1538, are used in combination with two R factor *S. typhunium* strains, TA100 and TA98. Experimental compounds were examined in the absence and presence of S-9 rat liver homogenates with elevated microsomal enzyme activity.

Quinolines **1a**, **1b**, **21**, and **24** demonstrated reactivity in strain TA1537 (Table III). Quinoline **24** gave a variable response, usually one per dose of a three-plate assay at higher doses. Mutagenic activity in tester strain TA1537 suggests that these compounds cause frameshift mutations and implies that these compounds are acting as intercalating agents and not as electrophilic agents.

Benzthiazole **2** and benzimidazole **4** were inactive in all of the tester strains both in the absence and presence of microsomal activation at dose ranges of 0.005–0.5 mg and 0.05–5.0 mg per plate, respectively.[11]

We have described the synthesis and biological activity of novel benzoheterocyclic[(methoxyphenyl)amino]oxoalkanoic acid esters as inhibitors of rat PMN 5-LO and as inhibitors of LTD_4- and OA-induced bronchospasm in the GP. Our initial goals of retaining the in vivo LTD_4 antagonist activity of **1** while eliminating mutagenic activity were successful in compounds **2** and **4**. Since the combination of activity constitutes a novel therapeutic approach with potential for the treatment of asthma and related allergies, further investigation with these compounds is warranted.

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 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.

4-[[3-(2-Benzthiazolylmethoxy)phenyl]amino]-4-oxobutanoic Acid Methyl Ester (2). (A) 2-(Chloromethyl)benzthiazole. To a solution of 2-aminothiophenol (7.5 g, 60 mmol) in methylene chloride at 0 °C was added methyl chloroacetimidate hydrochloride[7] (8.6 g, 60 mmol). The reaction mixture was allowed to warm to room temperature while being stirred overnight. The mixture was washed with water (3×), dried ($MgSO_4$), and concentrated to an oil. The oil was distilled [120–135 °C (0.5 mmHg)] to give 7.8 g (71% yield) of product.

(B) 3-(2-Benzthiazolylmethoxy)nitrobenzene. A mixture of 2-(chloromethyl)benzthiazole (17.6 g, 95 mmol), 3-nitrophenol (13.2 g, 95 mmol), cesium carbonate (30 g), sodium carbonate (10 g), potassium iodide (0.5 g), and acetone (600 ml) was heated at reflux overnight. The mixture was filtered and the resulting solution was partially concentrated. A crystalline solid formed, which was filtered and dried, giving 22.8 g (84% yield) of product, mp 157–158 °C.

(C) 3-(2-Benzthiazolylmethoxy)aniline. To a suspension of 3-(2-benzthiazolylmethoxy)nitrobenzene (18.6 g, 65 mmol) in ethanolic hydrochloric acid was added powdered iron. The reaction was stirred overnight at room temperature. After neutralization with saturated aqueous sodium bicarbonate, the mixture was extracted with methylene chloride (3×). The extract was dried ($MgSO_4$) and concentrated to give 15.1 g (90% yield) of product, mp 117–120 °C.

(D) Compound 2. To a solution of 3-(2-benzthiazolylmethoxy)aniline (14.1 g, 55 mmol) and triethylamine (5.6 g) in tetrahydrofuran (250 mL) at room temperature was slowly added a solution of 3-carbomethoxypropionyl chloride (8.3 g, 55 mmol) in tetrahydrofuran. The reaction mixture was stirred for 1 h. The mixture was filtered through a pad of Celite and silica gel, and the solvent was removed in vacuo, giving a solid. The solid was recrystallized from ethyl acetate, giving 17 g (83% yield) of product, mp 129–130 °C.

In a like manner as above with the appropriate combination of 2-aminothiophenol, 2-aminophenol, or *N*-methyl-2-phenylenediamine and 3-carbomethoxypropionyl chloride or 2-carbomethoxyacetyl chloride, compounds **3–6** were prepared. (See Table I for yield and melting point.)

Also as above with 1-acetanilinyl chloride (prepared from aniline and chloroacetyl chloride) and 2-(chloromethyl)benzthiazole, compound **13** was prepared.

Compound **14** was prepared as above except with use of 2-(chloromethyl)pyridine.

4-[[3-(2-Benzthiazolylmethoxy)phenyl]hydroxyamino]-4-oxobutanoic Acid Methyl Ester (7). (A) [3-(2-Benzthiazolylmethoxy)phenyl]hydroxylamine. To a solution of 3-(2-benzthiazolylmethoxy)nitrobenzene (1.6 g, 5.6 mmol) (see Experimental Section for compound **2**, part B) in tetrahydrofuran with 10% palladium on carbon was slowly added hydrazine hydrate (0.4 mL). The reaction mixture was stirred overnight at room temperature. The mixture was filtered through Celite and the solvent was removed in vacuo to give 1.3 g (87% yield) of product, mp 130–134 °C.

(B) Compound 7. Compound **7** was prepared by the method for compound **2**, part D, with [3-(2-benzthiazolylmethoxy)-phenyl]hydroxylamine. A white solid was obtained, mp 123–125 °C.

4-[*N*-Methyl[3-(2-benzthiazolylmethoxy)phenyl]-amino]-4-oxobutanoic Acid Methyl Ester (8). (A) *N*-Methyl-3-(2-benzthiazolylmethoxy)aniline. A mixture of 3-(2-benzthiazolylmethoxy)aniline (3.8 g, 14.88 mmol), prepared as in the Experimental Section for compound **2**, part C, triethyl orthoformate (25 mL), and 5–10 drops of trifluoroacetic acid was heated at reflux for 6 h. The mixture was concentrated, and the residual oil was dissolved in ethanol and treated with sodium borohydride (1.5 g) at 0 °C. The reaction mixture was allowed to warm to room temperature while being stirred overnight. The mixture was then refluxed for 3 h and concentrated. The remaining oil was dissolved in methylene chloride and washed with water, dried ($MgSO_4$), and concentrated. The remaining oil was crystallized from hexanes, giving 1.8 g (45% yield) of product, mp 93–95 °C.

(B) Compound 8. Compound **8** was prepared by the method of compound **2**, part D, but with *N*-methyl-3-(2-benzthiazolylmethoxy)aniline. A white solid was obtained, mp 107–110 °C.

4-[[3-(2-Benzthiazolylmethoxy)phenyl]amino]-4-oxobutanoic Acid (9). Compound **2** (2.2 g, 5.9 mmol) was dissolved in THF (30 mL) and treated with aqueous potassium hydroxide (1.0 g in 30 mL of H_2O). The reaction mixture was stirred overnight. The THF was removed in vacuo and 1 N HCl was added to give a pH of 2. A white precipitate formed, which was filtered and dried to give 2.1 g (99% yield) of product, mp 191–193 °C.

In the same manner with compound **4** as starting material, compound **10** was prepared, mp 213–214 °C.

4-[[3-(2-Benzthiazolyloxy)phenyl]amino]-4-oxobutanoic Acid Methyl Ester (11). (A) 3-(2-Benzthiazolyloxy)nitrobenzene. A mixture of 2-chlorobenzthiazole (17.0 g, 0.1 mol) and 3-nitrophenol (13.9 g, 0.1 mol) was heated at 140 °C for 6 h. After cooling, the remaining oil was crystallized from diisopropyl ether, giving 16.8 g (62% yield) of product, mp 86–88 °C.

(B) Compound 11. Compound **11** was prepared by the method of compound **2**, parts C and D, but with 3-(2-benzthiazolyloxy)nitrobenzene. A white solid was obtained, mp 78–82 °C.

4-[[3-(2-Benzofuranylmethoxy)phenyl]amino]-4-oxobutanoic Acid Methyl Ester (12). (A) 2-(Bromomethyl)benzofuran. A mixture of 2-methylbenzofuran (2.0 g, 15.1 mmol), *N*-bromosuccinimide (2.7 g, 15.1 mmol), and carbon tetrachloride was heated at reflux overnight. The reaction mixture was filtered and the solvent was removed in vacuo, giving 3.8 g (52% monobrominated) of product (62% yield).

(11) Benzthiazole **2** initially appeared to be active in tester strain TA1537. However, upon retesting in a liquid suspension assay, which permits treatment of the bacteria followed by removal of the test article during the incubation period, this compound was also found to be inactive.

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(B) Compound 12. Compound **12** was prepared by the method for compound **2**, parts B–D, but with 2-(bromomethyl)-benzofuran. A white solid was obtained, mp 112–113 °C.

***N*-[3-(2-Benzthiazolylmethoxy)phenyl]-2-oxopyrrolidine-4-carboxylic Acid Methyl Ester (18). (A) *N*-(3-Hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic Acid.** A mixture of 3-aminophenol (10.9 g, 0.1 mol) and itaconic acid (13.0 g, 0.1 mol) was heated to 120–130 °C for 5 min. After cooling, a solid formed, giving 21.5 g (97% yield) of product, mp 214–215 °C.

(B) *N*-(3-Hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic Acid Methyl Ester. A solution of *N*-(3-hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic acid (21.0 g, 94.9 mmol) in methanol with *p*-toluenesulfonic acid (0.1 g) was heated to reflux. The reaction mixture was refluxed for 4 days while water was removed with a Soxhlet extractor filled with 3A molecular sieves. The mixture was cooled and a solid formed, which was filtered and dried, giving 9.5 g (42% yield) of product, mp 178–180 °C.

(C) Compound 18. A mixture of 2-(chloromethyl)benzthiazole (see compound **2**, part A) (3.12 g, 17 mmol), *N*-(3-hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic acid methyl ester (4.0 g, 17 mmol), cesium carbonate (5.3 g, 17 mmol), sodium carbonate (1.8 g), potassium iodide (0.1 g), and acetone (200 mL) was heated at reflux overnight. The mixture was filtered and the resulting solution concentrated to an oil. The oil was triturated with ether, forming a solid, which was filtered and dried to give 4.0 g (62% yield), mp 99–102 °C.

Compounds **19** and **21** were prepared by following the procedures used in the preparation of compound **18** and employing 1-methyl-2-(chloromethyl)benzimidazole, 2-(chloromethyl)benzoxazole and 2-(chloromethyl)quinoline.

***N*-[3-(2-Benzthiazolylmethoxy)phenyl]pyrrolidine-2,5-dione (22). (A) 4-[(3-Hydroxyphenyl)amino]-4-oxobutanoic Acid Methyl Ester.** To an ice-cold solution of 3-aminophenol (21.8 g, 0.2 mol) and triethylamine (21.3 g, 0.2 mol) in THF (250 ml) was added a solution of 3-carbomethoxypropionyl chloride (30.1 g, 0.2 mol) in THF. The reaction mixture was allowed to warm to room temperature and was filtered through a pad of Celite and silica gel. The solvent was removed in vacuo to give a solid. Recrystallization from ethyl acetate gave 39.9 g (88% yield) of product, mp 144–146 °C.

(B) Compound 22. A mixture of 4-[(3-hydroxyphenyl)-amino]-4-oxobutanoic acid methyl ester (1.45 g, 6.5 mmol), 2-(chloromethyl)benzthiazole (1.20 g, 6.5 mmol) (see Experimental Section for compound **2**, part A), cesium carbonate (1.0 g), sodium carbonate (0.7 g), potassium iodide (5 mg), and acetone (60 ml) was heated at reflux for 2 h. The reaction mixture was filtered through a pad of Celite and silica gel, and the solvent was removed in vacuo. Recrystallization from acetone gave 1.34 g (56% yield) of product, mp 175–176 °C.

Compounds **23** and **24** were prepared by following the procedure used in the preparation of compound **22** and employing *N*-methyl-2-(chloromethyl)benzimidazole and 2-(chloromethyl)-quinoline.

Biological Test Procedures. Experimental detail for the rat PMN 5-LO and the GP LTD_4- and OA-induced bronchospasm model are provided in ref 1.

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Chemical Differentiating Agents. Differentiation of HL-60 Cells by Hexamethylenebis[acetamide] Analogues

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Hexamethylenebis[acetamide] (HMBA) is an agent in clinical trial that induces differentiation of certain types of tumor cells to nonmalignant phenotypes. In an attempt to discover a more potent compound, a number of bis-functionalized amides, imides, and hydrazine derivatives of HMBA were prepared and evaluated in vitro with the HL-60 human promyelocytic leukemia cell line. Among the compounds evaluated, the 5,5-dimethylhydantoin derivative is almost 10 times more potent than HMBA in inducing differentiation. The bis-imide, diacetyl-HMBA, is both more potent and effective than its parent compound. Six of the 16 compounds evaluated cause at least 20% differentiation. An inverse relationship between the degree of differentiation and the percentage of viable cells is described for HMBA and its analogues.

Compounds that induce cancer cells to differentiate to a less malignant phenotype provide an attractive area for the development of new anticancer drugs. Reduced toxicity relative to conventional chemotherapeutic agents is a distinct possibility since the mechanism of antitumor action is not based primarily on cytotoxicity.

The number of compounds that influence cell differentiation and growth characteristics continues to increase. These materials, which include simple organic molecules as well as proteins,[1-3] are thought to influence gene expression. An important aid in the ability to search for agents that induce terminal differentiation in malignant cells occurred when it was discovered that a virus-induced murine erythroleukemia cell line (MELC), when treated with Me_2SO, expressed many of the features common to terminally differentiated erythroid cells.[4,5] The development of the human HL-60 myeloid leukemia cell line in 1977 provided another important in vitro differentiation system.[6-8] While there are a number of cell lines now

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