(Phenylmethoxy)phenyl Derivatives of Ω-Oxo- and Ω-Tetrazolylalkanoic Acids and Related Tetrazoles. Synthesis and Evaluation as Leukotriene D_4 Receptor Antagonists

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Two series of (phenylmethoxy)phenyl compounds derived from the structure of LY163443 were synthesized and evaluated as leukotriene D_4 receptor antagonists. In the Ω-[(phenylmethoxy)phenyl]-Ω-oxoalkanoic acid series, 5-[4-[(4-acetyl-2-ethyl-3-hydroxyphenyl)methoxy]phenyl]-3,3-dimethyl-5-oxopentanoic acid (**8**) was the most potent antagonist of LTD_4-induced contractions of guinea pig ileum (pK_B of 7.60) and LTD_4 pressor response in pithed rats (ED_{50} of 1.4 mg/kg iv). Replacing the carboxylic acid function with 5-tetrazole gave slightly more potent compounds. In the Ω-[5-[[(phenylmethoxy)phenyl]alkyl]tetrazolyl]alkanoic acid series, replacing the carboxylic acid with 5-tetrazole gave compounds that were equally effective in the guinea pig ileum but more potent in vivo against the LTD_4 pressor response in rat. The pK_B value in the guinea pig ileum for 1-[2-hydroxy-3-propyl-4-[[4-[[2-[3-(1*H*-tetrazol-5-yl)propyl]-2*H*-tetrazol-5-yl]methyl]phenoxy]methyl]phenyl]ethanone (**25**) was 7.87 and the ED_{50} for antagonism of the LTD_4 pressor response was 4.0 mg/kg iv. The sodium salts of **8** (**9**) and **25** (**26**) given by the iv route of administration antagonized LTD_4-induced cardiovascular alterations in anesthetized rat and LTD_4-induced bronchoconstriction in guinea pig in a dose-dependent manner. Oral activity was also demonstrated against the LTD_4-induced bronchoconstriction in guinea pig.

Introduction

Extensive biological studies of cysteinyl leukotrienes C_4, D_4, and E_4 (LTC_4, LTD_4, and LTE_4) suggest that these products of arachidonic acid metabolism are important mediators of asthma.[1] Several laboratories are investigating the effectiveness of a variety of structural types of LTD_4/LTE_4 antagonists in treating this disease.[2] Leukotrienes also have been shown to alter cardiovascular function when administered to animals, particularly the rat.[3] The general profile of cardiovascular alterations is similar to that seen with endotoxins,[4] suggesting that the leukotrienes might be important mediators of septic shock.[5] Our group has developed leukotriene receptor antagonists containing the acetophenone moiety found in FPL 55712. LY171883[6] and LY163443[7] are representive examples of this structural type (Figure 1). The present report[8] describes modification of the structure of LY163443 and some studies with resultant compounds assessing their ability to block the effects of LTD_4 in pulmonary and cardiovascular systems.

As previously described,[9] the acetophenone portion of FPL 55712 can be connected to acidic functions with a variety of atoms. A particularly effective connecting function discovered in this laboratory and used in LY163443 was methoxyphenyl.[10] We have extended our investigation of compounds containing a (phenylmethoxy)phenyl group and have examined different ways of modifying the 5-tetrazolylmethyl group of LY163443. This gave rise to two series of compounds, the Ω-[(phenylmethoxy)phenyl]-Ω-oxoalkanoic acids and corresponding tetrazoles and the Ω-[5-[[(phenylmethoxy)phenyl]alkyl]-tetrazolyl]alkanoic acids and corresponding tetrazoles (Figure 1).

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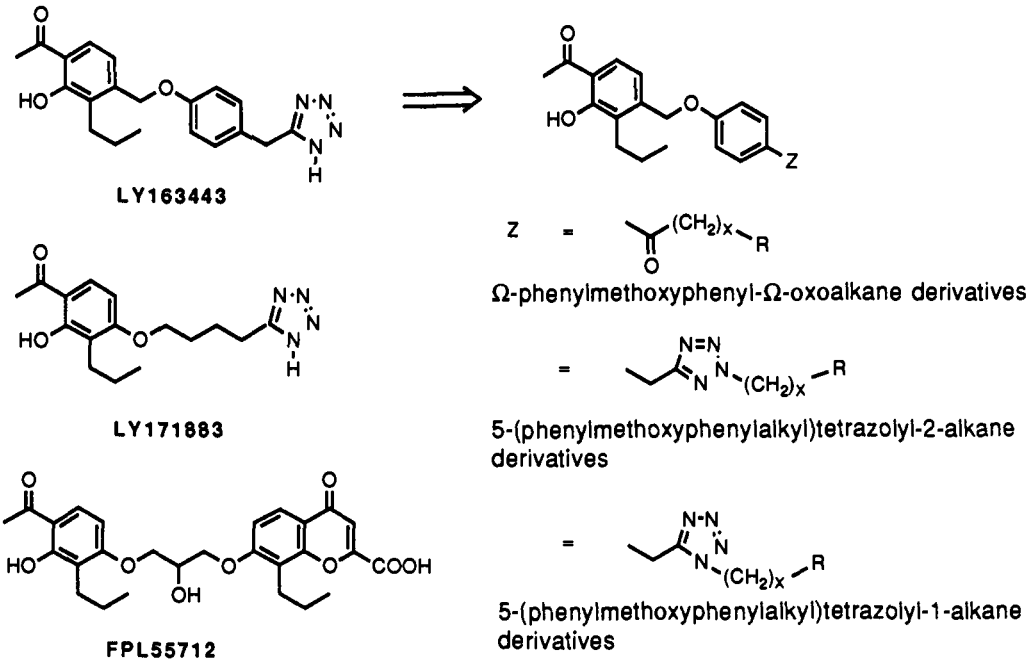
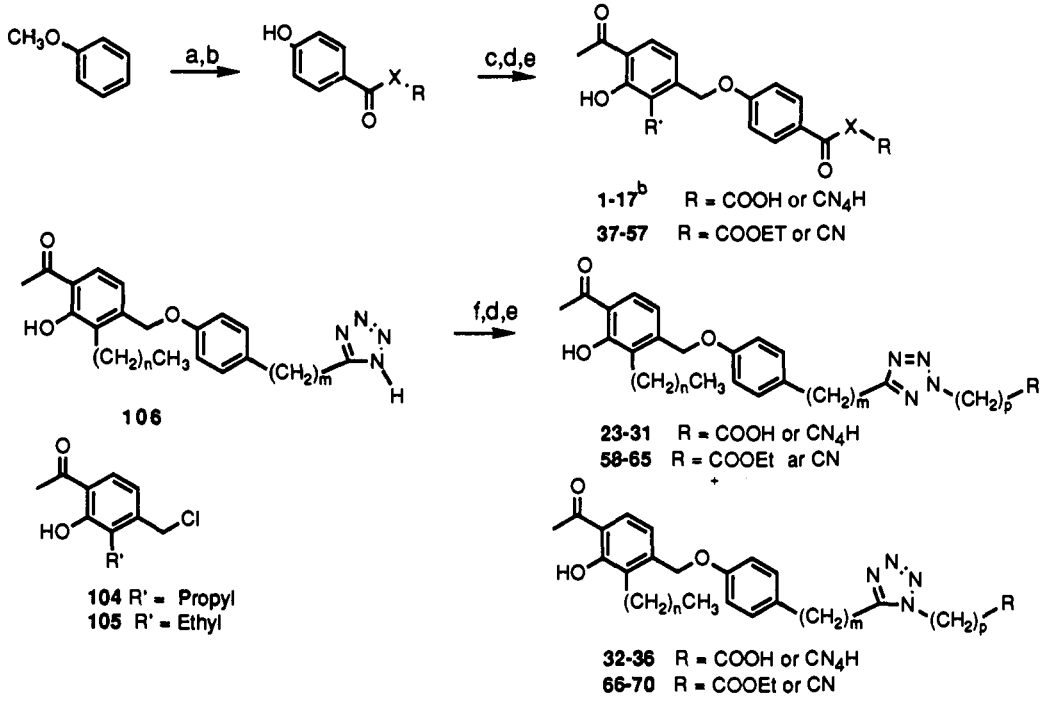


Figure 1. Selected leukotriene antagonists containing the acetophenone group and proposed structural modifications of the 5-tetrazolylmethyl moiety of LY163443.

Scheme I[a]



[a] (a) Carbocyclic anhydride or alkanoyl chloride, $AlCl_3$, dichloromethane; (b) $AlCl_3$, dichloromethane, heat or pyridine hydrochloride, heat; (c) **104** or **105**, ethanol, potassium *tert*-butoxide or sodium ethoxide; (d) NaOH, aqueous ethanol, reflux, R = COOH; (e) tributyltin azide, 1,2-dimethoxyethane, heat, R = 5-tetrazolyl; (f) bromoalkanenitrile or bromoalkanoic acid ester, potassium *tert*-butoxide or NaOEt, EtOH, R = CN or COOEt. [b] CN_4H = 5-tetrazolyl.

LTD_4-induced contraction of guinea pig ileum, a procedure previously described,[11] represented the initial biological evaluation of new compounds. Early studies in our laboratory indicated that compounds of this type equally inhibited LTD_4 and LTE_4 on the ileum, whereas none of them antagonized contractions to LTC_4. Compounds showing interesting activity in ileum were evaluated further by using lung tissue, e.g., trachea, parenchyma, and bronchus. In vivo evaluation of antagonist efficacy was done in the pithed rat by determining their ability to block the initial pressor effect produced by LTD_4 given intravenously. Selected compounds were studied further on cardiovascular parameters of the anesthetized rat and in the guinea pig, where the ability of the compounds to antagonize LTD_4-induced bronchoconstriction was measured.[9]

Chemistry

The general synthetic approach for making the Ω-[(phenylmethoxy)phenyl]-Ω-oxoalkanoic acids and tetra-

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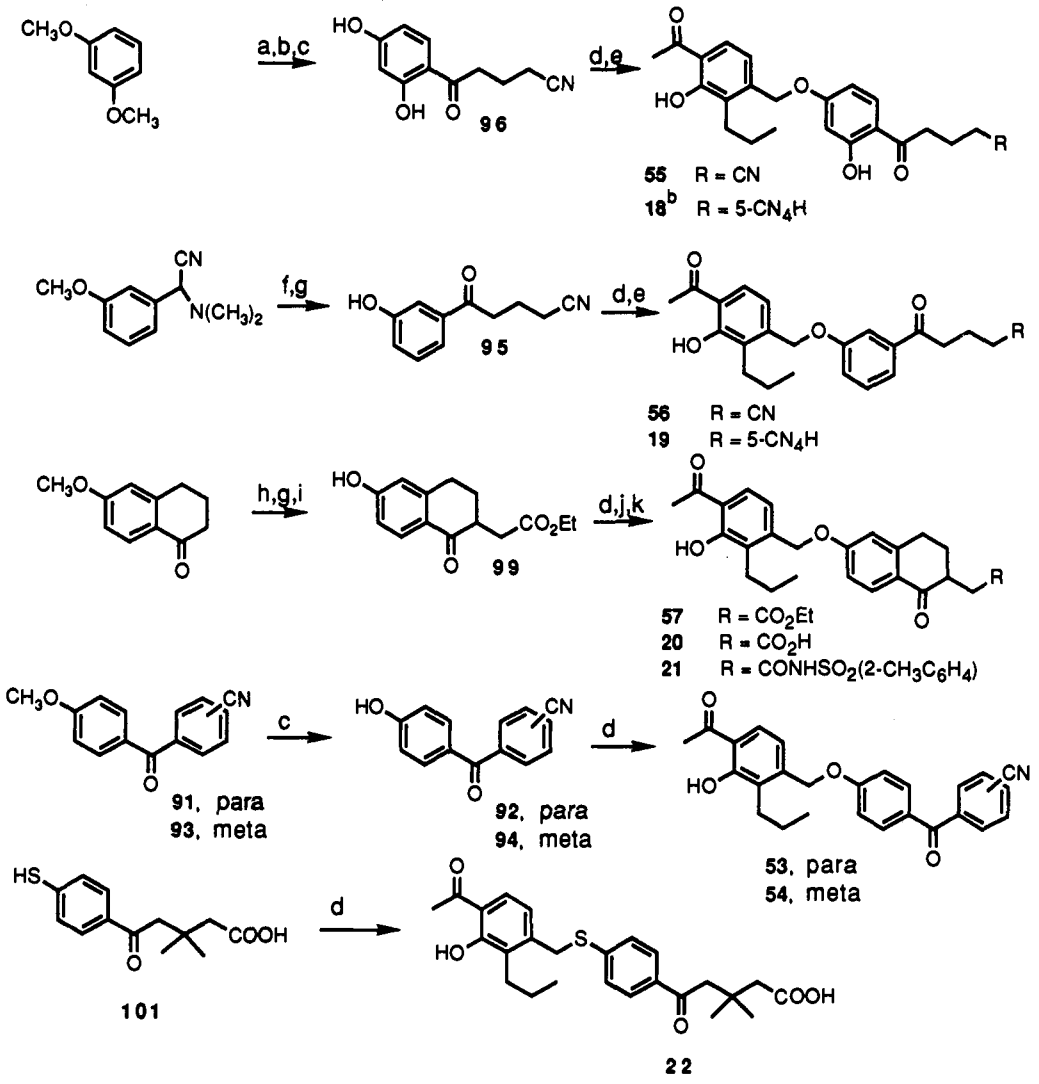
Scheme II[a]



[a] (a) Glutaric anhydride, $AlCl_3$, dichloromethane; (b) methanesulfonyl chloride, ammonia, pyridine; (c) $AlCl_3$, dichloromethane, heat; (d) **104**, potassium *tert*-butoxide, ethanol; (e) tributyltin azide, 1,2-dimethoxyethane, heat; (f) 4-bromobutanenitrile, lithium diisopropylamide, THF/HMPA; (g) pyridine hydrochloride, heat; (h) ethyl bromoacetate, lithium diisopropylamide; THF/HMPA; (i) methanesulfonic acid, ethanol; (j) NaOH, aqueous EtOH, heat, R = COOH; (k) *o*-toluenesulfonamide, DCC, 4-(dimethylamino)pyridine, dichloromethane, R = $CONHSO_2(CH_3C_6H_4)$. [b] 5-CN_4H = 5-tetrazolyl.

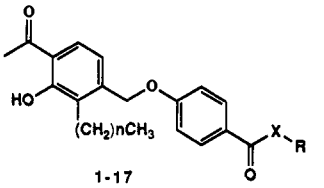
zoles **1–20** and **22** was to couple the appropriately substituted phenolic esters or nitriles with 1-[3-alkyl-4-(chloromethyl)-2-hydroxyphenyl]ethanone (**104**, alkyl = propyl, or **105**, alkyl = ethyl)[10] in ethanol, using sodium or potassium ethoxide as base (Scheme I). Subsequent basic hydrolysis converted the esters to carboxylic acids. The keto acid derivative where the two phenyl rings are connected with a methylenethio (**22**) was obtained by using the same sequence of reactions starting with the thiophenol **101**. Treatment with tributyltin azide in refluxing 1,2-dimethoxyethane converted the nitriles to tetrazoles. When the *gem*-dimethyl group was present β to the nitrile, the higher boiling 1,2-diethoxyethane was preferred. The sulfonamide **21** was prepared by condensation of *o*-toluenesulfonamide and the carboxylic acid **20** with DDC and 4-(dimethylamino)pyridine in dichloromethane. The β-oxopropanenitrile **37** was obtained by condensation of acetonitrile with the ethyl benzoate derivative **103** using sodium amide as base in liquid ammonia.

The intermediate phenolic nitriles and esters (Table V) were generally prepared by aluminum chloride catalyzed acylation of anisole with the appropriate anhydride or acid chloride followed by O-demethylation with aluminum chloride or heating in pyridine hydrochloride. Treatment of the halides **39** and **50**, prepared by aluminum chloride catalyzed acylation of phenoxy derivative **102**, with potassium cyanide in DMF at 60 °C gave the corresponding oxoalkanenitriles **40** and **51**.

The lithium salt of α-(3-methoxyphenyl)-α-(dimethylamino)acetonitrile was alkylated with 4-bromobutanenitrile. This product was O-demethylated and the dimethylamino nitrile function converted to ketone by heating at 185 °C in pyridine hydrochloride to give the meta-substituted phenolic intermediate **95**. Coupling and conversion to the tetrazole afforded the keto tetrazole **19** (Scheme II).

The sodium salt of LY163443 (**106**)[10] and a bromoalkanenitrile or bromoalkanoic acid ester in refluxing THF or DMF at room temperature gave a mixture of tetrazole 1- and 2-substituted products that were easily separated by silica chromatography. The isomeric tetrazole–alkanes **58–65** (Table IV) were identified by comparing chemical shifts in the proton NMR spectra of the N-substituted methylene hydrogens α to the tetrazole ring. Chemical shifts determined in deuteriochloroform for the 2-alkane derivatives were at approximately 0.4–0.5 ppm higher field

Table I. Chemical and Biological Data of Ω-[(Phenylmethoxy)phenyl]-Ω-oxoalkanoic Acids and Corresponding Tetrazoles

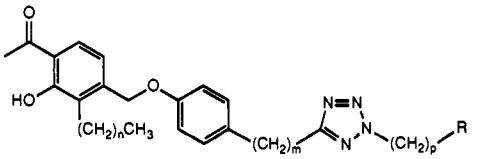


1-17

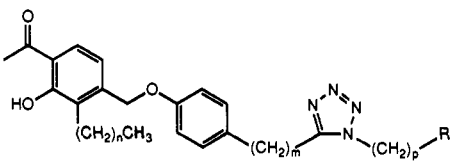
compd	n	X	R	synth[a] method	purifctn[b] method	yield, %	mp, °C	formula[c]	guinea pig[d] ileum pK_B values	LTD_4 pressor[e] response in rats: ED_{50} iv
1	2	CH_2	CN_4H[f]	A	A	50	207–209	$C_{21}H_{22}N_4O_4$	7.71 ± 0.13	24.1 (13.3–43.3)
2	2	CH_2CH_2	CO_2H	B	B	68	175–176	$C_{22}H_{24}O_6$	6.75 ± 0.06	18.2 (8.7–38.1)
3	2	CH_2CH_2	CN_4H	A	A	83	204–206	$C_{22}H_{24}N_4O_4$	7.09 ± 0.13	10.1 (8.0–12.9)
4	2	$CHCH_3CH_2$	CN_4H	A	B	41	140–142	$C_{23}H_{26}N_4O_4$	7.18 ± 0.10	9.2 (4.2–20.0)
5	2	$C(CH_3)_2CH_2$	CN_4H	A	A	31	44–50	$C_{24}H_{28}N_4O_4$	6.93 ± 0.05	10.8 (6.8–17.5)
6	2	$CH_2CH_2CH_2$	CO_2H	B	B	62	127–128	$C_{24}H_{28}O_6$	6.81 ± 0.11	9.5 (5.6–16.3)
7	2	$CH_2CH_2CH_2$	CN_4H	A	C	56	178–180	$C_{23}H_{26}N_4O_4$	7.67 ± 0.02	7.2 (1.4–37.4)
8	1	$CH_2C(CH_3)_2CH_2$	CO_2H	B	C	79	127–128	$C_{24}H_{28}O_6$	7.60 ± 0.11	1.4 (0.8–2.4)
9	1	$CH_2C(CH_3)_2CH_2$	CO_2Na	C	D	94	148–153	$C_{24}H_{27}O_6Na$	7.46 ± 0.06	
10	1	$CH_2C(CH_3)_2CH_2$	CN_4H	A	A	78	123–125	$C_{24}H_{28}N_4O_4$	8.09 ± 0.09	1.1 (0.5–2.3)
11	2	$CH_2C(CH_3)_2CH_2$	CO_2H	B	E	85	103–105	$C_{25}H_{30}O_6$	7.73 ± 0.08	2.9 (2.1–3.8)
12	2	$CH_2C(CH_3)_2CH_2$	CN_4H	A	A	35	151–153	$C_{25}H_{30}N_4O_4$	7.86 ± 0.08	1.3 (0.8–2.1)
13	2	$(CH_2)_4$	CO_2H	B	B	56	118–119	$C_{24}H_{28}O_6$	7.28 ± 0.09	9.1 (6.2–13.2)
14	2	$(CH_2)_4$	CN_4H	A	A	51	163–166	$C_{24}H_{28}N_4O_4$	7.28 ± 0.07	2.4 (1.7–3.3)
15	2	$(CH_2)_6$	CN_4H	A	C	23	134–137	$C_{26}H_{32}N_4O_4$	7.33 ± 0.09	8.5 (3.8–18.9)
16	2	1,4-phenylene	CN_4H	A	F	54	183–185	$C_{26}H_{24}N_4O_4$	7.66 ± 0.11	*g*
17	2	1,3-phenylene	CN_4H	A	G	55	208–214	$C_{26}H_{24}N_4O_4$	7.56 ± 0.14	*g*
18[h]				A	A	32	170–174	$C_{23}H_{26}N_4O_5$	7.73 ± 0.16	4.4 (3.4–5.7)
19[h]				A	A	57	140–145	$C_{23}H_{26}N_4O_4$	7.50 ± 0.08	15.5 (10.1–23.6)
20[h]				B	E	44	147–149	$C_{24}H_{28}O_6$	7.71 ± 0.11	*g*
21[h]				D	H	79	90–95	$C_{31}H_{33}NO_7S$	7.93 ± 0.04	*g*
22[h]				E	I	27	106–108	$C_{25}H_{30}O_5S$	7.55 ± 0.10	4.6 (3.4–6.1)

[a] See Experimental Section. [b] Solvent of recrystallization or chromatography methods: A, EtOAc/hexane; B, EtOH; C, EtOH/H_2O; D, crude; E, toluene; F, MeOH; G, silica HPLC (9:1 CH_2Cl_2/MeOH), crystallized from EtOAc/hexane; H, R-18 HPLC (9:1 CH_2Cl_2/MeOH), crystallized from MeOH; I, silica HPLC (9:1 toluene/EtOAc). [c] Analyses were made for C, H, and N, where applicable, and were within ±0.4% of their theoretical values. [d] Mean values ± SEM of 4–6 tissue determination. [e] Values in parentheses represent 95% confidence limits. [f] CN_4H = 5-tetrazolyl. [g] Not tested. [h] Structure in Scheme II.

Table II. Chemical and Biological Data of 5-[[(Phenylmethoxy)phenyl]alkyl]tetrazole-1- and -2-alkanoic Acids and Corresponding Tetrazoles



23-31



32-36

compd	n	m	p	R	synth[a] method	purifctn[b] method	yield, %	mp, °C	formula[c]	guinea pig[d] ileum pK_B values	LTD_4 pressor[e] response in rats: ET_{50} iv
23	2	0	3	CN_4H[f]	A	A	80	194–196	$C_{23}H_{26}N_8O_3$	7.55 ± 0.10	*g*
24	2	1	1	CN_4H	A	B	30	137–139	$C_{22}H_{24}N_8O_3$	7.66 ± 0.13	12.2 (8.7–17.1)
25	2	1	3	CN_4H	A	A	72	118–121	$C_{24}H_{28}N_8O_3$	7.87 ± 0.05	4.0 (2.6–5.9)
26	2	1	3	CN_4Na	C	C	5	90–95	$C_{24}H_{27}N_8O_3Na$		
27	2	1	3	CO_2H	B	D	74	92–94	$C_{24}H_{28}N_4O_5$	7.64–0.04	19.6 (14.0–27.4)
28	2	1	4	CN_4H	A	B	64	119–123	$C_{25}H_{30}N_8O_3$	7.67 ± 0.20	4.0 (2.1–7.6)
29	2	1	4	CO_2H	B	E	63	40–43	$C_{25}H_{30}N_4O_5$	7.79 ± 0.27	7.0 (4.2–11.6)
30	2	1	6	CN_4H	A	A	38	110–113	$C_{27}H_{34}N_8O_3$	7.56 ± 0.15	7.0 (4.9–9.9)
31	1	2	3	CN_4H	A	E	44	155–156	$C_{24}H_{28}N_8O_3$	7.96 ± 0.14	*g*
32	2	1	1	CN_4H	B	B	77	148–150	$C_{22}H_{24}N_8O_3$	7.72 ± 0.05	*g*
33	2	1	3	CN_4H	A	E	55	121–123	$C_{24}H_{28}N_8O_3$	7.64 ± 0.06	*g*
34	2	1	3	CO_2H	B	E	53	106–108	$C_{24}H_{28}N_4O_5$	7.07 ± 0.06	*g*
35	2	1	4	CN_4H	A	B	49	96–98	$C_{25}H_{30}N_8O_3$	7.54 ± 0.08	*g*
36	2	1	4	CO_2H	A	E	72	oil	$C_{25}H_{30}N_4O_5$	7.09 ± 0.11	*g*

[a] See *a*, Table I. [b] Solvent of recrystallization or chromatography methods: A, EtOH/H_2O; B EtOAc/hexane; C, crude; D, EtOH; E, silica HPLC (19:1 CH_2Cl_2/MeOH). [c–g] See *c–g*, Table I.

than their corresponding 1-alkane derivatives. The above esters were hydrolyzed with sodium hydroxide or the nitriles treated with tributyltin azide to obtain the 5-[[(phenylmethoxy)phenyl]alkyl]tetrazolyl 1- and 2-alkanoic acids and corresponding tetrazoles **23–36** listed in Table II.

Biological Results

Test compounds were first evaluated in vitro for their ability to antagonize LTD_4-induced contractions of guinea

pig ileum by methods previously described.[7,9,10,11] The pK_B values are reported in Tables I and II. Considering first the Ω-oxoalkane derivatives, the tetrazoles were slightly more potent antagonists than the corresponding carboxylic acids: compare **2** with **3**, **6** with **7**, and **11** with **12**. The optimum length of the alkylene group connecting the tetrazole with the ketone function was three carbon atoms and adding a *gem*-dimethyl to the alkylene did not increase potency (**7** and **12**, pK_Bs of 7.67 and 7.86, respectively). For the carboxylic acids, there was an increase in activity by adding the *gem*-dimethyl (**6** and **11**, pK_Bs of 6.81 and 7.73, respectively). Additional modifications such as a hydroxy group on the second phenyl ring (**18**), having the oxoalkane group meta to the connecting oxygen atom on the second phenyl ring (**19**), or a bicyclic tetrahydronaphthalenone derivative (**20**) produced equally potent antagonists. Substituting the connecting oxygen atom with sulfur (**22**) or converting a carboxy group to an acyl sulfonamide (**21**) retained good activity. In the substituted-tetrazole series (Table II), compounds terminally substituted with tetrazole or carboxylic acid were equally active when the internal tetrazole ring was substituted in the 2 position (pK_Bs in the range of 7.55–7.87). However, when this tetrazole was substituted in the 1 position, the tetrazoles were more active than the carboxylic acids. Compare **33** (7.64) with **34** (7.09) and **35** (7.54) with **36** (7.04).

The ability of test compounds to block the pressor response to iv LTD_4 in pithed rat was then investigated (see Tables I and II). Considering first the Ω-oxoalkane series, the alkylene chain connecting the ketone function with the acidic group could be varied with retention of good antagonism. Optimal activity was obtained when this group was trimethylene with disubstitution on the β carbon as in **8**, **10**, **11**, and **12**, with ED_{50}s of 1.4, 1.1, 2.9, and 1.3 mg/kg iv, respectively. Comparable antagonist activity was obtained when the carboxylic acid function was replaced with 5-tetrazole. Meta substitution of the second phenyl ring decreased antagonist efficacy: the ED_{50} of **19** with 15.5 mg/kg iv, as compared to 7.2 mg/kg iv for the para-substituted compound **7**.

In the substituted tetrazole series (Table II), the compounds substituted in the 2 position of the internal tetrazole ring were effective antagonists. Connecting a terminal tetrazole with a trimethylene or tetramethylene group to the internal tetrazole ring gave the most active derivatives, **25** and **28**, with equal ED_{50}s of 4.0 mg/kg iv each.

Two compounds were selected for further pharmacological evaluation: the carboxylic acid **8** from the Ω-oxoalkane series and the tetrazole **25** from the substituted-tetrazole series. Their sodium salts were prepared to give **9** and **26**, respectively (see structures in Table VI), to facilitate testing.

The abilities of these two compounds to antagonize the LTC_4-, LTD_4-, or LTE_4-induced contractions of several guinea pig smooth muscles were determined and reported as pK_B values in Table VI. Against LTE_4, the tetrazole **26** was more potent than the carboxylic acid **9** in all tissues tested, and both were most potent in ileum (pK_Bs of 7.73 and 8.37). Against LTD_4, both were also most active in ileum; however, in this tissue and in the trachea, **9** and **26** were about equally active to each other (pK_Bs 7.46 vs 7.84 and 6.52 vs 6.69). The pK_B for LY163443 in the ileum was 8.02 ± 0.05 and in the trachea was 7.41 ± 0.05.[6] Only **26** gave minimal effects against LTC_4. Neither compound antagonized the effects of histamine, bradykinin, carbamylcholine, or the thromboxane mimic, U46619, illustrating selectivity of antagonism of leukotriene responses.

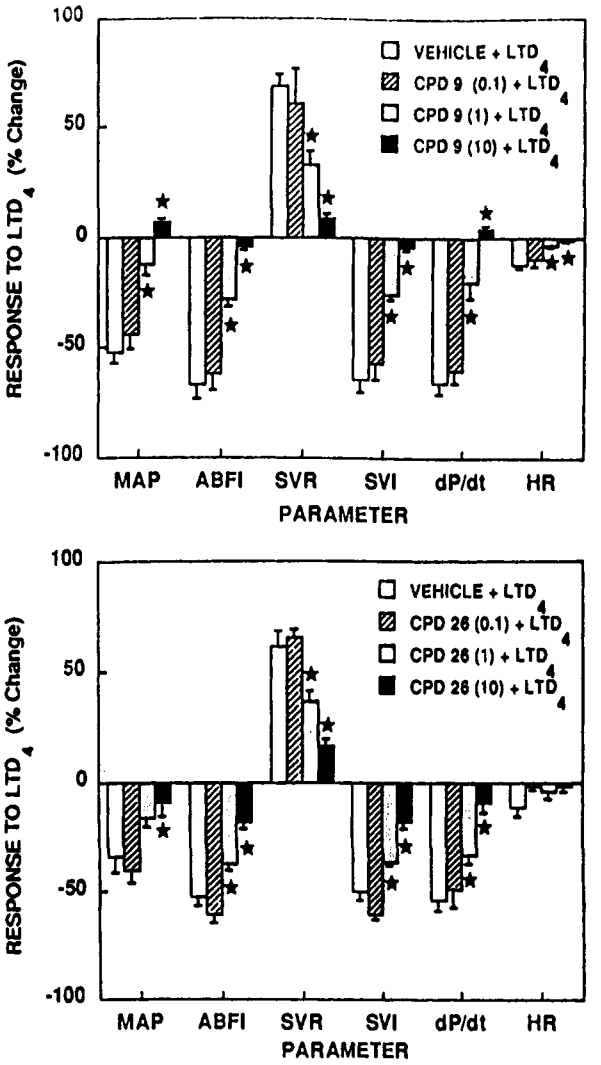


Figure 2. Inhibition of the LTD_4-induced (10 μg/kg, iv) cardiovascular alterations in anesthetized rat with compounds **9** and **26** (0.1–10.0 mg/kg, iv). MAP, mean arterial blood pressure; ABFI, aortic blood flow index; SVR, systemic vascular resistance; SVI, stroke volume index; dP/dt, myocardial contractility; HR, heart rate.

Hemodynamic alterations induced by iv LTD_4 were measured in the anesthetized rat and the antagonism of these effects by **9** or **26** was determined.[12] As shown in Figure 2, each compound antagonized decreases in mean arterial blood pressure, aortic blood flow index, stroke volume index, and myocardial contractility, and the increase in systemic vascular resistance induced by LTD_4 in a dose-dependent manner. The carboxylic acid **9** was slightly more effective, giving almost complete block at 10 mg/kg iv. Compounds **9** and **26** also antagonized the increase in hematocrit induced by LTD_4 in rat, as shown in Figure 3.

Modified Konsett–Rossler procedures[8,9] were used to determine the abilities of these two compounds to block the iv LTD_4-induced increases in total pulmonary impedance in guinea pig. As shown in Figures 4 and 5, 2 h post drug administration, **9** blocked the increases almost completely at 3 and 10 mg/kg orally and **26** at 10 mg/kg orally. Compound **9** also blocked ovalbumin-induced increases in pulmonary impedance in passively sensitized guinea pigs in a dose-dependent manner (Figure 6).

(12) Hahn, R. A.; MacDonald, B. R.; Martin, M. A. *J. Pharmacol. Exp. Ther.* **1983**, *224*, 206–214.

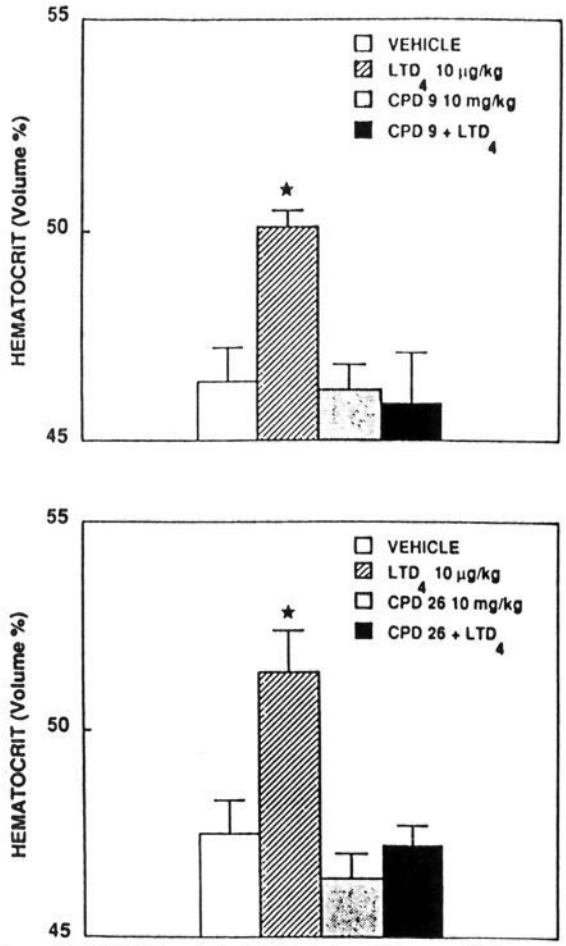


Figure 3. Inhibition of the LTD_4-induced increase in hematocrit in rat.

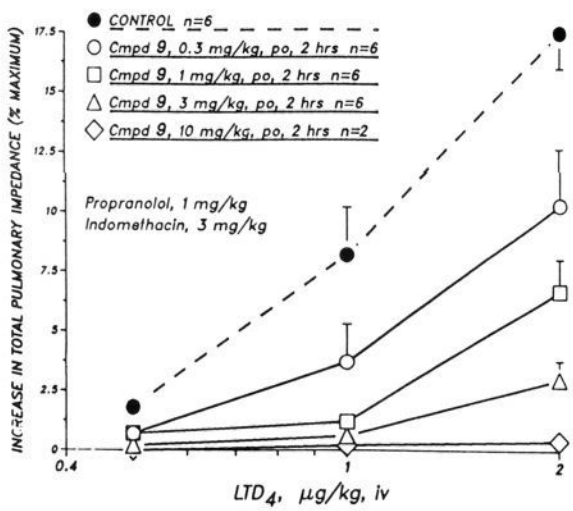


Figure 4. Inhibition of iv LTD_4-induced increases in total pulmonary impedance in guinea pig with compound **9**.

In summary, both the Ω-oxo- and the Ω-tetrazolyl-alkanoic acid structural modifications of the LY163443 gave potent compounds that antagonized LTD_4/LTE_4 receptors as measured in vitro on guinea pig ileum, trachea, and lung parenchymal strips. They also antagonized cardiovascular and pulmonary effects of LTD_4 in vivo either given by the iv or oral route of administration.

Experimental Section

Chemical Methods. Melting points were determined in open capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. NMR spectra were taken on GE QE-300 or Brucker WH-360 instruments. IR spectra were taken on a Nicole 10MX FT-IR instrument. Field-desorption mass spectra were determined on a Varian MAT-731 instrument. All compounds, unless otherwise indicated, had elemental analyses for carbon, hydrogen, and nitrogen where applicable within ±0.4% of theoretical values. Product yields were not optimized. The syntheses of 1-[4-(chloromethyl)-2-hydroxy-3-propylphenyl]ethanone (**104**), 1-[4-(chloromethyl)-3-ethyl-2-hydroxyphenyl]ethanone (**105**), 1-[2-hydroxy-3-propyl-4-[[4-(1*H*-tetrazol-5-ylmethyl)phenoxy]-methyl]phenyl]ethanone sodium salt (**106**), and 1-[2-hydroxy-3-propyl-4-[[4-(1*H*-tetrazol-5-yl)phenoxy]methyl]phenyl]ethanone (**107**) were previously described.[10]

Method A. 1-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)-methoxy]phenyl]-2-(1*H*-tetrazol-5-yl)ethanone (1). A solution of 2 g (0.005 mol) of **37** and 6.6 g (0.02 mol) of tributyltin azide in 20 mL of 1,2-dimethoxyethane was heated to maintain reflux for 96 h. It was allowed to cool to room temperature and poured into 75 mL of water containing 25 mL of concentrated HCl. The precipitate was filtered and crystallized from EtOAc/hexane to give 1.0 g of **1** (Table I).

For compounds **10**, **12**, and **14**, 1,2-diethoxyethane was used as the reaction solvent.

Method B. 4-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)-methoxy]phenyl]-4-oxobutanoic Acid (2). The ester **38** (19.7

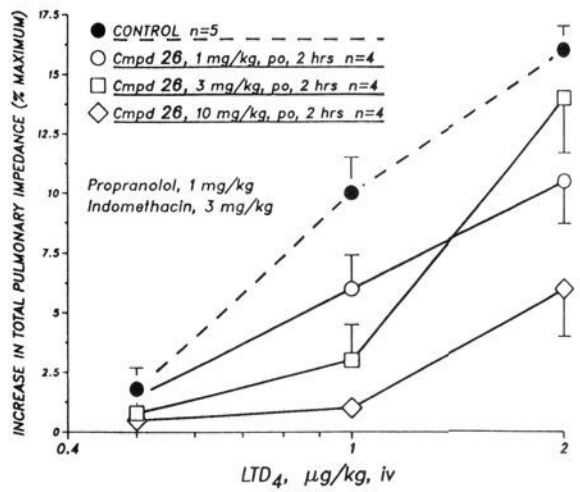


Figure 5. Inhibition of iv LTD_4-induced increases in total pulmonary impedance in guinea pig with compound **26**.

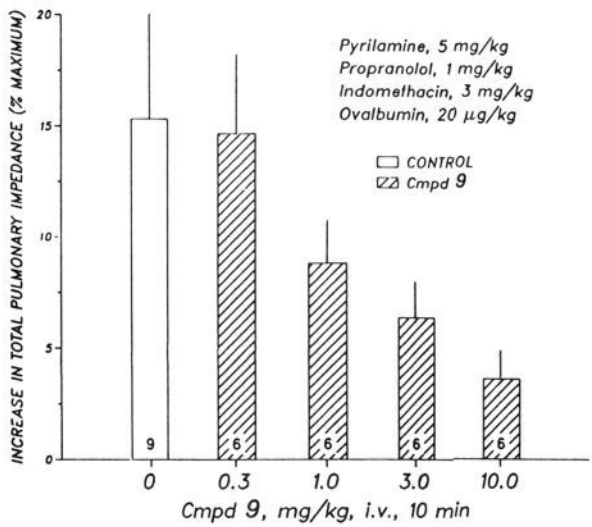
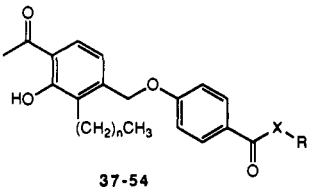


Figure 6. Inhibition of ovalbumin-induced increases in total pulmonary impedance in passively sensitized guinea pig with compound **9**.

Table III. Chemical Data of Ω-[(Phenylmethoxy)phenyl]-Ω-oxoalkanoic Acid Esters and Alkanenitriles



37-54

compd	n	X	R	synth[a] method	purifctn[b] method	yield, %	mp, °C	formula[c]
37	2	CH_2	CN	F	A	62	164–166	$C_{21}H_{21}NO_4$
38	2	CH_2CH_2	CO_2Et	G	A	59	134–136	$C_{24}H_{28}O_6$
39	2	CH_2CH_2	Cl	H	B	56	121–123	$C_{21}H_{23}ClO_4$
40	2	CH_2CH_2	CN	I	C	96	110–112	$C_{22}H_{23}NO_4$
41	2	$CHCH_3CH_2$	CN	J	D	76	82–84	$C_{23}H_{25}NO_4$
42	2	$C(CH_3)_2CH_2$	CN	J	A	68	83–86	$C_{24}H_{27}NO_4$
43	2	$CH_2CH_2CH_2$	CO_2Et	G	A	96	61–62	$C_{25}H_{30}O_6$
44	2	$CH_2CH_2CH_2$	CN	J	A	72	96–98	$C_{23}H_{25}NO_4$
45	1	$CH_2C(CH_3)_2CH_2$	CO_2Et	G	A	84	98–100	$C_{26}H_{32}O_6$
46	1	$CH_2C(CH_3)_2CH_2$	CN	J	A	65	95–97	$C_{25}H_{27}NO_4$
47	2	$CH_2C(CH_3)_2CH_2$	CO_2Et	G	A	78	oil	$C_{27}H_{34}O_6$
48	2	$CH_2C(CH_3)_2CH_2$	CN	J	C	73	92–94	$C_{25}H_{29}NO_4$
49	2	$(CH_2)_4$	CO_2Et	G	A	82	97–98	$C_{26}H_{32}O_6$
50	2	$(CH_2)_4$	Cl	H	D	77	96–98	$C_{23}H_{27}ClO_4$
51	2	$(CH_2)_4$	CN	I	D	59	100–102	$C_{24}H_{27}NO_4$
52	2	$(CH_2)_6$	CN	J	A	47	78–80	$C_{26}H_{31}NO_4$
53	2	1,4-phenylene	CN	J	E	44	142–144	$C_{28}H_{23}NO_4$
54	2	1,3-phenylene	CN	J	A	55	149–151	$C_{28}H_{23}NO_4$
55[d]				J	D	37	118–120	$C_{23}H_{25}NO_5$
56[d]				J	C	47	oil	$C_{23}H_{25}NO_4$
57[d]				J	D	76	oil	$C_{24}H_{30}O_6$

[a] See *a*, Table I. [b] Solvent of recrystallization or purification method: A, silica HPLC (19:1 toluene/EtOAc); B, toluene–hexane; C, crude product; D, silica HPLC (9:1 toluene/EtOAc); E, silica HPLC (49:1 methylene chloride/MeOH). [c] See *c*, Table I. [d] Structure in Scheme II.

g, 0.0478 mol) was heated with 30 mL of 5 N NaOH in 250 mL of EtOH for 2 h, diluted with water, and acidified with concentrated HCl. The resulting precipitate was collected and crystallized from EtOH to give **2** (Table I).

Method C. 5-[4-[(4-Acetyl-2-ethyl-3-hydroxyphenyl)methoxy]phenyl]-3,3-dimethyl-5-oxopentanoic Acid, Sodium Salt (9). A solution of 8.24 g (0.02 mol) of **8** in 200 mL of EtOH and 0.02 mol of 1 N NaOH was concentrated and then dried extensively at reduced pressure to give 8.2 g of **9** (Table I).

Method D. 6-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]-1,2,3,4-tetrahydro-*N*-[(2-methylphenyl)sulfonyl]-1-oxo-2-naphthaleneacetamide (21). A mixture of 1.0 g (0.0025 mol) of **20**, 0.336 g (0.00275 mol) of 4-(dimethylamino)pyridine, 0.9 g (0.0027 mol) of *o*-toluenesulfonamide, and 0.57 g (0.00275 mol) of DDC in 25 mL of CH_2Cl_2 was stirred at room temperature for 40 h. After addition of 10 mL of 1 N HCl, the mixture was stirred for 1 h, diluted with more solvent, and washed with water. The organic layer was concentrated and the product **21** (Table I) was purified by R-18 HPLC.

Method E. 5-[4-[[(4-Acetyl-3-hydroxy-2-propylphenyl)methyl]thio]phenyl]-3,3-dimethyl-5-oxopentanoic Acid (22). Potassium *tert*-butoxide (0.79 g, 0.00275 mol) was added to 0.9 g (0.0038 mol) of **101** in 10 mL of EtOH followed by 0.63 g (0.003 mol) of **104**. The mixture was stirred for 48 h and diluted with 25 mL of 2 N HCl, and the mixture was extracted with EtOAc. Compound **22** (Table I) was isolated by silica HPLC (9:1, toluene/EtOAc).

Method F. 3-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]-3-oxopropanenitrile (37). Sodium amide was made from 0.48 g of Na and 100 mL of liquid NH_3, and 2.5 g (0.0065 mol) of **103** and 3.0 g of acetonitrile in 25 mL of ether were added. The ammonia was replaced with ether over 3 h, and the mixture was washed with water. The aqueous wash was acidified with 5 N HCl and extracted with EtOAc. This extract was purified by silica HPLC (9:1, toluene/EtOAc) to give **37** (Table III).

Method G. 4-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]-4-oxobutanoic Acid, Ethyl Ester (38). Sodium (0.575 g, 0.025 g atom) was reacted with 50 mL of EtOH, 4.53 g (0.02 mol) of **104**, 6.7 g (0.03 mol) of **72**, and 3.8 g of NaI were added, and the mixture stirred for 72 h. The mixture was diluted with water and extracted with EtOAc, and the product was purified by silica HPLC (19:1, toluene/EtOAc) to give **38**, mp 134–136 °C (Table III).

Method H. 1-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]-3-chloropropanone (39). Aluminum chloride (0.027 equiv) was added to a cooled solution of 0.018 mol of 3-chloropropionyl chloride in 100 mL of CH_2Cl_2 followed by 5.0 g (0.018 mol) of **102** and the mixture stirred for 6 h. The mixture was treated with 6 N HCl, and EtOAc and the product were recovered from the organic solvent. This material was crystallized from toluene–hexane to give **39** (Table III).

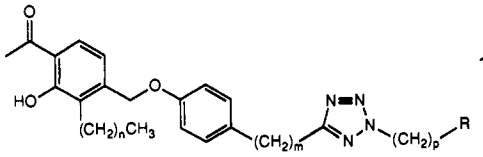
Method I. 4-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]-4-oxobutanenitrile (40). A mixture of 3 g (0.008 mol) of **39** and 5.2 g of KCN in 50 mL of DMF was stirred and heated at 60 °C for 6 h. The mixture was cooled, diluted with water, and extracted with EtOAc. The organic layer was dried (Na_2SO_4) and the solvent was removed by rotary evaporation to give 2.8 g of **40** (Table III).

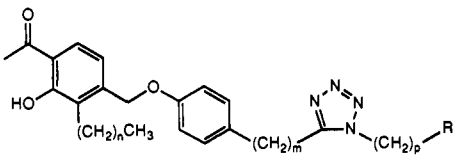
Method J. 4-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]-4-oxo-3-methylbutanenitrile (41). Potassium *tert*-butoxide (2.9 g, 0.026 mol) was dissolved in 50 mL of EtOH followed by the addition of 5.8 g (0.0631 mol) of **76**, 4.5 g (0.02 mol) of **104**, and 4 g of NaI. The mixture was stirred at room temperature for 30 h, poured into 100 mL of water, and extracted with EtOAc. The organic layer was concentrated and 5.8 g of **41** (Table III) was isolated by silica HPLC (9:1, toluene/EtOAc).

Method K. 5-[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]-2*H*-tetrazole-2-butanenitrile (58). A mixture of 10 g (0.026 mol) of **107**, 3 mL (0.03 mol) of 4-bromobutyronitrile, and 10 g of K_2CO_3 in 100 mL of acetone was stirred at room temperature for 20 h. The mixture was poured into water and extracted with EtOAc. The product recovered from EtOAc was purified by silica HPLC (9:1, toluene/EtOAc) to give 4.8 g of **58** (Table IV).

Method L. 5-[[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]methyl]-2*H*-tetrazole-2-acetonitrile (59) and 5-[[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methoxy]phenyl]methyl]-1*H*-tetrazole-1-acetonitrile (66). A mixture of 17.3 g (0.045 mol) of **106**, 1 mL of 0.5 N NaOH, and 3.5 mL (0.05 mol) of bromoacetonitrile in 200 mL of THF was heated

Table IV. Chemical Data of 5-[[(Phenylmethoxy)phenyl]alkyl]tetrazole-1- and -2-alkanoic Acid Esters and Alkanenitriles

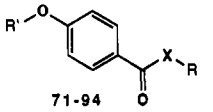




compd	n	m	p	R	synth[a] method	purifctn[b] method	yield, %	mp, °C	chem[c] shift of NCH_2 protons	formula[d]
58	2	0	3	CN	K	A	44	108–110	4.81	$C_{23}H_{25}N_5O_3$
59	2	1	1	CN	L	B	38	101–103	5.50	$C_{22}H_{23}N_5O_3$
60	2	1	3	CN	L	C	70	oil	4.72	$C_{24}H_{27}N_5O_3$
61	2	1	3	CO_2Et	M	D	62	oil	4.65	$C_{26}H_{32}N_4O_5$
62	2	1	4	CN	L	D	47	oil	4.62	$C_{25}H_{29}N_5O_3$
63	2	1	4	CO_2Et	M	D	63	oil	4.58	$C_{27}H_{34}N_4O_5$
64	2	1	6	CN	K	C	22	oil	4.56	$C_{27}H_{33}N_5O_3$
65	1	2	3	CN	K	A	61	90–91	4.71	$C_{24}H_{27}N_5O_3$
66	2	1	1	CN	L	B	47	89–92	4.98	$C_{22}H_{23}N_5O_3$
67	2	1	3	CN	L	C	32	oil	4.26	$C_{24}H_{27}N_5O_3$
68	2	1	3	CO_2Et	M	D	26	oil	4.25	$C_{26}H_{32}N_4O_5$
69	2	1	4	CN	L	D	33	oil	4.16	$C_{25}H_{29}N_5O_3$
70	2	1	4	CO_2Et	M	D	21	oil	4.14	$C_{27}H_{34}N_4O_5$

[a] See *a*, Table I. [b] Solvent of recrystallization or purification method: A, silica HPLC (9:1 toluene/EtOAc); B, silica HPLC (gradient toluene → 9:1 toluene/EtOAc); C, silica HPLC (gradient toluene → 8:2 toluene/EtOAc); D, silica HPLC (gradient toluene → 7:3 toluene/EtOAc). [c] NMR values given in ppm and were determined in deuteriochloroform. [d] See *c*, Table I.

Table V. Ω-Oxo-Ω-phenylalkane Intermediates



compd	R′	X	R	synth[a] method	purifctn[b] method	yield, %	mp, °C	formula[b]
71	CH_3	CH_2CH_2	CO_2H	N	A	55	146–148	$C_{11}H_{12}O_4$
72	H	CH_2CH_2	CO_2Et	O	B	33	108–110	$C_{12}H_{14}O_4$
73	CH_3	CH_2CH_2	CN	P	C	11	90–92	$C_{11}H_{11}NO_2$
74	H	CH_2CH_2	CN	Q	D	27	155–157	$C_{10}H_9NO_2$
75	CH_3	$CHCH_3CH_2$	CN	R	E	60	oil	$C_{12}H_{13}NO_2$
76	H	$CHCH_3CH_2$	CN	Q	F	62	140–143	$C_{11}H_{11}NO_2$
77	CH_3	$C(CH_3)_2CH_2$	CN	R	G	55	oil	$C_{13}H_{15}NO_2$
78	H	$C(CH_3)_2CH_2$	CN	Q	D	60	122–124	$C_{12}H_{13}NO_2$
79	CH_3	$CH_2CH_2CH_2$	CO_2H	N	H	32	132–135	$C_{12}H_{14}O_4$
80	H	$CH_2CH_2CH_2$	CO_2Et	O	E	37	74–75	$C_{13}H_{16}O_4$
81	CH_3	$CH_2CH_2CH_2$	CN	P	E	77	70–71	$C_{12}H_{13}NO_2$
82	H	$CH_2CH_2CH_2$	CN	Q	E	53	93–95	$C_{11}H_{11}NO_2$
83	CH_3	$CH_2C(CH_3)_2CH_2$	CO_2H	N	I	96	oil	$C_{14}H_{18}O_4$
84	H	$CH_2C(CH_3)_2CH_2$	CO_2H	S	J	76	112–114	$C_{13}H_{16}O_4$
85	H	$CH_2C(CH_3)_2CH_2$	CO_2Et	T	E	79	98–100	$C_{15}H_{20}O_4$
86	CH_3	$CH_2C(CH_3)_2CH_2$	CN	P	C	79	oil	$C_{14}H_{17}NO_2$
87	H	$CH_2C(CH_3)_2CH_2$	CN	Q	F	76	82–83	$C_{13}H_{15}NO_2$
88	CH_3	$(CH_2)_4$	CO_2Et	N	K	49	44–45	$C_{15}H_{20}O_4$
89	H	$(CH_2)_4$	CO_2Et	T	D	74	66–67	$C_{14}H_{18}O_4$
90	H	$(CH_2)_6$	CN	U	E	43	90–91	$C_{14}H_{17}NO_2$
91	CH_3	1,4-phenylene	CN	H	E	44	92–93	$C_{15}H_{11}NO_2$
92	H	1,4-phenylene	CN	Q	I	62	186–189	$C_{14}H_9NO_2$
93	CH_3	1,3-phenylene	CN	H	L	33	85–86	$C_{15}H_{11}NO_2$
94	H	1,3-phenylene	CN	Q	L	79	153–157	$C_{14}H_9NO_2$

[a] See *a*, Table I. [b] Solvent of recrystallization, bp, or chromatography method: A, EtOAc; B, toluene–EtOAc, C, silica (HPLC, 49:1 toluene/EtOAc); D, silica HPLC (4:1 toluene/EtOAc); E, silica HPLC (9:1 toluene/EtOAc); F, silica HPLC (7:3 toluene/EtOAc); G, 120–130 °C/0.1 Torr; H, EtOH; I, crude; J, toluene–CH_3CN; K, 142–155 °C/0.05 Torr; L, toluene–EtOAc. [c] See *c*, Table I.

to maintain reflux for 20 h. After concentrating at reduced pressure, water was added to the concentrate, and the products were extracted into EtOAc. These were separated by silica HPLC using a gradient (toluene → 9:1 toluene/EtOAc) to give 6.9 g of **58** and 8.6 g of **66** (Table IV).

Method M. 5-[[4-[(4-Acetyl-3-hydroxy-2-propylphenyl)-methoxy]phenyl]methyl]-2*H*-tetrazole-2-butanoic Acid, Ethyl Ester (61) and 5-[[4-[(4-Acetyl-3-hydroxy-2-propyl-phenyl)methoxy]phenyl]methyl]-1*H*-tetrazole-1-butanoic Acid, Ethyl Ester (68). A solution of 15.0 g (0.038 mol) of **106** and 15.7 mL (0.11 mol) of ethyl 4-bromobutyrate in 150 mL of DMF was stirred at room temperature for 48 h. The mixture was poured into water and the products were recovered in EtOAc. These were separated by silica HPLC (9:1 toluene/EtOAc → 7:3 toluene/EtOAc) to give 11.2 g of **61** and 4.8 g of **68** (Table IV).

Method N. 4-(4-Methoxyphenyl)-4-oxobutanoic Acid (71). Aluminum chloride (26.7 g, 0.1 g atom) was added in portions to 10 g (0.1 mol) of succinic anhydride and 16.2 g (0.15 mol) of anisole in 250 mL of CH_2Cl_2 with ice–ethanol bath cooling and stirring maintained for 3 h. The reaction mixture was poured into 20% HCl and the recovered product crystallized from EtOAc to give 11.5 g of **71** (Table V).

Method O. 4-(4-Hydroxyphenyl)-4-oxobutanoic Acid, Ethyl Ester (72). A solution of 100.0 g (0.48 mol) of 71, 500 mL of 48% HBr, and 1000 mL of acetic acid was heated to maintain reflux for 20 h. After the addition of 1 L of ethanol, the mixture was concentrated at reduced pressure and the addition of EtOH followed by concentration repeated. The crude product was recrystallized twice from toluene–EtOAc to give 35 g of **72**, mp 108–110 °C (see Table V).

Method P. 3,3-Dimethyl-5-(4-methoxyphenyl)-5-oxopentanenitrile (86). Methanesulfonyl chloride (25 mL, 0.32 mol) was added dropwise to 80 g (0.32 mol) of **83** in 1500 mL of pyridine with ice-bath cooling. The mixture was stirred for 1 h and allowed to warm to room temperature. Anhydrous NH_3 was bubbled into the reaction for 0.5 h and excess NH_3 was removed at reduced pressure. The mixture was cooled to 0 °C and 125 mL (1.63 mol) of methanesulfonyl chloride added dropwise. It was stirred and allowed to warm to room temperature over 2 h. The mixture was poured into 2 L of water and extracted with EtOAc. The organic layer was dried and concentrated, and 58.1 g of **86** (Table V) was isolated by silica HPLC (98:2 toluene/EtOAc).

Method Q. 4-(4-Hydroxyphenyl)-4-oxobutanenitrile (74). Aluminum chloride (24 g, 0.13 g atom) was added in portions to 11 g (0.06 mol) of **73** in 250 mL of CH_2Cl_2. The mixture was heated to maintain reflux for 20 h and then allowed to cool to room temperature. It was poured into ice/5 N HCl and extracted with EtOAc. The organic layer was dried and concentrated. The product was purified by silica HPLC (4:1 toluene/EtOAc) to give 2.8 g of **74** (Table V).

Method R. 4-(4-Methoxyphenyl)-3-methyl-4-oxobutanenitrile (75). A solution of 1.6 N *n*-butyllithium (1.1 mol) in hexane was slowly added to a cold (–60 °C) solution of 168 mL (1.1 mol) of diisopropylamine in 500 mL of THF. After 0.5 h, 164 g (1.0 mol) of 4-methoxypropiophenone in 50 mL of THF was added dropwise, followed by the addition of 77 mL (1.1 mL mol) of bromoacetonitrile. The temperature was allowed to warm from –60 °C to room temperature and the reaction was stirred for 16 h. It was diluted with water and extracted with EtOAc. The organic layer was concentrated and 121.8 g of **75** (Table V) was isolated by silica HPLC (toluene → 9:1 toluene/EtOAc).

Method S. 3,3-Dimethyl-5-(4-hydroxyphenyl)-5-oxopentanoic Acid (84). A mixture of 440 g of pyridine hydrochloride and 94 g (0.376 mol) of **83** was heated in an oil bath at 185 °C for 20 h and allowed to cool. The solid was taken up in water and the mixture extracted with EtOAc. The organic layer was concentrated and the product crystallized from toluene–acetonitrile to give 67.5 g of **84** (Table V).

Method T. 3,3-Dimethyl-5-(4-hydroxyphenyl)-5-oxopentanoic Acid, Ethyl Ester (85). A solution of 10 g (0.043 mol) of **84** and 3 mL of methanesulfonic acid in 100 mL of ethanol was stirred at 25 °C for 25 h and diluted with water, and the product was extracted into EtOAc. This material was purified by silica HPLC to give **85** (see Table V), mp 98–100 °C.

Method U. 8-(4-Hydroxyphenyl)-8-oxooctanenitrile (90). A solution of 0.11 mol of 1.6 N *n*-butyllithium in hexane was added to a solution of 15.4 mL (0.1 mol) of diisopropylamine in 70 mL of THF with ice–ethanol cooling and stirred 0.5 h. This solution was added to 19.0 g (0.1 mol) of α-(4-methoxyphenyl)-α-(dimethylamino)acetonitrile in 75 mL of THF and 35 mL of HMPA at –55 to –65 °C followed by the dropwise addition of 19 g (0.1 mol) of 7-bromoheptanenitrile in a equal volume of THF. The mixture was stirred for 1 h and allowed to warm to 25 °C, and stirring was maintained for 2 h. It was then poured into water and extracted with EtOAc, and the material was recovered from EtOAc (crude weight, 29 g). This material and 300 g of pyridine hydrochloride were heated in an oil bath at 185 °C for 20 h and, after cooling, taken up in water. The material was recovered in EtOAc and purified by silica HPLC to give 10 g of **90**, mp 90–91 °C (see Table V).

Method V. 3,3-Dimethyl-5-oxo-5-(4-mercaptophenyl)pentanoic Acid (101). Sodium hydride, 60% in mineral oil (4.0 g, 0.1 mol), was added to a solution of **85** (26.5 g, 0.1 mol) in 250 mL of DMF, and the solution was stirred at 25 °C for 1 h. Dimethylthiocarbamoyl chloride (17.3 g, 0.14 mol) was added, warmed to 90 °C, and stirred for 3 h. The solution was allowed to cool to room temperature, diluted with water, and extracted with EtOAc. The organic layer was concentrated and the resulting oil (14.5 g) was heated in an oil bath at 200 °C for 6 h. After cooling, this material was dissolved in 200 mL of ethanol and 25 mL of 5 N NaOH and heated to maintain reflux for 3 h. The mixture was diluted with water, made acidic with 5 N HCl, and extracted with EtOAc. The organic layer was concentrated and the product **101** (9.0 g) was isolated by silica HPLC (49:1 CH_2Cl_2/MeOH). Anal. ($C_{15}H_{20}O_3S$) C, H.

5-(3-Hydroxyphenyl)-5-oxopentanenitrile (95). Treatment of 0.1 mol of α-(dimethylamino)-α-(3-methoxyphenyl)acetonitrile with 0.1 mol of 4-bromobutanenitrile as described by method U gave 5.3 g (28%) of **95**, mp 92–94 °C (EtOAc–hexane). Anal. ($C_{11}H_{11}NO_2$) C, H, N.

5-(2,4-Dihydroxyphenyl)-5-oxopentanenitrile (96). Treatment of 0.5 mol of 1,3-dimethoxybenzene with 0.75 mol of glutaric anhydride and 1.0 mol of $AlCl_3$ as described by method N gave 52 g (41%) of **5-(2,4-dimethoxyphenyl)-5-oxopentanoic acid (97)**, mp 110–118 °C (EtOAc–hexane). Anal. ($C_{13}H_{16}O_5$), C, H. Fourteen grams of this material was treated with methanesulfonyl chloride in pyridine as in method P to give 9.4 g (72%) of **5-(2,4-dimethoxyphenyl)-5-oxopentanenitrile (98)**, mp 60–63 °C (silica HPLC, 9:1 toluene/EtOAc). Anal. ($C_{13}H_{15}NO_3$) C, H, N. Bis O-demethylation of 28.5 g (0.12 mol) of **98** by method Q gave 12.5 g (51%) of **96**, mp 105–106 °C (EtOAc–hexane). Anal. ($C_{11}H_{11}NO_3$) C, H, N.

6-Hydroxy-1,2,3,4-tetrahydro-1-oxonaphthalene-2-acetic Acid, Ethyl Ester (99). Alkylation of 6-methoxy-1,2,3,4-tetrahydro-1-oxonaphthalene with ethyl bromoacetate by method R gave **6-methoxy-1,2,3,4-tetrahydro-1-oxonaphthalene-2-acetic acid, ethyl ester (100)** (bp 152–156 °C/0.05 Torr) in 56% yield. Anal. ($C_{15}H_{18}O_4$) C, H. O-Demethylation of **100** by method Q gave **99** in 33% yield, mp 100–101 °C (toluene–hexane). Anal. ($C_{14}H_{16}O_4$) C, H, N.

1-[[(2-Hydroxy-3-propyl-4-phenoxy)methyl]phenyl]-ethanone (102). Coupling of 0.066 mol of phenol and 0.043 mol of **103** by method G gave 6.2 g (51%) of **102**, mp 53–55 °C. Anal. ($C_{18}H_{20}O_3$) C, H.

4-[(4-Acetyl-3-hydroxy-2-propylphenyl)methyl]benzoic Acid, Ethyl Ester (103). Ethyl 4-hydroxybenzoate (0.04 mol) was coupled with 0.02 mol of **104** by method G to give 5.6 g (78%) of **103**, mp 78–80 °C (silica HPLC, 9:1 EtOAc/toluene). Anal. ($C_{21}H_{24}O_5$) C, H.

Biological Methods. Guinea Pig Ileum, Trachea, and Parenchyma. The procedures described in refs 6 and 8 were used. Results are expressed as pK_B values, which are the –log of that antagonist concentration producing a 2-fold rightward shift of the LTD_4 concentration–response curve.[13] The guinea pig ileum results listed in Tables I and II are the mean ± the standard error of the mean values of 4–6 tissue determinations. Guinea pig smooth muscle results for compounds **9** and **26** are listed in Table VI.

Guinea Pig Total Pulmonary Impedance.[9] Guinea pigs were anesthetized with 45 to 50 mg/kg of pentobarbital sodium given ip. The left jugular vein as cannulated with a polyethylene catheter (PE 50) for administration of drugs and the right carotid artery was cannulated for the measurement of arterial blood pressure. A third cannula was inserted into the trachea and the animal ventilated with a tidal volume of 1 mL/100 g of body weight 50 times/minute with room air. Succinylcholine, 5 mg/kg, was given iv to suppress spontaneous respiration. The total pulmonary impedance (TPI) was measured with a Statham pressure transducer (P23ID) connected to a T-tube on the tracheal cannula. This procedure is a modification of the Konzett-Rossler[14] technique. Guinea pigs were pretreated with propranolol, 1 mg/kg, and indomethacin, 3 mg/kg, iv.

A separate group of guinea pigs were passively sensitized against ovalbumin by ip administration of 0.2 mL of antiserum 2 days preceding the experiment. The hyperimmune serum had been previously prepared by actively sensitizing male Hartley guinea pigs with 2 mg of ovalbumin in 50% complete Freund's adjuvant

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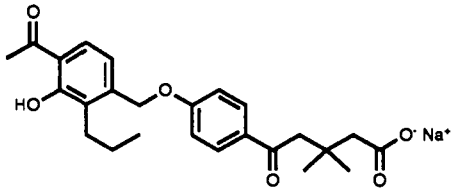
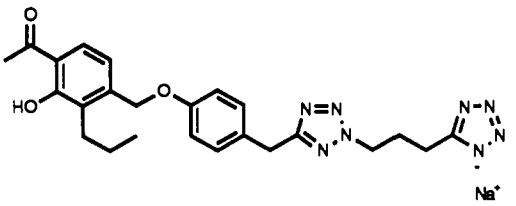
Table VI. pK_B Values against Various Agonists in Guinea Pig Smooth Muscles



9



26

antagonist	agonist	tissue	pK_B	slope of Schild plot	pA_2
9	LTE_4 (20)[a]	ileum	7.73 ± 0.15	0.81 ± 0.15	8.01
26	LTE_4 (16)	ileum	8.37 ± 0.05	0.95 ± 0.08	8.44
9	LTE_4 (17)	trachea	7.09 ± 0.08	0.92 ± 0.15	7.20
26	LTE_4 (16)	trachea	7.57 ± 0.09	1.03 ± 0.16	7.57
9	LTE_4 (11)	parenchyma	7.13 ± 0.07	0.81 ± 0.22	7.44
9	LTD_4 (20)	ileum	7.46 ± 0.06	0.80 ± 0.09[b]	
26	LTD_4 (16)	ileum	7.84 ± 0.06	0.93 ± 0.09	7.92
9	LTD_4 (16)	trachea[c]	6.52 ± 0.11	0.52 ± 0.17[b]	
26	LTD_4 (12)	trachea[c]	6.69 ± 0.07	0.96 ± 0.16	6.74
9	LTD_4 (15)	parenchyma	6.74 ± 0.09	0.88 ± 0.17	6.86
9	LTC_4 (7)	ileum[d]	<5		
26	LTC_4 (5)	ileum[d]	5.39 ± 0.12		
9	LTC_4 (5)	trachea[e]	<5		
26	LTC_4 (6)	trachea[e]	5.67 ± 0.12		
9	histamine	ileum	<5		
26	histamine	ileum	<5		
9	bradykinin	ileum	<5		
9	carbamylcholine	trachea	<5		
9	U46619	trachea	<5		

[a] Number of determinations. [b] Slope of Schild plot statistically different from 1, $p < 0.05$. [c] In the presence of 3 mM *l*-cysteine. [d] In the presence of 3×10^{-6} M FPL 55712; corrected for control shift. [e] In the presence of 45 mM *l*-serine borate.

given ip on days 1 and 5. On day 21, the animals were bled and the serum collected and stored at −20 °C. In addition, to propranolol and indomethacin, 5 mg/kg of pyrilamine, an antihistamine, was given iv. Under these conditions the antigen-induced increase in TPI is primarily mediated by the leukotrienes.[6]

Rat Preparations.[12] Adult male Sprague-Dawley rats, weighing approximately 320–380 g, were anesthetized with pentobarbital sodium (65 mg/kg, ip). The trachea was cannulated and the rats were ventilated with room air delivered from a rodent respirator (Harvard; tidal volume 1 mL/100 g of body weight, 60 cycles/min). Pulsatile arterial blood pressure was measured from a cannulated carotid artery by using a Statham transducer (P23ID). Mean arterial blood pressure was calculated as diastolic pressure plus one-third pulse pressure. Cardiac rate was monitored by a cardiotachometer, which was triggered by the systolic pressure pulse. Drug solutions were administered iv through a catheter placed in a jugular vein.

In certain experiments, rats were pithed by passing a steel rod through the right orbit and down the entire length of the spinal column. The rod was left in position for the duration of the experiment.

For hemodynamic studies, the left thorax was entered at the insertion of the ribs with the sternum. The ribs were gently retracted and the ascending aorta was isolated. A calibrated electromagnetic flowprobe (Carolina Medical Electronics) was placed around the root of the aorta and connected to a flowmeter (Carolina) to record mean aortic blood flow. Phasic left ventricular pressure was measured (Statham transducer, P231D) via a catheter placed in the ventricular cavity through a small apical puncture. The left ventricular pressure signal was electronically differentiated to obtain dP/dt_{max} as an index of myocardial contractility. Directly measured parameters were continuously recorded on a multichannel oscillograph (Beckman). In addition, aortic blood flow index, systemic vascular resistance, and stroke volume index were calculated.

LTD_4-Induced Pressor Response in Rats. The relative potencies of test compounds as antagonists of cardiovascular LTD_4 receptors were determined in pithed rats. Following an equilibration period, separate groups of pithed rats were pretreated iv with various doses of test compound or vehicle 15 min prior to LTD_4 (10 μg/kg, iv) challenge. Pressor responses to LTD_4 were then compared and used to calculate ED_{50} (95% confidence interval) values for each compound tested. ED_{50} is the dose of compound (mg/kg, iv) required to decrease the pressor response to LTD_4 by 50%. Results are listed in Tables I and II.

Cardiovascular Measurements in Anesthetized Rats. Myocardial and systemic hemodynamic responses to LTD_4 (10 μg/kg, iv) were recorded in normal anesthetized rats and in anesthetized rats pretreated with test compound (0.1–10.0 mg/kg, iv). Four to six rats were used for each data point. Hemodynamic alterations were continuously monitored for 30 min following LTD_4 administration. Results with compounds **9** and **26** are reported in Figure 2. The effect of 15-min pretreatment with compounds **9** and **26** on the LTD_4-induced (10 μg/kg, iv) elevation of hematocrit in rat are reported in Figure 3.

Registry No. 1, 119348-40-2; **2**, 119348-34-4; **3**, 119348-41-3; 4, 119348-46-8; **5**, 119348-44-6; **6**, 119348-39-9; **7**, 119348-42-4; **8**, 119348-30-0; **9**, 135312-25-3; **10**, 119348-47-9; 11, 119348-29-7; **12**, 119348-28-6; **13**, 119348-38-8; **14**, 119348-43-5; **15**, 119348-60-6; **16**, 118909-15-2; **17**, 118909-06-1; **18**, 135312-26-4; **19**, 119348-49-1; **20**, 119348-58-2; **21**, 135312-27-5; **22**, 135312-28-6; **23**, 122268-06-8; **24**, 122267-93-0; **25**, 122267-95-2; **26**, 122268-12-6; **27**, 122268-03-5; **28**, 122267-97-4; **29**, 122268-05-7; **30**, 122267-98-5; **31**, 135312-29-7; **32**, 122267-92-9; **33**, 122267-94-1; **34**, 122268-02-4; **35**, 122267-96-3; **36**, 122268-04-6; **37**, 119348-67-3; **38**, 119348-33-3; **39**, 135312-30-0; **40**, 119348-68-4; **41**, 119348-73-1; **42**, 119348-71-9; **43**, 119348-37-7; **44**, 119348-69-5; **45**, 119348-51-5; **46**, 119348-32-2; **47**, 135312-31-1; **48**, 119348-31-1; **49**, 119348-35-5; **50**, 135312-32-2; **51**, 119348-70-8; **52**, 119348-78-6; **53**, 135312-33-3; **54**, 118909-05-0; **55**, 119348-72-0; **56**, 119348-75-3; **57**, 119348-56-0; **58**, 122290-42-0; **59**, 122267-73-6; **60**, 122267-77-0; **61**, 122267-69-0; **62**, 122267-79-2; **63**, 122267-67-8; **64**, 122267-83-8; **65**, 135312-34-4; **66**, 122267-73-6; **67**, 122267-78-1; **68**, 122267-70-3; **69**, 122267-80-5; **70**, 122267-68-9; **71**, 3153-44-4; **72**, 66123-43-1; **73**, 55234-56-5; **74**, 7182-43-6; **75**, 135312-35-5; **76**, 135312-36-6; **77**, 135312-37-7; **78**, 135312-38-8; **79**, 4609-10-3; **80**, 66123-78-2; **81**, 26823-02-9; **82**, 135312-39-9; **83**, 31526-44-0; **84**, 135312-40-2; **85**, 119348-66-2; **86**, 135312-41-3; **87**, 135312-42-4; **88**, 42916-80-3; **89**, 119348-65-1; **90**, 135339-55-8; **91**, 27645-60-9; **92**, 27645-61-0; **93**, 60694-67-9; **94**, 118909-04-9; **95**, 135312-43-5; **96**, 135312-44-6; **97**, 4654-07-3; **98**, 135312-45-7; **99**, 105806-38-0; **100**, 50558-96-8; **101**, 135312-46-8; **102**, 135312-47-9; **103**, 97582-47-3; **104**, 97582-36-0; **105**, 97582-38-2; **106**, 107223-78-9; **107**, 97581-69-6; *o*-toluenesulfonamide, 88-19-7; 3-chloropropionyl

chloride, 625-36-5; 4-bromobutyronitrile, 5332-06-9; bromoacetonitrile, 590-17-0; ethyl 4-bromobutyrate, 2969-81-5; anisole, 100-66-3; succinic anhydride, 108-30-5; 4-methoxypropylphenone, 4160-51-4; α-(4-methoxy)-α-(dimethylamino)acetonitrile, 15190-05-3; 7-bromoheptanenitrile, 20965-27-9; dimethylthiocarbamoyl chloride, 16420-13-6; α-(dimethylamino)-α-(3-methoxyphenyl)-acetonitrile, 15189-99-8; 1,3-dimethoxybenzene, 151-10-0; glutaric anhydride, 108-55-4; 6-methoxy-1,2,3,4-tetrahydronaphthalen-1-(2*H*)-one, 1078-19-9; phenol, 108-95-2; ethyl 4-hydroxybenzoate, 120-47-8; leukotriene D_4, 73836-78-9.

Pregnanes That Bind to the Digitalis Receptor: Synthesis of 14-Hydroxy-5β,14β-pregnane Glycosides from Digitoxin and Digitoxigenin

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The preparation of the mono-, bis-, and trisdigitoxosides of 14-hydroxy-5β,14β-pregnan-20-one and 14,20β-dihydroxy-5β,14β-pregnane by two routes, based on the conversion of the α,β-unsaturated γ-lactone in digitoxin to the 20-ketone and 20β-alcohol by ozonolysis and zinc–acetic acid treatment followed by lithium tri-*tert*-butoxyaluminum hydride reduction, are described. Synthesis of the α-L-rhamnoside derivatives is described also. Structures were confirmed by ^1H and ^{13}C NMR spectra. These derivatives show strong interaction with the cardiac glycoside receptor of heart muscle in an [^3H]ouabain radioligand binding assay. Structure–activity relationships which are reported for glycosides and genins show that the α-L-rhamnoside derivatives are more potent than the β-D-digitoxoside or the β-D-glucoside and that the β-D-glucosides are more potent than the mono-, bis-, and trisdigitoxosides. Potency is not increased by the addition of the second and third digitoxose units.

Certain pregnanes bind to the cardiac glycoside recognition site on Na^+,K^+-ATPase and inhibit the enzyme (the sodium pump) in membranes, cells, and tissues.[1] The most potent derivatives identified, thus far, are pregnane C-3 glycosides that are cardiotonic and exert certain potentially useful effects on heart and kidney not shared by the digitalis drugs.[1] We now report on the contribution of some C-3 substituted sugars to binding potency of the pregnanes.

We have shown that the 3β-β-D-glucoside and 3β-α-L-rhamnoside of 14,20β-dihydroxy-5β,14β-pregnane bind with high affinity to the cardiac glycoside receptor in dog heart muscle as determined in a [^3H]ouabain binding assay.[1] The mono-, bis-, and trisdigitoxosides of 14β-hydroxypregnane were synthesized and tested on receptor binding to compare the effect of the number of sugar units and to compare the monodigitoxoside with the corresponding β-D-glucoside and α-L-rhamnoside.

Chemistry

Synthesis of these compounds was carried out by means of our recently demonstrated method[2] for conversion of the α,β-unsaturated γ-lactone in the cardiac glycosides into the acetyl group using ozone and excess zinc–acetic acid (Scheme I). Degradation of the sugars to the mono and bis derivatives was achieved by the method of Satoh et al.[3] except lithium tri-*tert*-butoxyaluminum hydride (LTBA) was substituted for sodium borohydride in the dialdehyde reduction. Two synthetic routes to mono- and bisdigitoxosides **9** and **6** were carried out. First, ozonolysis of digitoxin (**1**) followed by reduction with zinc–acetic acid gave 21-methyl ketone **2** which was reduced with LTBA to give 20β-alcohol **3**. The terminal digitoxose was removed by sodium periodate oxidation, LTBA reduction, and mild acid hydrolysis to give bisdigitoxoside **6**; the second digitoxose unit was removed in a similar manner to give monodigitoxoside **9**. Second, digitoxin (**1**) was converted to bis- and monodigitoxosides **4** and **7** and these were then treated with ozone and zinc–acetic acid to give the corresponding 21-methyl ketones **5** and **8**. LTBA reduction of **5** and **8** also gave 20β-alcohols **6** and **9**, respectively.

The preparation of pregnane glycosides **11**, **14**, and **15** was carried out in a similar manner (Scheme II). The β-D-glucoside (**10**) and the α-L-rhamnoside (**13**) of digitoxigenin (**16**) were treated with ozone followed by excess zinc–acetic acid to yield 21-methyl ketone derivatives **11** and **14**; reduction of **14** with LTBA gave 20β-alcohol **15**.

Alternatively **14** and **15** were prepared by treatment of 3β-alcohol **23**, prepared by hydrolysis of 3β-acetate **22**, with bromorhamnose triacetate and Fetizons reagent to give the triacetyl rhamnoside, which on removal of the *tert*-butyldimethylsilyl protecting group with fluoride ion followed by hydrolysis gave 20β-alcohol **15**, or after neutral oxidation followed by mild hydrolysis gave 20-ketone **14**.

Structures were established by their ^1H and ^{13}C NMR spectra (see Tables I and II) which are consistent with published data.[4,5]

Results and Discussion

Receptor binding affinity of digitoxigenin possessing three digitoxose units [i.e. digitoxin (**1**)] is not altered significantly by the progressive removal of the sugars since potency of bis- and monodigitoxosides **1** and **7** is unchanged and similar to that of β-D-glucoside **10** (Table III). However, α-L-rhamnoside **13** binds more strongly than monodigitoxoside **7**. A similar comparison of 14β-hydroxypregnan-20-one derivatives **2**, **5**, **8**, **11**, and **14**, which may be viewed as a truncated lactones, shows a large decrease in binding. The increase in binding for β-D-

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