may occur in other drug–receptor systems. For example, opioid phenylmorphans and 3-phenyl- and 4-phenylpiperidines also form such pairs.[27,45-47] A possible molecular explanation for the opposite activity of enantiomers may be the delocalization of charge that occurs when amines are protonated.[48] That is, rather than being concentrated on the ammonium hydrogen, the positive charge is spread over the adjacent C–N bonds so that the "back" of the N–H bond also contains significant positive charge. Thus, (6a*S*)-NPA may be able to bind to the same receptor site as (6a*R*)-NPA since the overall shapes of these molecules are similar and there is sufficient charge at the back of the N–H bond to allow binding to a complementary electrostatic site in the receptor. Antagonists may bind to the same receptor site as agonists, but it appears that agonist activity requires the proper N–H orientation.

To summarize, two ((6a*S*)-NPA and (1*S*,2*R*)-5-OH-MDAT) of the three postsynaptic DA antagonists can be closely superimposed with the juxtaposition of the phenyl *m*-hydroxyl, the N-substituent, and the direction of the N–H bond. The third antagonist, (3*S*)-PPP, can also assume this conformation with only a moderate energy penalty possibly accounting for its relatively low potency. A similar juxtaposition occurs for the three DA agonists. These observations point out the importance of the orientation of the ammonium hydrogen (or lone-pair electrons) in determining agonist and antagonist activity at DA receptors. This view is consistent with a previous proposal[17] that the 6a-hydrogen at the chiral center of aporphines is responsible for the enantiomeric selectivity of such aporphines since the chiral center also determines the orientation of the ammonium hydrogen.

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Registry No. (3*R*)-PPP, 85976-54-1; (3*S*)-PPP, 85966-89-8; 3-PPP, 75240-91-4; (6a*S*)-NPA, 79703-31-4; NPA, 18426-20-5; (1*R*,2*S*)-5-OH-MDAT, 96148-66-2; (1*S*,2*R*)-5-OH-MDAT, 96148-67-3; 5-OH-MDAT, 90295-44-6.

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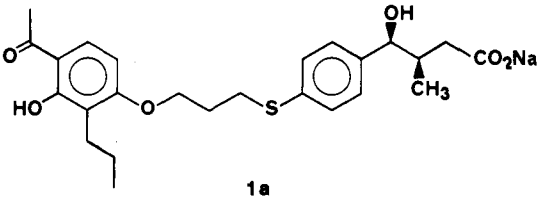
Design and Synthesis of Sodium ($\beta R^*,\gamma S^*$)-4-[[3-(4-Acetyl-3-hydroxy-2-propylphenoxy)propyl]thio]-γ-hydroxy-β-methylbenzenebutanoate: A Novel, Selective, and Orally Active Receptor Antagonist of Leukotriene D_4

Sir:

The leukotrienes C_4, D_4, and E_4 are peptido–lipid conjugates derived from the 5-lipoxygenase pathway of arachidonic acid metabolism that collectively account for the biological activity known as slow-reacting substance of anaphylaxis (SRS-A).[1-5] These products have been ascribed an important role in the etiology of human asthma on the basis of their demonstrated release upon antigenic stimulation from human and animal lung tissue,[6,7] their potent and long-lasting contractile effects on airway smooth muscle[8,9] and their abilities to promote mucus production,[10] decrease mucocillary clearance[11] and modulate vascular permeability.[12,13] From these observations has evolved the hypothesis that a potent and specific leukotriene antagonist should offer an effective new treatment for asthma. This hypothesis remains to be tested in clinical trials, when a suitably safe and potent drug is available.[14]

In this paper we describe the research that has led to the discovery of sodium ($\beta R^*,\gamma S^*$)-4-[[3-(4-acetyl-3-hydroxy-2-propylphenoxy)propyl]thio]-γ-hydroxy-β-methylbenzenebutanoate (L-649,923) (1a), a potent and orally active antagonist of LTD_4 that has the potential to help define the role of leukotriene D_4 in human disease.



1a

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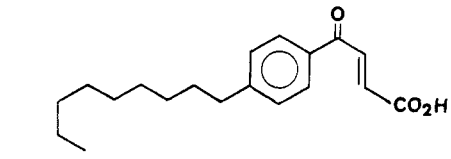
Table I. Summary of Biological Activity of Substituted Phenylketobutyric Acids and Related Metabolites

compd no.	X	R^1	R^2	in vitro activity[16] (% inhibition at 0.1 μg/mL)	in vivo activity (guinea pig)[22] iv (ED_{50},[a] mg/kg)	in vivo activity (guinea pig)[22] id (effective dose,[a] mg/kg)
4a	O	H	H	49 ± 10	1.1	5 (2)
4b	O	3-F	H	81 ± 7	1.0	5–10 (13)
4c	O	3-Me	H	53 ± 3	NT	>10
4d	S	H	H	63 ± 14	1.1 ± 0.3 (8)	5–10 (30)
4e	S	3-F	H	82 ± 10	1.1	10
4f	S	3-F	OH	32 ± 3	0.9	>10
4g	S	3-n-Pr	H	37 ± 7	0.2	5–10 (3)
5				65 ± 4	0.5 ± 0.16 (9)	10 (1)
6				65 ± 9	1.3 ± 0.13 (8)	10 (1)
3 (FPL-55712)				83 ± 10	0.10 ± 0.02 (6)	>10

[a] Values were determined in anesthetized guinea pigs against bronchoconstrictions induced by LTD_4 (0.2 μg/kg, iv) following iv or id administration of the drug. ED_{50} values (iv) are based on one to two determinations with drug administered in cumulative doses 5 min prior to each subsequent LTD_4 challenge. For multiple determinations, the mean ED_{50} ± SEM is given with the number of determinations in parentheses. Effective dose values (id) are given (with the number of experiments in parentheses) as minimum doses of compound that produced greater than 50% inhibition of bronchoconstriction within 50 min after a single id dose. LTD_4 challenges were repeated 10, 30, and 50 min after dosing.

Early in our program we noted that 4-alkylphenylketobutenoic acid derivatives (e.g., 2)[15] showed weak but significant activity in vitro in our primary screen, the LTD_4 challenged guinea pig ileum.[16,17] Noting similarities between 2 and the LTD_4 antagonist FPL-55712[18] (3), that is, that both are arylketobutenoic acid derivatives with an appended lipophilic chain of 9 or 10 atoms, we were prompted to prepare a series of compounds, 4, incorporating what we surmised to be the key components of 2 and 3.



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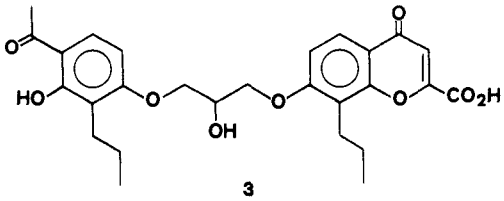
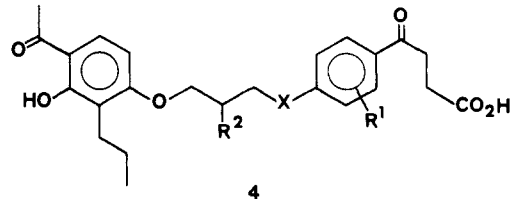


3



4

The synthesis of these compounds was straightforward (vide infra) and led to the pleasing observation that these compounds not only exhibited good in vitro activity but also showed significant in vivo activity in the guinea pig, by both the intravenous (iv) and intraduodenal (id) routes of administration (Table I). On the basis of the examples shown, substitution, either in the linking chain or on the aryl ring, gave no further advantage.

During more detailed studies, we observed that thioether analogues generally exhibited more consistent id and oral absorption as indicated by biological activity and by blood levels of the compounds as detected by reversed-phase HPLC analysis of blood samples (data not shown).[19] In blood level studies on **4d**, we noted that the ketobutyric acid chain was rapidly metabolized in the dog and the rat via reduction to **5** and β-oxidation to **6** (Scheme I).[20] Both metabolites retained intrinsic biological activity, but were less active by the id route than the parent compound (Table I). Wishing to minimize the potential for interspecies differences in metabolism and to further optimize pharmacodynamic properties of **4d**, we prepared a series of analogues of **4d** wherein β-oxidation was blocked by methyl substitution or by substituting a tetrazole for the carboxyl group. The results are presented in Table II. While these analogues generally retained full in vitro activity, the (βR*,γS*)-γ-hydroxy-β-methyl analogue, **1a**, exhibited a superior profile of in vivo activity by both the iv and id routes of administration. Notably, the βR*,γR* analogue **1b** was much less active in vivo although comparably active in vitro. These differences must therefore reflect differing pharmacodynamic properties between the two diastereomers.

Compound **1a** is a racemate and being a competitive antagonist (vide infra) its two enantiomers might be expected to exhibit differences in activity. To test this hypothesis, **1a** was resolved by esterification (CH_2N_2), conversion of the γ-hydroxy group to the γ-(*d*-α-methoxyphenylacetate esters (DCC, DMAP, *d*-α-methoxyphenylacetic acid, ether, room temperature, 5 h), separation by preparative HPLC, and subsequent base hydrolysis. Both

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(16) Isolated terminal ileum segments (2.5 cm) in aerated Krebs–bicarbonate solution in the presence of atropine (10^{-6} M) and Timolol maleate (0.5 μg/mL) are mounted under 1 g of passive force. After equilibration, several submaximal control responses to LTD_4 (0.3 ng/mL) were obtained, and following 30-s exposure to test substance, the control concentration of LTD_4 was added to the bath and tension response was measured; percent inhibitions quoted are at the screening dose of 0.1 μg/mL and are the mean (±SEM) of three determinations.

(17) Compound **2** inhibited 60% in this assay at 1 μg/mL final bath concentration (n = 1).

(18) Augstein, J.; Farmer, J. B.; Lee, T. B.; Sheard, P.; Tattersall, M. L. *Nature (London), New Biol.* **1973**, *245*, 215.

(19) Blood samples (rat, dog, or rhesus monkey) collected in heparinized tubes were centrifuged, diluted with an equal volume of acetonitrile, and centrifuged, and the supernatant was analyzed directly by reversed-phase HPLC (C-18, eluant 65–70% methanol in pH 7.0 (0.1 N) phosphate buffer).

(20) A similar pattern of metabolism has been observed for fenbufen. Vanhear, G. E.; Chiccarelli, F. S.; Bonenfant, P. A.; Barr, A. *J. Pharm. Sci.* **1977**, *67*, 1662.

Scheme I

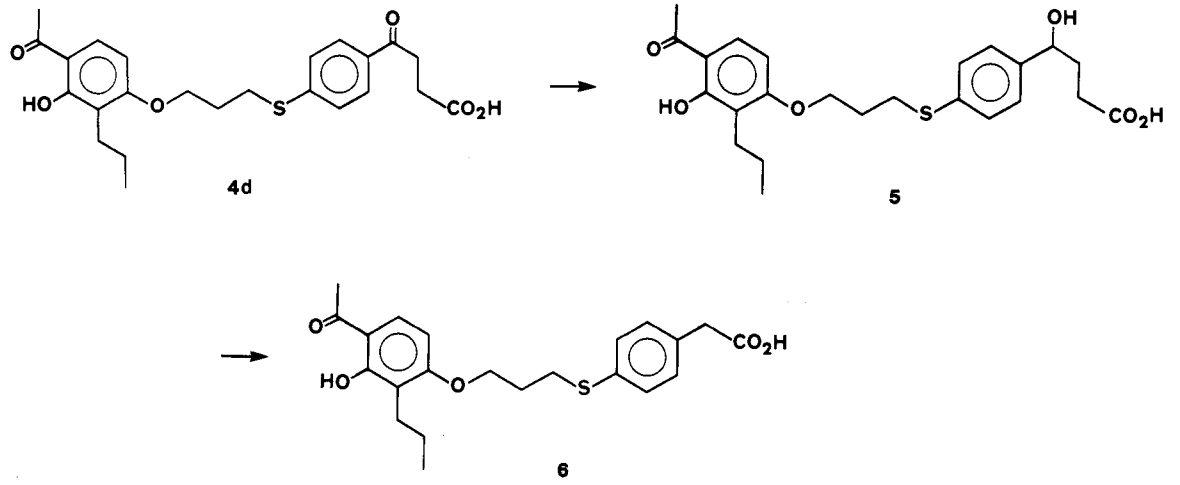
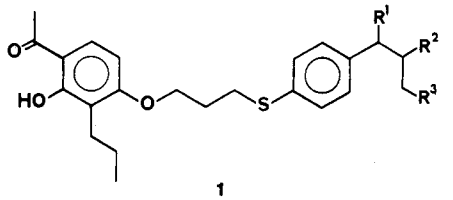


Table II. Summary of Activity of "Metabolically Blocked" Analogues of 4d



compd no.	R^1	R^2	R^3	in vitro activity[16] (% inhibition)	in vivo activity (guinea pig)[22] iv (ED_{50},[a] mg/kg)	in vivo activity (guinea pig)[22] id (effective dose,[a] mg/kg)
1a	OH ($\beta R^*,\gamma S^*$)	CH_3	COONa	73 ± 7	0.26 ± 0.06 (10)	5 (7)
1b	OH ($\beta R^*,\gamma R^*$)	CH_3	COONa	62 ± 5	1.5	>10
1c	OH	$(CH_3)_2$	COONa	45 ± 12	0.5	5 (2)
1d	=O	H	tetrazole	63 ± 6	0.6	>10

[a] See footnote *a*, Table I.

enantiomers ((+) isomer, $[\alpha]_D$ +9.5°, and (−) isomer, $[\alpha]_D$ −9.2°) exhibited quite similar properties both in vitro and in vivo when compared to **1a** (see summary of pharmacology). It thus appears that these β and γ substituents, while serving to impart significant advantages in terms of the absorption, distribution, and perhaps elimination profiles of these compounds, are not crucial for receptor recognition, at least as judged by our animal and tissue models.

Chemistry.[21] Compounds **4a–f** were prepared by reaction of the appropriate anisole with succinic anhydride ($AlCl_3$, dichloroethane, 0 °C) followed by demethylation (HBr, HOAc, reflux), esterification (HCl, MeOH), and either direct coupling with 3-(4-acetyl-3-hydroxy-2-propylphenoxy)propyl bromide (**7**) (K_2CO_3, MEK, reflux, 4 h) or prior conversion of the phenolic intermediate to the mercapto analogue ((1) $Me_2NC(S)Cl$, NaH, DMF; (2) 200 °C, neat; (3) NaOMe, MeOH; (4) HCl) followed by coupling with **7**, or in the case of **4f** with 3-(4-acetyl-3-hydroxy-2-propylphenoxy)-1,2-epoxypropane (conditions as above), and subsequent hydrolysis of the esters (NaOH, MeOH, THF) in each case. Compound **5** was prepared by reduction ($NaBH_4$) of **4d**. Compound **6** was prepared as the sodium salt from 4-methoxyphenylacetic acid in a procedure analogous to that for **4d**. Compound **1d** was prepared by coupling 4-mercapto-γ-oxobenzenebutyronitrile[22] with **7** followed by conversion to the tetrazole (n-Bu_3SnN_3, THF, reflux 24 h; HCl, ether). Compound **1c** was prepared from thioanisole via Friedel–Crafts reaction (propionyl chloride, $AlCl_3$, CH_2ClCH_2Cl, 0 °C, subsequent alkylation (LDA, THF, −78 °C; $BrCH_2COOMe$), hydrolysis of the ester (NaOH, THF, MeOH), second alkylation (KH, MeI, THF–Me_2SO, −40 to 0 °C) followed by esterification (CH_2N_2), and deblocking of the thioanisole group[22] while the resultant thiolate anion was trapped in situ with 1,3-dibromopropane ((1) mCPBA, 0 °C, 1 h; (2) trifluoroacetic anhydride, 40 °C, 0.25 h; (3) K_2CO_3, MEK, H_2O trace, $BrCH_2CH_2CH_2Br$, room temperature, 24 h) to provide methyl 4-[(3-bromopropyl)-thio]-γ-oxo-β,β-dimethylbenzenebutanoate. Reduction ($NaBH_4$, $CeCl_3$, MeOH), coupling with 2,4-dihydroxy-3-propylacetophenone (K_2CO_3, MEK), and hydrolysis provided **1c**. Compounds **1a** and **1b** were prepared by alkylation of methyl 4-(*N*,*N*-dimethylthiocarbamoyl)-γ-oxobenzenebutanoate (vide supra) (KH, MeI, THF) followed by hydrolysis (NaOH, MeOH), reduction of the keto acid ($NaBH_4$), lactonization (TFA, CH_2Cl_2), separation of the cis and trans lactones by flash chromatography, hydrolysis of the thiocarbamate (NaOMe, MeOH), coupling with **7**, and basic hydrolysis (NaOH, MeOH, THF) to provide **1a** and **1b** as hygroscopic sodium salts.

(21) All compounds were characterized by IR and NMR spectroscopy. Elemental analyses were within ±0.4% of the theoretical values unless otherwise noted.

(22) Young, R. N.; Gauthier, J. Y.; Coombs, W. *Tetrahedron Lett.* *1984*, *25*, 1753.

Summary of Pharmacology of 1a and Its Enantiomers.[23,24] In vitro in the guinea pig, 1a exhibited pA_2 values and slopes vs. LTD_4 from Schild plot analysis of 8.1 ± 0.3 (slope = 1.0 ± 0.2) on the isolated ileum strip and 7.2 ± 0.2 (slope = 0.7 ± 0.1) on the isolated trachea. Selectivity of 1a was indicated by its inability to significantly antagonize other spasmogens such as acetylcholine, serotonin, $PGF_{2\alpha}$, or histamine on tracheal tissues. In receptor binding studies,[25] 1a inhibited [^{3}H]-LTD_4 binding to guinea pig lung membranes with a K_i of 400 ± 213 nM and [^{3}H]-LTC_4 binding with a K_i of 8.6 ± 0.9 μM.

In vivo 1a antagonized bronchoconstriction induced by LTD_4 (0.2 μg/kg iv) in artificially ventilated anesthetized guinea pigs with an ED_{50} of 0.26 ± 0.06 mg/kg when administered intravenously and was also consistently active (>50% inhibition) at 5.0 mg/kg when administered intraduodenally 10–50 min prior to challenge with LTD_4. In addition, 1a has shown significant activity vs. antigen-induced dyspnea in hyperreactive rats[26] and vs. LTD_4 and antigen challenge in conscious squirrel monkeys[26] following oral doses of 1–10 mg/kg.[27]

The (+) enantiomer of 1a exhibited a pA_2 of 7.2 ± 0.2 (slope = 0.8 ± 0.1) from Schild plot analysis on the isolated guinea pig trachea while the (−) enantiomers of 1a gave a pA_2 of 6.9 ± 0.2 (slope = 0.6 ± 0.1). The ED_{50} values iv in the guinea pig vs. LTD_4 were 0.28 ± 0.05 and 0.79 ± 0.15, respectively, for the (+) and the (−) enantiomers.

Thus a new series of leukotriene D_4 antagonists has been discovered. In particular 1a has been shown to be a potent, selective, and orally active receptor antagonist of leukotriene D_4. This compound should allow the evaluation of the role of LTD_4 in asthma and other allergic diseases. Clinical trials now in progress will define its potential role as a novel therapy for such diseases in humans.

Registry No. (±)-**1a**, 100443-69-4; (+)-**1a**, 91541-76-3; (−)-**1a**, 103346-41-4; (±)-**1a** (free acid), 91542-58-4; **1b**, 103365-84-0; **1b** (free acid), 103365-85-1; **1c**, 91541-73-0; **1c** (free acid), 103305-28-8; **1d**, 103305-30-2; **4a**, 91541-00-3; **4b**, 91540-93-1; **4c**, 91541-04-7; **4d**, 91540-81-7; **4e**, 91540-89-5; **4f**, 91540-75-9; **4g**, 91542-00-6; **5**, 103321-16-0; **6**, 91361-94-3; **7**, 40786-20-7; MeSPh, 100-68-5; *p*-$MeOC_6H_4CO(CH_2)_2CO_2H$, 3153-44-4; *p*-$HSC_6H_4CO(CH_2)_2CN$, 91358-98-4; *p*-$Br(CH_2)_3SC_6H_4COC(Me_2)CH_2CO_2Me$, 91541-70-7; *p*-$Me_2NC(S)C_6H_4CO(CH_2)_2CO_2Me$, 103305-29-9; 3-(4-acetyl-3-hydroxy-2-propylphenoxy)-1-mercaptopropane, 97384-33-3; γ-(3-fluoro-4-methoxy)-γ-oxobenzenebutanoic acid, 347-63-7; γ-(3-methyl-4-methoxy)-γ-oxobenzenebutanoic acid, 33446-14-9; γ-(3-propyl-4-methoxy)-γ-oxobenzenebutanoic acid, 100972-66-5; 3-(4-acetyl-3-hydroxy-2-propylphenoxy)-1,2-epoxypropane, 57161-85-0; succinic anhydride, 108-30-5; 2,4-dihydroxy-3-propylacetophenone, 40786-69-4; leukotriene D_4, 73836-78-9.

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(23) Error calculations are quoted as ± standard error of the mean. For a description of methodology, see: Carrier, R.; Cragoe, E. J.; Ethier, D.; Ford-Hutchinson, A. W.; Girard, Y.; Hall, R. A.; Hamel, P.; Rokach, J.; Share, N. N.; Stone, C. A.; Yusko, P. *Br. J. Pharmacol.* **1984**, *82*, 389.

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