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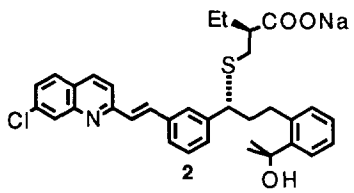
DISCOVERY OF MK-0476, A POTENT AND ORALLY ACTIVE LEUKOTRIENE D_4 RECEPTOR ANTAGONIST DEVOID OF PEROXISOMAL ENZYME INDUCTION

M. Labelle*, M. Belley, Y. Gareau, J.Y. Gauthier, D. Guay, R. Gordon, S.G. Grossman†, T.R. Jones, Y. Leblanc, M. McAuliffe, C. McFarlane, P. Masson, K.M. Metters, N. Ouimet, D.H. Patrick†, H. Piechuta, C. Rochette, N. Sawyer, Y.B. Xiang, C.B. Pickett, A.W. Ford-Hutchinson, R.J. Zamboni and R.N. Young

Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe Claire-Dorval, Québec, Canada, H9R 4P8 †Department of Safety Assessment, Merck and Co., West Point, PA

Abstract. Structure-activity studies leading to the discovery of **1 (MK-0476)** are described. The initial compound of this series, **2**, was a potent leukotriene D_4 (LTD_4) antagonist, but was also a peroxisomal enzyme inducer in the mouse. Structure-activity relationships around the thioether chain were explored to remove this undesirable feature. It was found that alkyl substituents in the ß position relative to the carboxylic acid reduce the potency as a peroxisomal enzyme inducer while preserving the LTD_4 antagonistic properties. Dialkyl substitution essentially eliminates the enzyme induction. The optimal styryl quinoline **1** exhibited high *in vitro* potency and *in vivo* activity on oral dosing without significant liver enzyme induction in the mouse.

Recent studies on specific orally active leukotriene D_4 (LTD_4) receptor antagonists[1,2] have demonstrated clinical activity in human bronchial asthma. We recently described a new series of styryl quinoline thioethers exemplified by **2** and **3**,[3] which are potent LTD_4 receptor antagonists and are orally active in animal models of asthma. The isomer **3**, which was devoid of peroxisomal enzyme induction (PEI) properties in rodents, has been further optimized to give **4**.[4] On the other hand the isomer **2**, along with all the compounds tested thus far with the (R) configuration at the benzylic centre, induced significant PEI and liver weight increase (LWI) when administered at high doses p.o. to mice.[5] Such activity has been associated with liver tumor formation in rodents for peroxisomal proliferators in general,[6] as well as for LTD_4 antagonists as a subclass.[5,7,8] This (R)



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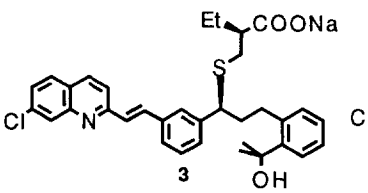

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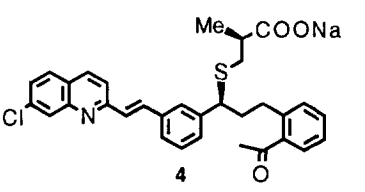

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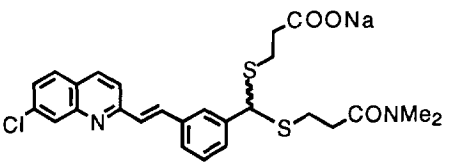
stereochemical series included LTD_4 receptor antagonists of tantalizingly superior *in vitro* and *in vivo* potencies, and we therefore embarked on a comprehensive structure-activity relationship study which has led to a unique understanding of the structural requirements for the elimination of the PEI and LWI properties from a chemical series. These efforts culminated in the identification of the clinical candidate **1** (MK-0476).

Peroxisomes recognize and ß-oxidize fatty acids.[9] In our studies, the enzymatic activity monitored to assess PEI was fatty acyl Co-A oxidase (FACO).[5] The relationship of fatty acid metabolism to PEI suggested particular emphasis on the modification of the thioalkanoic acid side chain of **2**, and especially of the ß-position relative to the carboxylic acid. Previously published attempts along these lines have been unsuccessful, the 3-thia-fatty acids[10] and 3-phenoxyacetic acids[11] being stronger enzyme inducers in rats than the corresponding fatty acids in spite of being free of ß-oxidation. The observed correlation of PEI with the (R)-benzylic stereochemistry in the aforementioned series as well as the similar stereoselectivity observed for the enantiomers of MK-571[5] (**16**, **17**) is compatible with either a receptor mediated control of enzyme induction, or a stereospecific disruption of lipid homeostasis in the hepatocytes. In light of the recent identification of a nuclear receptor, termed the peroxisome proliferator activated receptor (PPAR),[12] which is believed to be involved in the induction of FACO,[13] it appeared probable that receptor recognition rather than metabolic disruption could be critical to PEI in our series. Since the factors controlling recognition at the LTD_4 receptor and at the PPAR are likely different, it should be possible to design a leukotriene antagonist in the (R) stereochemical series that would not cause PEI and the associated LWI. The aforementioned 3-thia-fatty acids and phenoxyacetic acids, although resistant to enzymatic ß-oxidation by virtue of the presence of a heteroatom at the ß-position, offer a topology and electronic aspect very similar to that of the corresponding fatty acids which act as agonists at the PPAR. We reasoned that addition of steric bulk on the acid side chain may be an effective way to prevent an agonistic interaction between our compounds and the PPAR.

The table shows the LTD_4 binding *in vitro* potency, LWI and PEI data for a series of propionic and butyric acids incorporating alkyl substituents that were prepared to test this hypothesis. All the compounds show high affinity for the LTD_4 receptor, superior to the previous generation of LTD_4 antagonists exemplified by **16** (MK-571) and **17** (MK-0679).

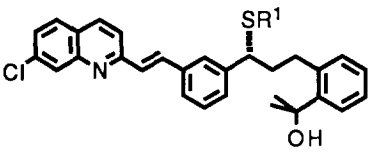


16: racemate (MK-0571)
17: (R)-isomer (MK-0679)

In the propionic series (entries **2-12**), the stereochemistry at the ß center of the chain may have some influence on LTD_4 receptor binding (compare **7** and **8**), but this effect is certainly not as important as that demonstrated for the α and benzylic centers.[3] Disubstitution at the ß center did not alter potency at the LTD_4 receptor. In contrast, the LWI and PEI potency of the compounds were markedly affected by the substitution

pattern of the side chain. Whereas the (S)-α-ethyl compound **2** induces important LWI and PEI even at 200 mg/kg, the (R,S) ß-ethyl compound **6** showed a lesser response at higher dose. Comparison of the pair **7** and

TABLE. Peroxisomal enzyme induction in the mouse



Compound	Thioether chain SR^1	LTD_4 binding IC_{50}[a](nM)	LWI[b]	PEI[b]
2	(α-S) $SCH_2CHEtCO_2H$	0.06; 0.2	+63%[c,d,e]	+608%[c,d,e]
5	(ß-RS) $SCHMeCH_2CO_2H$	0.79±0.13	+5%[c]	+22%[c]
6	(ß-RS) $SCHEtCH_2CO_2H$	0.58±0.21	+9%	+188%[e]
7	(ß-S) $SCHMeCH_2CO_2H$	0.33±0.09	+11%[d]	+24%
8	(ß-R) $SCHMeCH_2CO_2H$	0.40;0.60	+12%	+109%[e]
9	(ß-RS,α-RS) $SCHMeCHMeCO_2H$	0.44±0.05	+27%[d,e]	+673%[d,e]
10	(ß-R)(α-R) $SCHMeCHMeCO_2H$	0.30±0.08	+10%[d]	+91%[d,e]
11	(ß-S)(α-S) $SCHMeCHMeCO_2H$	2.3±0.5	+32%[d,e]	+393%[d,e]
12	$SCMe_2CH_2CO_2H$	0.68±0.3	+3%	-10%
13	(R,S) $SCH_2CHMeCH_2CO_2H$	0.43±0.18	+10%	+18%[d]
14	$SCH_2CMe_2CH_2CO_2H$	0.30±0.04	+2%	+25%
1	$SCH_2C(CH_2CH_2)CH_2CO_2H$	0.50±0.12	0%	+16%
16	-[f]	1.5±0.1	+38%[d,e]	+318%[d,e]
17	-[f]	3.1±0.5	0%	65%

a) Inhibition of binding of [^{3}H]LTD_4 to guinea pig lung membrane.[16] Values are mean ± S.E.M. or individual determinations. **b)** Average of 4 male and 4 female mice percentage liver weight increase (LWI) and peroxisomal enzyme induction (PEI) over control animals[5], after 4 days dosing at 400 mg/kg p.o., unless otherwise indicated. In all cases, blood levels were measured to confirm good bioavailability and validate the *in vivo* comparison. The fatty acyl Co-A oxidase activity increase is used as a measure of the PEI. **c)** Mice dosed at 200 mg/kg dose. **d)** Statistically significant difference (pairwise t-test) from the control group in males. **e)** Statistically significant difference (pairwise t-test) from the control group in females. **f)** Structures unrelated to the table header, see drawings in the text.

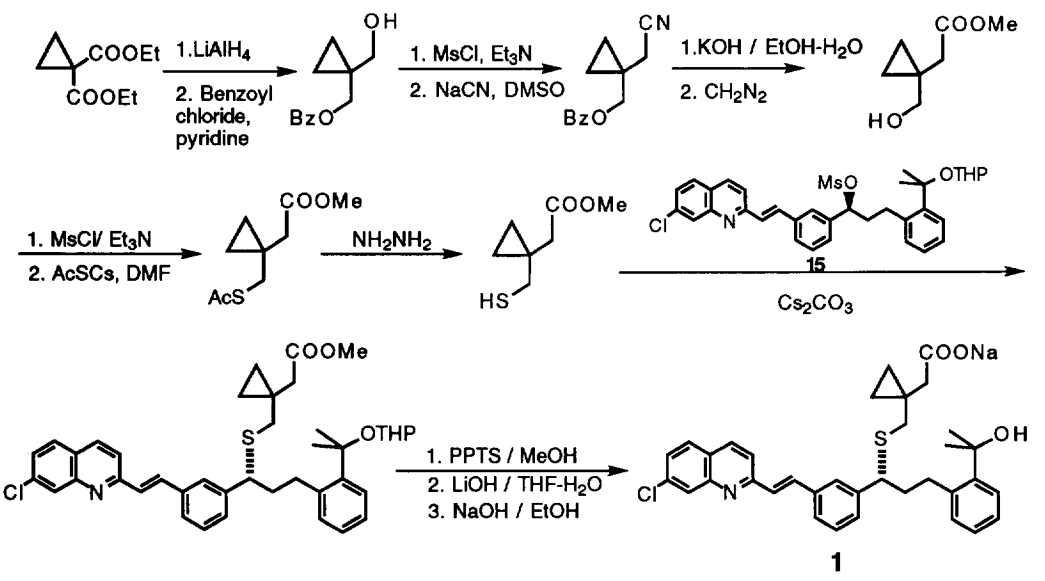
11, which differ by the presence of an (S)-methyl group at the α-center, shows marked differences in LWI and PEI for the latter. On the other hand, the pair **8** and **10** show similar low level LWI and PEI in spite of the addition of an (R)-α-methyl group in **10**. The presence of an (S)-α-alkyl substituent thus appears to be an

important factor in LWI and PEI in this series. A ß-methyl group has less effect on LWI and PEI. The diastereomeric mixture **5** gave no statistically significant LWI or PEI at 200 mg/kg, and the two separate isomers **7** and **8** were at worst weak inducers at 400 mg/kg. Most notably, compound **12** with dimethyl substitution at the ß-center was devoid of LWI and PEI even at 400 mg/kg.

In a series of butyric acid analogs (**13**,**14**,**1**), the same high potency on the LTD_4 receptor was observed. Compound **13**, with the side chain bearing a single methyl in the ß-position, gave small LWI and PEI somewhat akin to that observed for the homologous compounds **7** and **8** at the same dose. However, compounds bearing ß-dialkyl substitution (**14** and **1**) showed no significant LWI and PEI, again in keeping with the observations made in the propionic acid series. Amongst the compounds devoid of LWI and PEI, **1** (Sodium 1-(((1(R)-3)-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)phenyl)-3-(2-(1-hydroxy-1-methylethyl)phenyl)-propyl)thio)methyl)cyclopropane acetate, **L-706,631**, **MK-0476**) was selected as a development candidate based on superior bioavailability (42%) and terminal half-life (4 h) in the squirrel monkey (data not shown).

The compounds were synthesized by coupling the methanesulfonate **15** with the appropriate thiol, as exemplified with **1** in the Scheme. The synthesis of **15** was performed as previously described for its enantiomer,[3] but using (R)-enantiomer of the oxazaborole[14] as the catalyst in the reduction of the precursor ketone.

Scheme



In a guinea-pig lung membrane binding assay[16] where **MK-571** and **ICI 204,219** had IC_{50} values of 2.5 ± 0.5 nM and 0.44 ± 0.15 nM respectively, **1** competed with [^{3}H]LTD_4 with an IC_{50} of 0.5 ± 0.12 nM.

1 was shown to be a potent and competitive antagonist of leukotriene D_4-induced contractions of non-tonal guinea-pig trachea, with a pA_2 value of 9.3 ± 0.5 and a slope of 0.8. Oral administration of 0.01 mg/kg of **1** as the sodium salt in 1.0% methocel to squirrel monkeys,[17] followed 4 h later by a LTD_4 challenge, produced a 56% ($p<0.05$) reduction of the increase in pulmonary resistance and a 78% ($p<0.02$) reduction in the decrease in dynamic compliance, relative to those produced by a control leukotriene D_4 challenge in the same animals treated with vehicle alone. A more detailed pharmacological profile of **1** has been reported.[18]

Conclusion. A new series of potent, selective, orally active leukotriene D_4 antagonists has been optimized to remove undesirable side effects such as LWI and PEI in rodents, while retaining potent LTD_4 antagonist activity. These SAR studies show correlation of ß-substitution with reduction or elimination of PEI and LWI. This, along with the correlation of PEI and stereochemistry, indicate that PEI in rodents is a receptor mediated event and that with appropriate modifications to the structures, this induction can be abolished.

The optimal compound **1** was found to be potent both *in vitro* on human and animal preparations, and *in vivo* in a variety of animal models of asthma, while being devoid of peroxisomal enzyme induction and liver weight increase in the mouse. In keeping with these observations, recent clinical studies have demonstrated that **1** induces bronchodilatation and potent blockade of LTD_4-induced bronchoconstriction in asthmatic patients.[19]

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