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2-ALKYL-4-ETHYL-5-[6-METHYL-6-(2H-TETRAZOL-5-YL)HEPTYLOXY]PHENOL LEUKOTRIENE B4 RECEPTOR ANTAGONISTS

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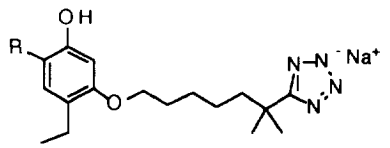
Abstract: A series of 2-n-alkyl-4-ethyl-5-[6-methyl-6-(2H-tetrazol-5-yl)heptyloxy]phenols were prepared and shown to be potent leukotriene B4 (LTB4) receptor antagonists. They bound to the human neutrophil and guinea pig lung LTB4 receptors with high affinity. Each compound was also shown to be effective at antagonizing the effects of LTB4-induced integrin up-regulation on human neutrophils and on LTB4-mediated contraction of guinea pig lung parenchyma.

Leukotriene B4 (LTB4), a product of the 5-lipoxygenase pathway of arachidonic acid metabolism, has held a prominent place as a potential pro-inflammatory eicosanoid.[2] Consequently, within the last several years a number of structurally diverse LTB4 receptor anatagonists were developed with the hope of identifying a novel anti-inflammatory therapy.[3] Our efforts in this area focused primarily on the development of the 1,2,4,5-substituted phenol class of antagonists. Analysis of the structure activity relationships (SAR) within this class of LTB4 receptor antagonists revealed that the nature of *ortho*-phenolic substituent had a dramatic effect on receptor binding affinity, functional antagonistic potency and in vivo efficacy.[4] Because we were interested in further defining the structure activity relationships within this class of leukotriene antagonists, we chose to evaluate 2-n-alkyl-4-ethyl-5-[6-methyl-6-(2H-tetrazol-5-yl)heptyloxy]phenols as antagonists of LTB4 and compare their efficacy to the previously reported hydroxyacetophenone (**1**) and isosteric *ortho*-alkoxyphenols (**2**).[4]



R = C(O)CH3 **1**

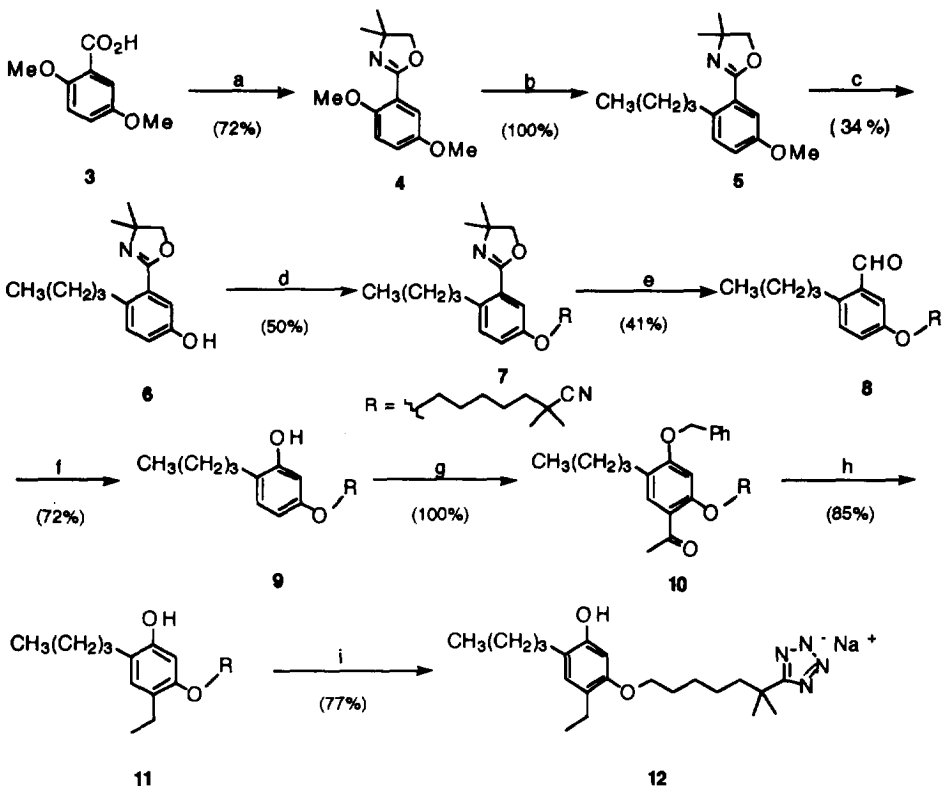
R = O-Alkyl **2**

The preparation of the desired *ortho*-n-alkyl derivatives required that we develop a general approach to the synthesis of 2,4-dialkyl-1,5-dioxygenated aromatic systems. Our initial strategy for synthesis of the desired tetrasubstituted aromatic systems envisioned using regioselective aromatic substitution chemistry in which the aromatic directing group would also act as a masked C-1 phenol. As the *ortho*-directing system, we chose the *ortho*-methoxyphenyloxazoline moiety.[5] *Ortho*-methoxyphenyloxazoline chemistry provided the required versatility necessary for the introduction of structural diversity, and equally important, the oxazoline could be converted to an aldehyde and then via peracid oxidation, into the desired phenol.[4,5] Execution of this approach is exemplified in Scheme 1 with the preparation of the *ortho*-butylphenol LTB4 receptor antagonist **12**. The

synthesis began with 2,5-dimethoxybenzoic acid which already provided oxygenation at C-5 that was needed for attachment of the ether-linked acidic side chain. Formation of the oxazoline **4** from 2,5-dimethoxybenzoic acid proceeded under standard conditions in 72% yield.[5] *n*-Butyl Grignard displacement of the methyl ether of **4** provided the desired *ortho*-alkyloxazoline **5**. The next step required cleavage of the remaining methyl ether to allow attachment of the 6-cyano-6-methylheptyl side chain precursor of the tetrazole acid moiety. Cleavage of the methyl ether by heating methyl ether **5** with NaCN in DMSO provided the phenol **6** in reproducible but low yield (34%).[6] Attachment of the nitrile side chain was accomplished by stirring phenol **6** in DMF with solid K_2CO_3 and 1-iodo-6-cyano-6-methylheptane at room temperature.[4] This gave the ether **7** in 86% yield. Next, conversion of the phenyloxazoline to the phenol was completed as planned. Degradation of the oxazoline moiety to the aldehyde proceeded under standard conditions.[5] Subsequent Baeyer-Villager oxidation and hydrolysis of the resulting formate ester provided the desired phenol **9** in 72% yield. The phenol was protected as its benzyl ether, and then the C-4 substituent was appended via Friedel-Crafts acylation with acetyl chloride and $SnCl_4$. The protection and acetylation steps provided the ketone **10** in 93% yield. Reduction of the ketone to the desired C-4 ethyl substituent with triethylsilane in trifluoroacetic acid and hydrogenolysis of the benzyl ether gave the fully elaborated phenol **11**.[7] Treatment of the nitrile **11** with NaN_3 and dimethylaminoethanol hydrochloride in diglyme at 135 °C for several days provided the crude tetrazole acid.[4] The tetrazole acid **12** was subsequently purified as its sodium salt by medium pressure chromatography on CHP-20 resin.

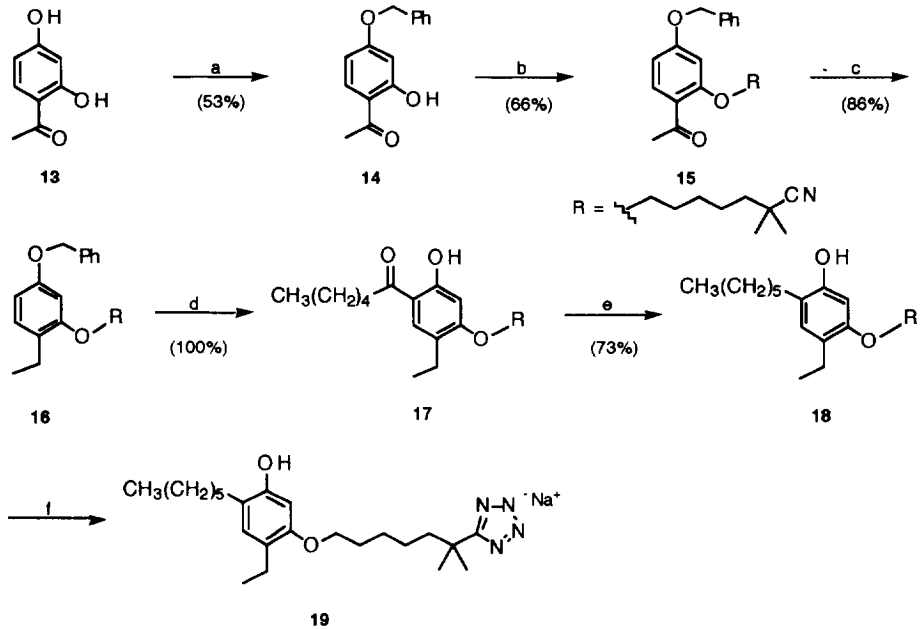
An alternative and more efficient approach to the synthesis of *ortho-n*-alkylphenol antagonists is demonstrated in Scheme 2 with the synthesis of the *n*-hexyl LTB_4 receptor antagonist **19**. In contrast to our earlier approach, the strategy depicted in Scheme 2 attached the *ortho*-alkyl (i.e., *n*-hexyl) substituent later in the synthesis. This approach allowed us to construct a general intermediate **16** which could be used for the synthesis of subsequent derivatives. Also, the starting material contained three of the four required aromatic functionalizable substituents and therefore, this approach required only one carbon-carbon bond formation step, i.e. attachment of the *ortho*-phenol substituent. Selective benzylation of 2,4-dihydroxyacetophenone at the C-4 hydroxyl group took advantage of the reduced nucleophilicity of the strongly hydrogen-bonded *ortho*-hydroxyacetophenone moiety. The remaining free phenol was alkylated with 1-chloro-6-cyano-6-methylheptane providing **15** in 66% yield.[4] As before, reduction of the ketone **15** with Et_3SiH in trifluoroacetic acid provided the ethyl compound in 86% yield. The next step required attachment of the substituent which would ultimately become the *ortho*-alkylphenol group. This was accomplished via Friedel-Crafts acylation using hexanoyl chloride with $SnCl_4$ as the Lewis acid. Concomitant with acylation, we also observed clean cleavage of the benzyl ether, a transformation which we ultimately wished to affect. This debenzylation most likely occurred after the acylation step since in the presence of Lewis acids, *ortho*-ketone substituents greatly facilitate the cleavage of neighboring benzylethers to afford the free phenols.[6] The next step required reduction of the ketone group of **17** to give the methylene derivative **18**. We accomplished this by first forming the carbonate of the phenol with ethyl chloroformate and then treating the reaction with aqueous $NaBH_4$.[8] The desired alkane was obtained in 73% purified yield. Preparation of tetrazole **19** proceeded as described in Scheme 1.

Scheme 1



Reagents: (a) i) $SOCl_2$, CH_2Cl_2, ii) dimethylaminoethanol, iii) $SOCl_2$; (b) n-butylMgBr, THF, 25 °C; (c) NaCN, DMSO, 80 °C; (d) K_2CO_3, DMF, 6-cyano-1-iodoheptane, 25 °C; (e) i)MeI, CH_2Cl_2, 25 °C, ii) $NaBH_4$, EtOH, 25 °C, iii) HCl, THF; (f) mCPBA, CH_2Cl_2, 25 °C; (g) i) benzylbromide, K_2CO_3, DMF, ii) acetyl chloride, CH_2Cl_2, $SnCl_4$; (h) i) Et_3SiH, TFA, CCl_4, ii) 10%Pd/C, EtOAc, 50 psi; (i) i) NaN_3, diglyme, dimethylaminoethanol hydrochloride, 135 °C, ii) NaOH, CHP-20 chromatography.

Scheme 2



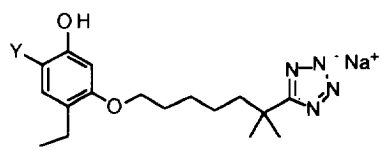
Reagents: (a) K_2CO_3, MEK, DMSO, benzylbromide; (b) K_2CO_3, DMF, 6-cyano-1-iodoheptane; (c) Et_3SiH, TFA, CCl_4; (d) hexanoyl chloride, $SnCl_4$, CH_2Cl_2; (e) ethylchloroformate, Et_3N, THF, 0 °C to 25 °C, ii) $NaBH_4$, H_2O, 0 °C to 25 °C; (f) i) NaN_3, diethylaminoethanol hydrochloride, diglyme, 135 °C, ii) NaOH, CHP-20 chromatography.

Receptor Binding: Receptor binding affinities were evaluated in both human neutrophil and guinea pig lung membrane radioligand binding assays (Table 1).[9] Each *ortho*-alkyl derivative was shown to bind with high affinity to each receptor type. The human neutrophil receptor binding affinity data for the *ortho*-alkyl series paralleled closely with the results observed for the alkoxy series. The most potent alkyl analogue, the *ortho*-ethyl analogue **23,** provided a 2.5-fold improvement in human neutrophil receptor affinity and a 3.8-fold improvement in the affinity for guinea pig lung membrane receptors when compared to the hydroxyacetophenone **1**. As in the case of the *ortho*-alkoxy series, a large n-hexyl chain ortho to the phenolic hydroxyl caused a notable reduction in human neutrophil receptor binding affinity. Analysis of the guinea pig lung membrane receptor binding data showed that the *ortho*-alkyl series was comparable to the *ortho*-alkoxy series in receptor affinity.

Functional Antagonism: Each of the *ortho*-alkyl derivatives were evaluated for functional antagonism of the effects of LTB4 on both the up-regulation of human neutrophil CD11b/CD18 adhesion molecules and on guinea pig lung parenchyma strip contraction (see Table 1).[9,10] Within the *ortho*-alkyl series, the SAR for

antagonism of human neutrophil adhesion molecule up-regulation correlated well with the relative binding affinities for human neutrophil LTB4 receptors (e.g., the six atom ortho substituents demonstrated reduced potency relative to the shorter chain analogues). Compared to the parent hydroxyacetophenone, *ortho*-alkylphenol derivatives were significantly more effective at antagonizing the LTB4-induced CD11b/CD18 adhesion molecule up-regulation , however, there was no clear difference in potency relative to the *ortho*-alkoxy analogues. The *ortho*-alkyl derivatives **12** and **24** were markedly more effective than either the hydroxyacetophenone **1** or *ortho*-alkoxy derivatives at antagonizing the effect of LTB4 on guinea pig lung parenchymal strips. The n-propyl analogue **24** provided a 6-fold improvement in antagonism of LTB4-induced guinea pig lung parenchymal strip contraction relative to its ether isostere **21**. This is in contrast to only a 1.3-fold improvement for **24** versus **21** in the antagonism of the up-regulation of human neutrophil CD11b/CD18 adhesion molecules.

Table 1. Human Neutrophil and Guinea Pig Lung Receptor Binding and Functional Antagonism



Cmpd No.	Y	Human Neutrophil Receptor Binding Ki (nM)	Guinea Pig Lung Membrane Receptor Binding Ki (nM)	Human Neutrophil CD11b/CD18 Integrin Up-regulation IC_{50} (nM)	Guinea Pig Lung Parenchyma Strip Contraction K_B (nM)
20	CH_3O	3.53	25.1 ± 9.2	282	222 ± 38
21	CH_3CH_2O	4.75 ± 0.2	14.2 ± 2.9	206	264 ± 17
22	$CH_3(CH_2)_4O$	14.3	22.6 ± 5.5	395	216 ± 41
23 [a]	CH_3CH_2	5.14 ± 2.4	17.1 ± 3.4	179	167 ± 40
24	$CH_3(CH_2)_2$	5.53	14.2 ± 6.3	161	34 ± 14
12	$CH_3(CH_2)_3$	6.51	10.7 ± 0.9	308	53 ± 7
19	$CH_3(CH_2)_5$	28.4	33.1 ± 0.9	878	688 (n = 2)
1 [a]	$CH_3C(O)$	12.8 ± 1.4	65.7 ± 10.7	2874 ± 470	197 ± 43

[a] Tested as the free acid.

The evaluation of 2-n-alkyl-4-ethyl-5-[6-methyl-6-(2H-tetrazol-5-yl)heptyloxy]phenols has demonstrated that these derivatives are potent as both receptor binders and functional antagonists of human neutrophil and guinea pig lung tissue LTB4 receptors. We have also shown that an alkyl group ortho to the phenol in the 1,2,4,5 substituted phenol class of antagonists can effectively substitute for an alkoxy or acyl substituent without loss in potency. By the fact that substituting an alkyl group for an alkoxy substituent provides receptor ligands with essentially identical receptor affinities, seems to indicate that the critical recognition phenomenon associated with the ortho phenol substituent is a hydrophobic interaction and that little or no associated electronic contribution is involved. Interestingly, although isosteric alkyl and alkoxy derivatives were essentially indistinguishable when comparing their binding affinities for human neutrophils or guinea pig lung membrane receptors or when comparing their ability to antagonize LTB4 induced human neutrophil integrin up-regulation, several alkyl derivatives were clearly superior to alkoxy derivatives at antagonizing guinea pig parenchymal strip contraction. These results thus raise the issue of whether species differences in receptor structure and/or tissue or cell specific receptor subtypes are associated with the observed antagonist effects.[11]

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