Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 19, pp. 2349-2354, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00424-6

SYNTHESIS AND PHARMACOLOGICAL PROFILE OF NEW 1, 3-DISUBSTITUTED CYCLOHEXANES AS LEUKOTRIENE B_4 RECEPTOR ANTAGONISTS

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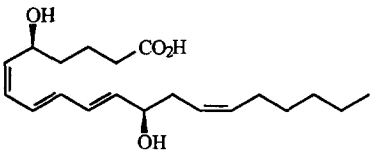
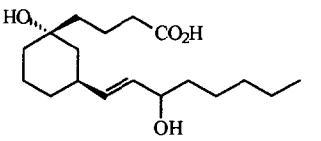
Abstract: In the course of developing stable leukotriene B_4 antagonists, we synthesized a novel non aromatic series of compounds containing a 1, 3-disubstituted cyclohexane ring in place of the conjugated double bonds of the natural eicosanoid. The Structure-Activity Relationship (SAR) studies leading to the identification of the acid **1d** are described.

Leukotriene B_4 (LTB_4) is a 5-lipoxygenase product of arachidonic acid metabolism which stimulates human polymorphonuclear leukocyte (PMN) functions. LTB_4 is a potent inducer of neutrophil chemotaxis[1], aggregation[2], degranulation[3] and respiratory burst[4] in vitro. It has been postulated to be a major inflammatory mediator[5] in a number of disease states such as rheumatoid or spondyloarthritis[6], psoriasis[7], ulcerative colitis[8] and some respiratory diseases[9]. Consequently, a number of potent and selective LTB_4 receptor antagonists have been developed in the past few years, the large majority of them being aromatic compounds[10]. Both LY-223982[11] and SC-41930[12] are structurally related to the LTD_4 receptor antagonist FPL-55712, while SM-9064[13] and U-75302[14] are related to LTB_4, but exhibit partial agonist activity. Recent reports described the high affinity of ONO 4057[15] and RG-14893[16], which are pure antagonists.

As part of our studies directed towards the identification of LTB_4 antagonists based on the structure of the natural ligand itself, we thought it was of interest to check the hypothesis that designed cyclohexylic compounds could mimic the conjugated double bonds of leukotriene B_4. This concept offered numerous opportunities for obtaining analogs with restricted conformational freedom, with the goal of identifying a stable LTB_4 receptor antagonist.



LTB_4



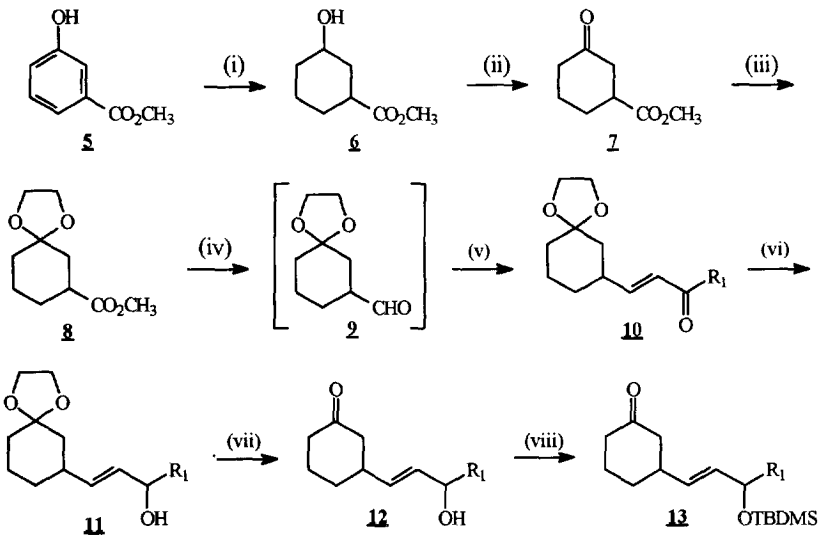
1a

During the course of that research, compound **1a** was identified as an early development[17] and became the focus of SAR studies. We wish to describe here our initial efforts in this area with the synthesis of the diastereoisomer compounds **1**, **2**, **3**, and **4** and the isolation of the enantiomers of **1d** (**1d1** and **1d2**).

Chemistry:

The general synthetic route to compounds **1**-**4** is illustrated in schemes I and II[18]. The various ω chains of these compounds were introduced in good yields on the cyclohexylic ring by Horner-Wadsworth-Emmons reactions[19] between the crude unstable aldehyde **9** and the ylides of the suitable β-ketophosphonates (Scheme I). The required methyl 3-oxo-cyclohexanecarboxylate **7** was prepared[20] by successive hydrogenation and oxidation of methyl 3-hydroxy benzoate **5**. The reduction of the protected keto-ester **8** with diisobutylaluminium hydride at -78°C was monitored by Gas Chromatography in order to control the formation of alcohol (over reduction). As soon as traces of alcohol appeared (after 30 min), the resulting crude aldehyde **9** was added on the ylide of the β-ketophosphonate (NaH, THF) at -40°C. ^{1}H NMR proved the enones **10**, obtained in 75-85% yields, to be only of E configuration[21], no trace of Z analogue being detected.

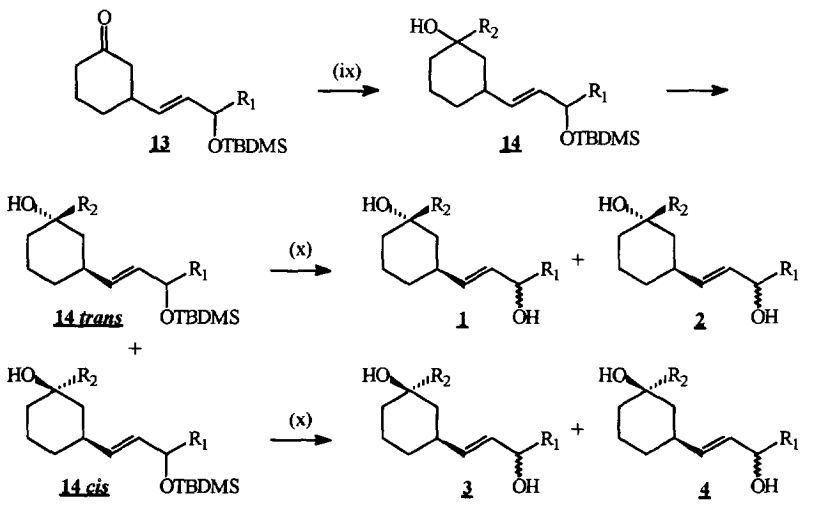
Scheme I



Keys: (a) $R_1 = (CH_2)_4CH_3$ (b) $R_1 = (CH_2)_4CH_3$ (c) $R_1 = (CH_2)_7CH_3$ (d) $R_1 = (CH_2)_4Ph$.
Reagents and conditions (isolated yield):
(i) H_2/Ru, 100 bars, 120°C, ethanol (93%); (ii) CrO_3, H_2SO_4 (82%); (iii) Ethylene glycol, APTS, toluene reflux (95%); (iv) DIBAL-H, toluene, -78°C; (v) β-ketophosphonate, HNa, -40°C (70-86%); (vi) $NaBH_4$, $CeCl_3$, methanol (92-99%); (vii) SiO_2, H_2SO_4 (76-77%); (viii) TBDMSCl, DBU, CH_2Cl_2 (85-89%).

These enones were then reduced to the corresponding enols **11** by treatment with sodium borohydride in the presence of cerium chloride[22], in order to avoid simultaneous hydroboration of the double bond. After deprotection of the carbonyl function[23], the allylic alcohols **12** were protected as their *tert*-butyldimethylsilyl ethers **13**. This key intermediate synthon allowed the homologation of a large variety of α chains by 1,2-additions on the carbonyl function (Scheme II). The condensation of the ethyl acetate carbanion (LDA at -78°C) afforded compounds **1-4 b, c, d**, while the four carbons-homologated compounds **1-4 a** were obtained using the previously described lithiated OBO ortho-ester[24]. In each series (**a** to **d**) the ***14 trans*** and ***14 cis*** silylated ethers were separated by column chromatography, and their relative *trans* and *cis* configurations established by ^1H NMR[25]. As expected, the bulky group was locked in equatorial position. Deprotection of ***14 trans*** and ***14 cis*** hydroxy-esters, followed by careful preparative HPLC separation provided respectively and by order of elution the pure **2-1** and **4-3** diastereoisomers[26]. At this stage of the research, all the racemic compounds **1-4 a-d** were converted into their sodium salts (NaOH, methanol) for biochemical/pharmacological testing. The racemic mixture of compound **1d** was resolved by chiral HPLC[27] to afford successively by order of elution the enantiomers **1d1** and **1d2**.

Scheme II



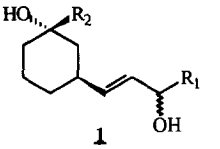
Keys: (a) $R_1 = (CH_2)_4CH_3$ $R_2 = (CH_2)_3CO_2H$; (b) $R_1 = (CH_2)_4CH_3$ $R_2 = CH_2CO_2Et$;
(c) $R_1 = (CH_2)_7CH_3$ $R_2 = CH_2CO_2Et$; (d) $R_1 = (CH_2)_4Ph$ $R_2 = CH_2CO_2Et$.

Reagents and conditions (isolated yield):
(ix a) OBO ortho ester, *t*BuLi, THF, -78°C (56%) or (ix b,c,d) Ethyl acetate, LDA, THF, -80°C (87-92%); (x) HCl 1N, THF (82-84%).

Biological results :

The diastereoisomers **1 a-d** to **4 a-d** were tested for their ability to inhibit the binding of LTB_4 to human neutrophil membranes[28]. In each series (**a** to **d**), the more polar **1** of the *trans* diastereoisomers **2** and **1** exhibited the highest competitive effect (Table I), compared to compounds **2**, **3** and **4**, which also competed for [^{3}H] LTB_4 binding but with lower affinities (not shown).

Table I: Inhibition of specific binding of [^{3}H] LTB_4[28] to human neutrophils



1

Compound	R_1	R_2	IC_{50} (μM) [a]	RBA [b]
1a	$(CH_2)_4CH_3$	$(CH_2)_3CO_2Na$	70	
1b	$(CH_2)_4CH_3$	CH_2CO_2Na	8	1/1000
1c	$(CH_2)_7CH_3$	CH_2CO_2Na	1	1/125
1d	$(CH_2)_4Ph$	CH_2CO_2Na	0.8	1/100
1d1	$(CH_2)_4Ph$	CH_2CO_2Na	0.3	1/40
1d2	$(CH_2)_4Ph$	CH_2CO_2Na	70	

[a] IC_{50} are extrapolated from mean competition curves obtained from at least three different experiments.

[b] RBA: Relative binding affinity: IC_{50} LTB_4 / IC_{50} compound.

Shortening of the carboxylic chain (**1a** to **1b**), lenghtening of the lipidic tail (**1b** to **1c**) and introduction of a phenyl group at the end of the lipophilic chain, in order to obviate ω-oxydation[29] as for LTB_4[30], led to the stable compound **1d** which exhibited substantial affinity for LTB_4 receptor (RBA=1/100). The first eluted enantiomer of **1d** in chiral HPLC (**1d1**) elicited as expected a higher affinity than the racemic **1d**, the other enantiomer (**1d2**) only demonstrating a very weak affinity for the receptor.

The antagonist properties were then evaluated against LTB_4-induced human neutrophils chemotaxis[31] and guinea pig lung parenchyma contraction[32]. It was very encouraging that none of the four isomers **1-4** demonstrated any significant LTB_4 receptor agonist activity at concentrations up to 50 μM, in opposition with U-75302[14], SC-45694[33], and potent disubstituted pyridines analogues[34], which were found to be partial functional agonists for LTB_4 receptor.

Compound **1d** inhibited the LTB_4-induced chemotaxis (pKB: 6.5) and the contractile effect of LTB_4 on guinea pig lung parenchyma strips (IC_{50}: 40nM). In the PMA (phorbol 12-myristate 13-acetate)-induced ear oedema test[35], **1d** exhibited a potent anti-inflammatory activity when topically applied on the skin (EC_{50} = 10

μg/ear). This effect is likely related to LTB_4 antagonism, since **1d** has been shown not to modify either cyclooxygenase or lipoxygenase activities[36] at the highest tested concentration (10^{-5} M).

In conclusion the replacement of the unstable triene unit of the natural eicosanoid for a cyclohexylic ring afforded compounds eliciting good affinities for the LTB_4 receptor and interesting antagonist activities. The designed compounds being stable rigid mimics of the natural ligand, they may provide useful tools which could lead to significant advances in our knowledge of the tridimensional structure of the receptor itself.

Finally, in this new series of 1, 3-disubstituted cyclohexane compounds, the resolution of the racemic mixture of compound **1d** afforded one enantiomer (**1d1**) being 200 fold more active than the other one (**1d2**). Work is currently under investigation in order to determine the absolute configuration of the enantiomerically pure **1d1** and try to correlate it to the known configuration of LTB_4. This attractive compound has been selected for further development and is currently subjected to functional modifications.

Acknowledgements:

Financial support and PhD fellowship to J.M. Poudrel from the Centre National de la Recherche Scientifique and Servier/ADIR Co. are gratefully acknowledged. The authors would also like to thank Mr Alain Chabaud for excellent technical support, Miss Christèle Glot for biological test assistance, Professor C. Roussel (URA-CNRS 1410, Marseille) for kind advices and Miss C. Popescu for preliminary tests on chiral chromatography columns.

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18. All new compounds exhibited satisfactory ^{1}H and ^{13}C NMR, IR, elemental analysis and/or high resolution mass spectra data. Diastereomeric mixtures of compounds **1**, **2**, **3** and **4** were not separated, except **1d**.
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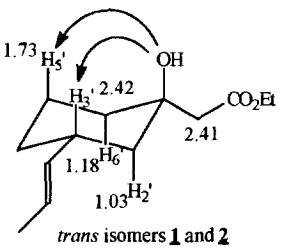
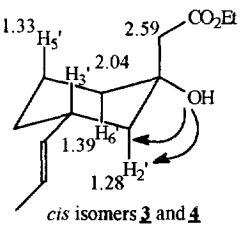
25. The respective *cis* and *trans* configurations were easily attributed, using the influence of the hydroxyl group position on the axial protons chemical shifts in ^{1}H NMR ($CDCl_3$, 360 Mhz).



trans isomers **1** and **2**



cis isomers **3** and **4**

Due to the field effect of the oxygen atom, the axial cyclohexylic protons $H_{5'}$ and $H_{3'}$ are more deshelded when the hydroxyl function is loocked in axial position.

26. Spectral data for compound **1d**: I.R.: 3410, 1720, 1185, 960; ^{1}H-NMR ($CDCl_3$): 0.85 - 1.80 (m; 14H); 1.26 (t; 3H; J = 7.1 Hz); 2.41 (s; 2H); 2.42 (m; 1H); 2.59 (t; 2H; J = 7.7 Hz); 4.00 (q; 1H; J = 6.4 Hz); 4.16 (q; 2H; J = 7.1 Hz); 5.40 (dd; 1H; J = 15.5 Hz, J = 6.7 Hz); 5.52 (dd; 1H; J = 15.5 Hz, J = 6.4 Hz); 7.15 (m; 3H); 7.25 (m; 2H);
27. Column used: Chiralcel® OD (Daicel), 20 µm, 250×50 mm. UV detection at 210 nm. Elution (100 ml/min) with heptane/isopropanol (90/10) afforded successively the enantiomers **1d1** (13 min) and **1d2** (21 min).
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31. Isolated human neutrophils labelled with ^{51}Cr (1 µCi/10^6 cells) for 1h at 37°C, were suspended at 10^7 cells/ml in Hank's buffer supplemented with 1% bovine serum albumin. LTB_4 and competitors in Hank's buffer were added to the bottom half of Boyden-Keller chambers; two 3 µm cellulose nitrate filters were placed over each well and the top part of the chambers was filled with cell suspension. The chambers were incubated at 37°C for 150 min. Radioactivity on the lower filter was measured by liquid scintillation spectrometry.
32. Strips of Dunkin Hartley guinea pig lung parenchyma were placed in organ baths containing oxygenated Tyrode solution at 37°C. Strips were recorded on a polygraph with an isometric tension of 400 mg. After a 1h equilibration mepyramine (1µM) and atropine (10 µM) were added to the Tyrode solution and the compound was tested for its agonist activity. The antagonist activities were tested by adding the drugs to the bath 2 min before LTB_4 (30 nM) stimulation.
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(*Received in Belgium* 12 *July* 1996; *accepted* 9 *September* 1996)