Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 811–814

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Leukotriene B_4 Photoaffinity Probes: Design, Synthesis and Evaluation of New Arylazide-1,3-Disubstituted Cyclohexanes

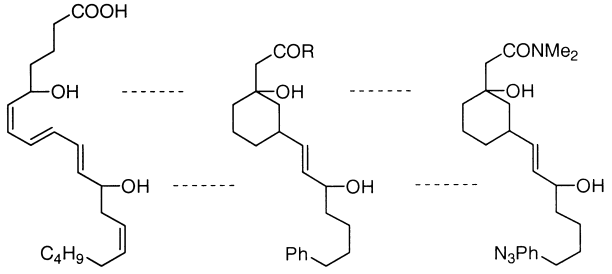
Denis Durand, Pierre Hullot, Jean-Pierre Vidal, Jean-Pierre Girard,* Jean Louis Banères, Joseph Parello, Agnès Muller, Claude Bonne and Jean-Claude Rossi

Laboratoire de Chimie Biomoléculaire et des Interactions Biologiques, UPRESA-CNRS 5074, Faculté de Pharmacie, 15 Avenue Flahault, 34060 Montpellier Cedex 2, France

Received 6 December 1999; accepted 10 February 2000

Abstract—The synthesis and the binding affinities of new leukotriene B_4 receptor photoaffinity probes, where a 1,3-disubstitued cyclohexane ring replaces the conjugated $\Delta^{6,7}$ and $\Delta^{8,9}$ double bonds of the natural eicosanoid, are described. One enantiomeric compound, **4bα**, is specifically cross-linked upon photolysis to the recombinant leukotriene B_4 receptor from human origin (h-BLTR) solubilized in a micellar medium. This probe appears as a good candidate for identifying the ligand binding site of this receptor.

Leukotriene B_4 (LTB_4), a product of 5-lipoxygenase induced arachidonic acid metabolism, has been shown to be a potent mediator of the inflammatory process and to be involved in the progression of a variety of inflammatory diseases.[1–12] At the cellular level, LTB_4 exerts its effect through recruitment and activation of neutrophils and eosinophils, through binding to a cell-surface receptor. Recently, the cloning of the complementary DNA (cDNA) encoding a cell-surface receptor of LTB_4 that is highly expressed in human leukocytes has been reported.[13] The amino acid sequence of this protein h-BLTR[14] is suggestive of a G-protein coupled receptor with seven transmembrane helices. We were particularly interested in mapping the BLTR ligand binding site through photoaffinity labelling. We have recently reported[15] the synthesis and structure–activity relationship of a novel series of compounds where a 1,3-disubstituted cyclohexane ring replaces two of the conjugated double bonds of the natural eicosanoid. The synthesized enantiomeric compounds, **1bα** and **2bα**, are more stable and more rigid mimics of the natural ligand and display good affinities ($IC_{50} = 0.3$ and 0.04 μM, respectively) for the LTB_4 receptor in human neutrophils (see Table 1).



We have now focused on the use of aryl azides[16] as photoreactive nitrene precursors which are structurally related to the LTB_4 antagonists **1bα** and **2bα** with the terminus of their lower chains replaced by a benzyl azide group. It was anticiped that these probes would bind to h-BLTR and establish a covalent crosslink with the receptor upon photolysis of the aryl azide, thus allowing us to map the h-BLTR ligand binding site. We describe here our initial efforts in this respect with the synthesis of the racemic diastereoisomers **3**, **4**, **5** and **6**, and the isolation of the enantiomers **4bα**, **4bβ** and **6bα**, **6bβ**.

Chemistry

The general synthetic route to compounds **3**–**6** is illustrated in Schemes 1 and 2. The ω chain of these compounds was introduced in good yields on the cyclohexylic

*Corresponding author. Fax: +33-6754-8625; e-mail: girard@pharma.univ-montpl.fr

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00103-7

ring by the Wadsworth–Horner–Emmons reaction between the crude unstable aldehyde[15] **12** and the ylide of the suitable β-ketophosphonate **11** (Scheme 1). This reaction gives **13** in 75% yield.

The reduction of the known keto-ester[15] to the keto-aldehyde **12** with diisobutylaluminium hydride at −78 °C was monitored by gas chromatography in order to control the formation of alcohol (overreduction). As soon as traces of alcohol appeared (after 30 min), the resulting crude aldehyde **12** was added at −40 °C on the ylide of the β-ketophosphonate **11** (NaH, THF). Compound **11** was prepared in five steps (Scheme 1). The first step, a Wittig reaction between *p*-nitrobenzaldehyde and the phosphonium salt **7**, afforded compound **8** in 62% yield. After esterification in acidic conditions, the protected amine **10** was obtained by successive hydrogenation and protection with trityl chloride in 90% yield. Addition of **10** to the ylide of methyldiethylphosphonate, generated in the presence of *n*-butyllithium, in a mixture of toluene/THF, gave the diethyl 2-oxo-6-(4-tritylamino)phenylhexylphosphonate **11** in 85% yield. The enone **13** was then reduced to the enol **14** by treatment with sodium borohydride in the presence of cerium chloride,[17] to avoid simultaneous hydroboration of the double bond. The latter was used as a precursor for synthesizing compounds **3** to **6**.

After deprotection of both carbonyl and amine functions under acidic conditions (Scheme 2), **15** reacted with $NaNO_2$ and NaN_3 to give, in a mixture of MeOH/HCl at 0 °C, the azido compound **16** in 95% yield.

The condensation of the ethyl acetate carbanion (LDA at −78 °C) afforded four diastereoisomers, as a mixture in 62% yield of two *trans* configuration (racemate **3a** and **3b**) and two *cis* configuration (racemate **3c** and **3d**). Using the same strategy, the condensation of the *N*,*N*-dimethylacetamide carbanion (LDA at −60 °C) gave four diastereoisomers **4a**–**d** in 90% yield. These compounds were isolated by column chromatography and their relative *trans* and *cis* configurations were readily established by ^1H NMR, using the influence of the hydroxyl group at C1 on the proton chemical shifts[15] of the cyclohexane ring.

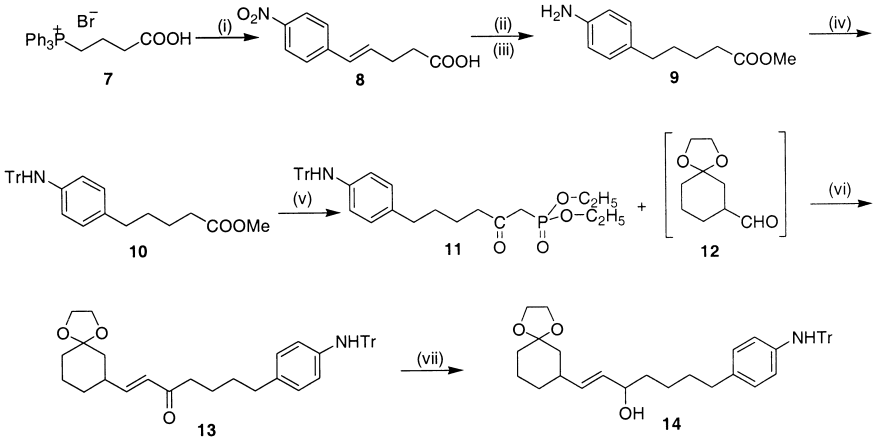
We also report the synthesis of two iodine substituted compounds **5** and **6** from precursor **15** (Scheme 2). The latter was treated with Chloramine T and sodium iodide, in a mixture of acetic acid/water, to give **17** in 55% yield. The phenyl amine was oxidized with sodium nitrite in aqueous HCl and methanol at 0 °C and treated with NaN_3 to afford **18** in 95% yield. Using the same strategy, we previously reported that the condensation of the ethyl acetate carbanion (LDA at −78 °C) to **16** afforded four diastereoisomeric compounds, two *trans* and two *cis* compounds (**5a**–**d**) as racemic mixtures, in 70% yield. Similarly, the condensation of the *N*,*N*-dimethylacetamide carbanion (LDA at −60 °C) gave four diastereoisomers **6a**–**d** in 89% yield. Each diastereoisomer was isolated by preparative HPLC[18a] chromatography.

At this stage, all the racemic compounds **3a**–**d** and **5a**–**d** were converted into their sodium salts (NaOH, methanol) for biochemical/pharmacological testing (see below). The racemic compounds **4b** and **6b** were resolved by chiral HPLC[18b] to afford by order of elution the enantiomers **4bα**[19] and **4bβ** for the former, **6bα** and **6bβ** for the latter.

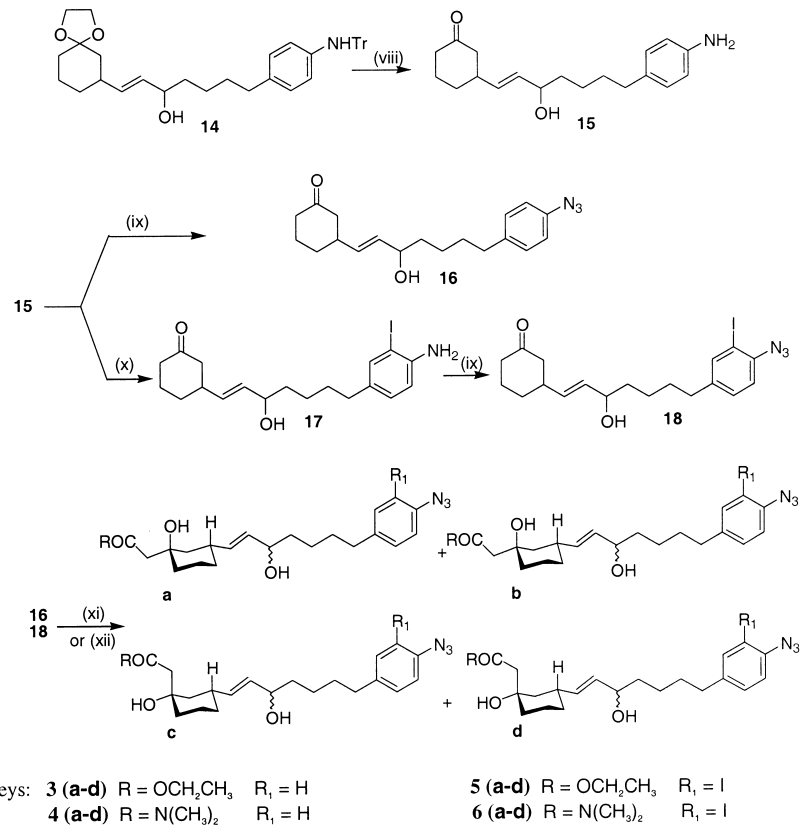
Biological Results

The diastereoisomers **3a**–**d** to **6a**–**d**, used here as racemic mixtures, were tested for their ability to inhibit the binding of LTB_4 to human neutrophil membranes.[20–22] In each series (**1** to **6**), the HPLC more polar *trans* diastereoisomers **b** exhibited the highest competitive effect (Table 1) compared to compounds **a**, **c** and **d**, which also competed for $[^3H]LTB_4$ but with lower affinities (IC_{50} values not given).

We note that incorporation of a *p*-azido group is systematically associated with a reduced affinity. Furthermore, the iodination in the *meta*-position results in a



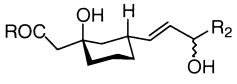
Scheme 1. Reagents and conditions (isolated yield): (i) *p*-nitrobenzaldehyde, *t*-BuOK, THF (62%); (ii) MeOH, H_2SO_4 (98%); (iii) H_2, Pd/C 5%, 55 psi (100%); (iv) TrCl, Pyridine (90%); (v) $(C_2H_5O)_2P(O)–CH_3$, *n*-BuLi, Toluene/THF (85%); (vi) NaH, −40 °C (75%); (vii) $NaBH_4$, $CeCl_3$, Methanol (99%).



Scheme 2. Reagents and conditions (isolated yield): (viii) HCl 1N, THF (98%); (ix) $NaNO_2$, NaN_3, MeOH, 0 °C (95%); (x) NaI, Chloramine T, AcOH:H_2O 9:1 (55%); (xi) Ethyl acetate, LDA, THF, −78 °C (62 to 70%); (xii) *N*,*N*-dimethylacetamide, LDA, THF, −60 °C (90%).

further decrease of the affinity. This decrease is significantly marked for the amide series **6a**–**d**. The first eluted enantiomers from racemates **4b** and **6b** by chiral HPLC (**4bα** and **6bα**) resulted, as expected, in higher affinities than the racemic **4b** and **6b**. The other enantiomers (**4bβ** and **6bβ**) only demonstrated a very weak affinity for the receptor.

Table 1. Binding of the synthetic competitors to the LTB_4 receptor in human neutrophils



Compound	R	R_2	IC_{50} (μM)[a]
1bα	OH[b]	$(CH_2)_4Ph$	0.3[15]
2bα	$N(CH_3)_2$	$(CH_2)_4Ph$	0.04[15]
3b	OH[b]	$(CH_2)_4PhN_3$	2
4b	$N(CH_3)_2$	$(CH_2)_4PhN_3$	2
4bα	$N(CH_3)_2$	$(CH_2)_4PhN_3$	0.7
5b	OH[b]	$(CH_2)_4PhN_3I$	>10
6b	$N(CH_3)_2$	$(CH_2)_4PhN_3I$	>10
6bα	$N(CH_3)_2$	$(CH_2)_4PhN_3I$	6

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

The enantiomer **4bα** was then used to define the ligand binding site of h-BLTR. This receptor has been recently produced in our laboratory as a recombinant protein in *Escherichia coli* and isolated as a micelle-solubilized protein. The latter was characterized by circular dichroism thus affording 45–50% of helical residues in agreement with a protein including seven transmembrane helices (labelled TM-I to TM-VII). Photolysis[23] of **4bα** in the presence of h-BLTR at 1:1 molar ratio resulted in covalent cross-linking of about 40% of the photoactivable antagonist to the receptor, as established by electrospray mass spectrometry based on measurements with the reaction mixture of the photoadduct and the unreacted protein. Analysis combining amino acid sequencing and mass spectrometry of the generated h-BLTR/**4bα** photolabelled peptides, after trypsin proteolysis, establishes that cross-linking occurs at the level of specific residues in two of the seven putative transmembrane helices, namely Cys 97, Ser 100, Met 101 and Ser 104 in TM-III, as well as Trp 234 and Tyr 237 in TM-VI. Our preliminary results with the isolated receptor thus establish that compound **4bα** is a good candidate for mapping the ligand binding site of the LTB_4 membrane receptor (full details to be published elsewhere). The iodinated compound **6bα**, although displaying a reduced affinity (Table 1), is intended as a radiolabelled probe to investigate the potential heterogeneity of the LTB_4 receptor.

References and Notes

1. Ford-Hutchinson, A. W.; Bray, M.; Doig, M.; Schipley, M.; Smith, M. J. *Nature* **1980**, *286*, 264.
2. Goldman, D. W.; Gifford, L. A.; Marotti, T.; Koo, C. H.; Goetzl, E. J. *Fed. Proc.* **1987**, *46*, 200.
3. Palmblad, J.; Malmster, C.; Uden, A.; Radmark, O.; Engstedt, L.; Samuelson, B. *Blood* **1981**, *58*, 658.
4. Showell, H. J.; Naccache, P. H.; Borgeat, P.; Picard, S.; Vallerand, P.; Becher, E. L.; Sha'afi, R. I. *J. Immunol.* **1982**, *128*, 811.
5. Kragballe, K.; Voorhees, J. *Acta Derm. Venereol.* **1985**, (Suppl. 120), 12.
6. Stenson, D. W. *J. Gastroenterol.* **1990**, *25* (Suppl. 172), 13.
7. Davidson, E. M.; Rae, S. A.; Smith, M. J. H. *Annu. Rheum. Dis.* **1983**, *43*, 677.
8. Rae, S. A.; Davidson, E. M.; Smith, M. J. H. *Lancet* **1982**, *2*, 1122.
9. Wardlaw, J. J.; Hay, H.; Cromwell, O.; Collins, J. V.; Kay, A. B. *J. Allergy Clin. Immunol.* **1989**, *84*, 12.
10. Cromwell, O.; Walport, M. J.; Morris, H. R.; Taylor, G. W.; Hodson, M. E.; Batten, J.; Kay, A. B. *Lancet* **1981**, *2*, 164.
11. Antonelli, M.; Buffi, M.; De Blasi, R. A.; Crimi, G.; Conti, G.; Mattia, C.; Vivino, G.; Lenti, L.; Lombardi, D.; Dotta, A. *Intensive Care Med.* **1989**, *15*, 296.
12. Katsura, K.; Minamisawa, H.; Katayama, Y.; Shimizu, J.; Goto, T.; Urushiyama, K.; Terashi, A.; Kanda, Y.; Yoshino, Y. *Prostaglandins* **1988**, *36*, 655.
13. Yokomizo, T.; Izumi, I.; Chang, K.; Takuwa, Y.; Shimizu, T. *Nature* **1997**, *387*, 620.
14. Alexander, S. P. H.; Peters, J. A. *Trends Pharmacol. Sci.* (Ion Channel Nomenclature Supplement) **1999**, 53.
15. Poudrel, J. M.; Hullot, P.; Vidal, J. P.; Girard, J. P.; Rossi, J. C.; Muller, A.; Bonne, C. *J. Med. Chem.* **1999**, *42*, 5289.
16. For review: Bayley, H. *Photogenerated Reagents in Biochemistry and Molecular Biology*; Elsevier: New York, **1983**.
17. Gemal, A. L.; Luche, J. L. *J. Am. Chem. Soc.* **1981**, *103*, 5454.
18. (a) Column used: Licrospher® 5 μm, 250×10 mm. UV detection at 250 nm. Elution (6 mL/min) with cyclohexane: ethyl acetate (25:75) afforded successively the diastereoisomers **4a** (22 min), **4b** (30 min), **4c** (36 min) and **4d** (45 min); (b) Column used: Chiralcel® OD (Daicel), 5 μm, 250×10 mm. UV detection at 250 nm. Elution (1.35 mL/min) with heptane: isopropanol (70:30) afforded successively the enantiomers **4bα** (23 min) and **4bβ** (33 min).
19. Compound **4bα**: (1*R**,3*R**)-1-Hydroxy-3-((3*R***S**, E)-3-hydroxy-7-(4-azidophenyl)hept-1-en-1-yl)cyclohexane-1-*N*,*N*-dimethyl acetamide: ^{1}H NMR ($CDCl_3$, 360 MHz): 0.90 (m, 1H, $H_{4'a}$); 0.94 (t, 1H, $H_{2'a'}$ $J_{2',3'}=12.6$ Hz); 1.11 (m, 1H, $H_{6'a}$); 1.33 (m, 2H, $H_{5''}$); 1.39 (m, 2H, $H_{4''}$); 1.54 (m, 1H, $H_{5'e}$); 1.57 (m, 2H, $H_{6''}$); 1.64 à 1.87 (m, 4H, $H_{4'e'}$ $H_{5'a'}$ $H_{6'e'}$ $H_{2'e}$); 2.35 (s, 2H, H_2); 2.46 (m, 1H, $H_{3'}$); 2.56 (t, 2H, $H_{7''}$, $J_{7'',6''}=10.2$ Hz); 2.94 (s, 3H, Me); 2.98 (s, 3H, Me); 3.97 (q, 1H, $H_{3''}$, $J_{3'',2''}=J_{3'',4''}=6.5$ Hz); 5.14 (s, 1H, OH); 5.40 (q, 1H, $H_{2''}$, $J_{2'',1''}=15.7$ Hz, $J_{2'',3''}=6.5$ Hz); 5.52 (q, 1H, $H_{1''}$, $J_{1'',2''}=15.6$ Hz, $J_{1'',3'}=6.5$ Hz); 6.92 (d, 2H, $H_{9''}$, $J_{9'',10''}=8.6$ Hz); 7.13 (d, 2H, $H_{10''}$, $J_{10'',9''}=8.6$ Hz). ^{13}C NMR ($CDCl_3$, 90 MHz): 20.8 ($C_{5'}$), 24.9 ($C_{5''}$), 31.3 ($C_{6''}$), 32.2 ($C_{4'}$), 35.0 ($C_{3'}$), 35.0 (N-CH_3), 35.2 ($C_{7''}$), 37.1 ($C_{4''}$), 37.2 ($C_{6'}$), 37.2 (N-CH_3), 43.5 (C_2), 43.5 ($C_{2'}$), 69.9 ($C_{1'}$), 73.0 ($C_{3''}$), 118.8 ($2C_{10''}$), 129.7 ($2C_{9''}$), 130.1 ($C_{2''}$), 137.3 ($C_{1''}$), 138.3 ($C_{11''}$), 139.4 ($C_{8''}$), 182.1 (C_1). Mass spectrometry (FAB$^+$; GT; PM = 414): $m/z=437$ ($M+Na^+$; 10); 415 ($M+H^+$; 30); 397 (M-H_2O+H+; 20); 307 (M-2H_2O-CH_2CONMe; 15); 91 ($PhCH_2^+$; 35); 72 ($CONMe_2^+$, 55). UV λ_{max} (EtOH)nm (ε): 250 (13600).
20. Neutrophil isolation: Polymorphonuclear leukocytes (PMN) were purified from freshly drawn human blood by standard techniques using Dextran T500 sedimentation and centrifugation on Ficoll/Paque (Pharmacia) followed by hypotonic lysis of erythrocytes. The purified PMN were washed in HBSS (Hank's balanced salt solution, Sigma) without Ca^{++} and Mg^{++} and cell viability was assessed by trypan blue exclusion. [^{3}H] LTB_4 binding assays: Binding to PMN was performed in HBSS without Ca^{++} and Mg^{++} containing 5 mmol/l HEPES buffer. PMN (10^6 cells) were incubated 20 min at 4 °C with 1 nmol/L [^{3}H] LTB_4 (7.4 TBq/mmol from Amersham) in the presence or absence of competitors at various concentrations. Binding was determined by the filtration technique previously described.[22]
21. Lin, A. H.; Ruppel, P. L.; Gorman, R. R. *Prostaglandins* **1984**, *28*, 837–849.
22. Muller, A.; Ghiglieri-Bertez, C.; Modat, G.; Bonne, C. *Prostaglandin Leukotriene Med.* **1987**, *26*, 233.
23. Photolysis experiment: A mixture of h-BLTR and **4bα** at 1 μM each was incubated at 4 °C for 30 min and then irradiated for 20 min at 4 °C using a 150-watt ultraviolet lamp (Heraeus TQ 150 Z3) with a 3 mm thick glass plate as a filter (active emission spectrum: λ >315 nm).