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Synthesis and Biological Evaluation of New LTA4 Analogs

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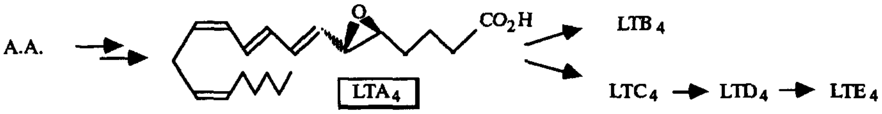
Abstract : New stable leukotriene A_4 analogs with the epoxide ring replaced by isoxazole or isoxazoline have been synthesized. These derivatives have proven to be weak leukotriene biosynthesis inhibitors or receptor antagonists.

Leukotriene A_4 (LTA_4) is produced from Arachidonic Acid (AA) in human leukocytes by the 5-lipoxygenase pathway. It is converted by LTA_4 hydrolase into the proinflammatory LTB_4, or alternatively by Glutathione-S-transferase and transpeptidases into LTC_4, LTD_4 and LTE_4, which are potent spasmogenic agents implicated in the pathology of allergic diseases (1). Since LTA_4 is the *last common intermediate* in this 5-LO pathway it appeared as a particularly attractive model for the design of new leukotriene biosynthesis inhibitors or receptor antagonists.



Various LTA_4 analogs have already been described (2) : - LTA_3, for instance, is a potent LTA_4 hydrolase irreversible inhibitor (3) and several other derivatives containing the allylic epoxide moiety have also been reported (2) ; - epithio corresponding to LTA_4 has been described as moderately potent (4) and aziridine was also reported without mention of its biological properties (5) ; - an acyclic LTA_4 analog lacking the epoxide ring was also shown to be a 5-LO inhibitor (6) ; - replacement of the labile oxirane by a cyclopropane yielded the stable methano LTA_4, this compound being also a 5-LO inhibitor (4). Modulations on both lateral chains did not improve the potency of these derivatives (2).

It is noteworthy that very few analogs where the oxirane is replaced by more stable five membered heterocycles have been reported until now. Of special interest are the dihydrofuran described as a weak inhibitor of LTB_4 formation (7) and thiophene derivatives recently reported as LTA_4 hydrolase inhibitors (8) and LTB_4 receptor antagonists (9).

As part of our research program in this field, we have prepared several new five-membered heterocyclic LTA_4 analogs as described in Figure 1.

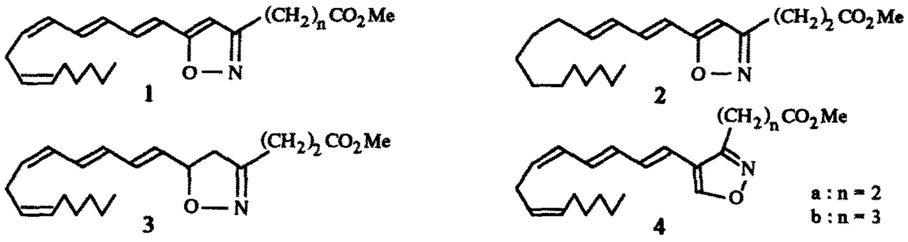
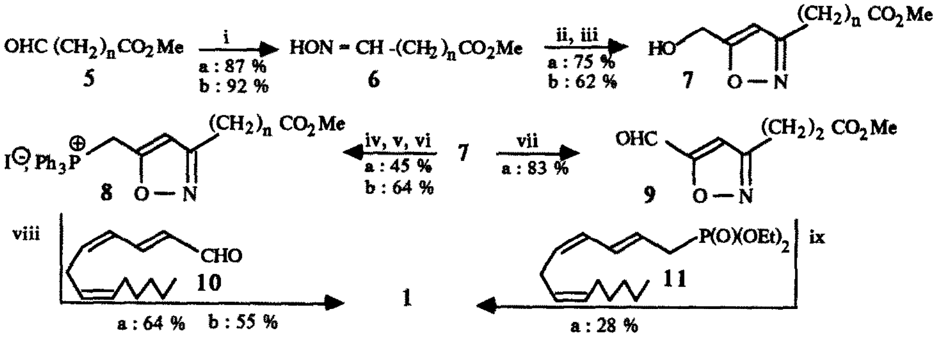


Figure 1

These compounds were synthesized by convergent strategies using Wittig reactions for the construction of the carbon framework with the double bonds located at the proper positions and 1,3-dipolar cycloadditions for the regioselective elaboration of the heterocycles. Furthermore, a new method for the synthesis of 3-substituted 4-formyl isoxazoles was developed during the preparation of analogs **4**.

Synthesis

3,5-Disubstituted isoxazoles are conveniently prepared by cycloadditions of nitrile oxides with monosubstituted alkynes (10), and this method was used for the preparation of analogs 1 (Scheme 1).

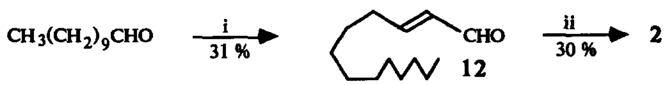


Scheme 1 : i : NH_2OH, HCl, Na_2CO_3, H_2O, RT (a : 90 min, b : 2 h.) ; ii : Cl_2, $CHCl_3$, -30°C ; iii : $H\text{-}C\equiv C\text{-}CH_2OH$, Et_3N, Tol., RT (a : 45 min, b : 90 min) ; iv : TsCl, KOH, ether, 0°C, 90 min ; v : NaI, acetone, 30°C (a : 17 h., b : 25 min) ; vi : Ph_3P, CH_3CN reflux (a : 14 h., b : 3 h.) ; vii : PCC, 3Å mol. sieves, CH_2Cl_2, RT, 30 min) ; viii : LDA, THF, 0°C, 15 min then **10**, -10°C, 1 h. ; ix : n-BuLi, -80°C, 30 min, then **9**, -80°C to RT in 1 h., then RT 20 h..

The crude chloroximes obtained by chlorination of oximes **6** were directly reacted with triethylamine in the presence of propargyl alcohol, giving isoxazoles **7** as the only isolated cycloadducts. The high regioselectivity in these cycloadditions is in agreement with literature data (11). Starting from these key intermediates, two different routes were followed. In the first one, isoxazoles **7** were transformed, in three classical steps, into the phosphonium salts **8**. The corresponding ylides reacted with dienal **10** (12) to give the desired analogs **1** (overall yields from **5** : **1a** : 19 % ; **1b** : 20 %) ; these compounds were obtained as (≈ 1/1) mixtures of E and Z isomers at the C_6-C_7 double bond and separated by HPLC : column Merck Hibar Si60 7 µm (250-10), eluent ethylacetate-petroleum ether 1: 9, 4 mL/min, $\lambda = 280$ nm. The second route was more selective : oxidation of isoxazole **7a** gave aldehyde **9a** which reacted with the carbanion of phosphonate **11** (13) to give **1a**, essentially as the E isomer, albeit in a lower yield.

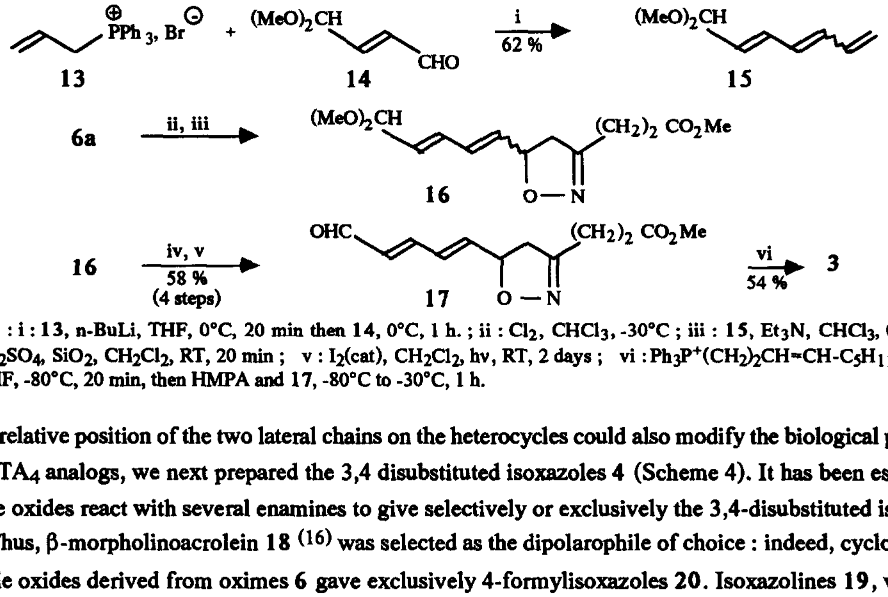
The tetrahydro derivative **2** was also prepared in order to evaluate the possible role of the two terminal double bonds on the biological activity. This compound was synthesized (Scheme 2) by reaction of the ylide derived

from phosphonium salt 8a with enal 12. Analog 2 was obtained again as a mixture of E,E and E,Z isomers separated by flash chromatography on SiO_2 (60 % overall yield).



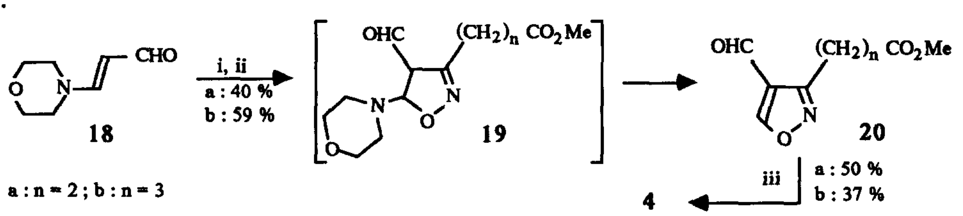
Scheme 2 : i : $Ph_3PCHCHO$, Tol., 80°C, 14 h. ; ii : **8a**, LDA, THF, 0°C, 15 min, then **12**, -10°C, 1 h.

Since the hybridization of the C_6 carbon atom could eventually be of importance for the biological activity of LTA_4 analogs of this type, we prepared also the corresponding isoxazoline **3** (Scheme 3). Triene **15**, easily accessible as a (≈ 1/1) mixture of E,E and E,Z isomers from **14** (14), was chosen as the starting material. The cycloaddition of nitrile oxide derived from **6a** took place exclusively on the less substituted double bonds, in agreement with literature data on similar reactions (10). After acetal hydrolysis and isomerization by iodine to the thermodynamically more stable E,E dienal **17**, a final Wittig reaction gave the desired analog **3** in a 31 % overall yield from **6a**.



Scheme 3 : i : **13**, n-BuLi, THF, 0°C, 20 min then **14**, 0°C, 1 h. ; ii : Cl_2, $CHCl_3$, -30°C ; iii : **15**, Et_3N, $CHCl_3$, 0°C, 2 h. ; iv : 5 % H_2SO_4, SiO_2, CH_2Cl_2, RT, 20 min ; v : I_2(cat), CH_2Cl_2, hν, RT, 2 days ; vi : $Ph_3P^+(CH_2)_2CH{=}CH\text{-}C_5H_{11}$, I^-, n-BuLi, THF, -80°C, 20 min, then HMPA and **17**, -80°C to -30°C, 1 h.

Since the relative position of the two lateral chains on the heterocycles could also modify the biological properties of such LTA_4 analogs, we next prepared the 3,4 disubstituted isoxazoles **4** (Scheme 4). It has been established that nitrile oxides react with several enamines to give selectively or exclusively the 3,4-disubstituted isoxazoles (10)(15). Thus, β-morpholinoacrolein **18** (16) was selected as the dipolarophile of choice : indeed, cycloadditions with nitrile oxides derived from oximes **6** gave exclusively 4-formylisoxazoles **20**. Isoxazolines **19**, which are the plausible intermediates during these reactions, have never been isolated nor spectroscopically detected. Condensation with the anion of phosphonate **11** led, after purification by flash-chromatography on SiO_2, to the desired analogs as the E isomers at the newly created double bonds (overall yields from **6** : **4a** : 20 % ; **4b** : 22%).



Scheme 4 : i : **6** and Cl_2, $CHCl_3$, -30°C then ii : **18**, Et_3N, $CHCl_3$, RT, 2 h. ; iii : **11** and LDA (for **4a**) or n-BuLi (for **4b**), THF, -80°C, 30 min, then **20**, -80°C to RT, 1 h., then RT (a : 6 h., b : 15 h.).

Biological studies

The biological activity of these new LTA_4 analogs has been studied on arachidonic acid metabolism in human polymorphonuclear leukocytes (PMN) stimulated by the calcium ionophore A23187 (17). All compounds have been assayed as their potassium salt derivatives after saponification of the ester moiety and the amount of both 5-HETE and LTB_4 have been measured in each case. All compounds appeared to be inactive at 10 μM and only derivatives **3**, **4a** and **4b** displayed a modest activity at 100 μM leading to a 50 to 70 % inhibition of the formation of 5-HETE and LTB_4.

Compounds were also tested for potential inhibition of LTD_4-induced contraction of isolated guinea pig ileum using a classical test system for evaluating LTD_4 antagonists (18). Compounds **3**, **4a** and **4b** were shown to inhibit LTD_4-induced contraction with IC_{50} of 9.5, 6.0 and 5.6 10^{-5} M respectively, while compounds 1 and 2 had no significant effect at 5.10^{-5}M.(19)

In conclusion, using convergent strategies we have synthesized new LTA_4 analogs with isoxazoles or isoxazolines instead of the labile epoxide ring. These derivatives are only weak leukotriene biosynthesis inhibitors or receptor antagonists.

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REFERENCES AND NOTES

1. For a recent comprehensive review see : *"Leukotrienes and Lipoxygenases, Chemical, Biological and Clinical Aspects"*, Rokach, J. Ed., Elsevier **1989**.
2. Fitzsimmons, B.J. ; Rokach, J. in *"Leukotrienes and Lipoxygenases, Chemical, Biological and Clinical Aspects"*, Rokach, J. Ed., Elsevier **1989**, 427-512 and references cited therein ; Bellamy, F.D. ; Coquelet C. ; Grée, R. , Mioskowski, C. ; Rossi, J.C. *Actualité Chimique*, Juin-Juillet **1986**, 13-27.
3. Evans, J. ; Nathaniel, D. ; Charleson, S. ; Leveille, C. ; Zamboni, R. ; Leblanc, Y. ; Frenette, R. ; Fitzsimmons, B.J. ; Leger, S. ; Hamel, P. ; Ford-Hutchinson, A.W. *Prostaglandins Leukotrienes Med.* **1986**, *23*, 167-171.
4. Arai, Y. ; Shimoji, K. ; Konno, M. ; Konishi, Y. ; Okuyama, S. ; Iguchi, S. ; Hayashi, M. ; Miyamoto, T. ; Toda, M. *J. Med. Chem.* **1983**, *26*, 72-78.
5. Zamboni, R. ; Rokach, J. *Tetrahedron Lett.* **1983**, *24*, 331-334.
6. Patterson Jr., J.W. ; Murthy, D.V.K. *J. Org. Chem.* **1983**, *48*, 4413-4415.
7. Corey, E.J. ; Su, W.G. *Tetrahedron Lett.* **1990**, *31*, 2089-2092.
8. Labaudinière, R. ; Hilboll, G. ; Leon-Lomeli, A. ; Lautenschläger, H.H. ; Parnham, M. ; Kuhl, P. ; Dereu, N. *J. Med. Chem.* **1992**, *35*, 3156-3169.
9. Labaudinière R. ; Hilboll, G. ; Leon-Lomeli, A. ; Terlain, B. ; Cavy, F. ; Parnham, M. ; Kuhl, P. ; Dereu, N. *J. Med. Chem.* **1992**, *35*, 3170-3179.
10. Caramella, P. ; Grünanger, P. in *"1,3 dipolar Cycloaddition Chemistry"* Padwa, A., Ed. John Wiley and sons **1984**, 291-392.
11. Bastide, J. ; Henri-Rousseau, O. *Bull. Soc. Chim. Fr.* **1973**, 2294-2296.
12. This compound is easily obtained in two steps (Wittig reaction and acetal hydrolysis, 80 % overall yield) from **14**.
13. This compound is conveniently prepared in three steps (reduction to alcohol, bromination and reaction with $P(OEt)_3$, 48 % overall yield) from **10**. See also Buck, J.C. ; Ellis, F. ; North, P.C. *Tetrahedron Lett.* **1982**, 4161-4162.
14. Grée, R. ; Tourbah, H. ; Carrié, R. *Tetrahedron Lett.* **1986**, *27*, 4983-4987.
15. Grundmann, G. *Synthesis* **1970**, 344-359 and references cited therein.
16. This compound is easily obtained (66 % yield) by addition of morpholine to propynal following : Ficini, J. ; Normant, H. *Bull. Soc. Chim. Fr.* **1964**, 1294-1298.
17. Billah, M.M. ; Bryant, R.W. ; Siegel, M.I. *J. Biol. Chem.* **1985**, *260*, 6899-6906.
18. Holroyde, M.C. ; Ghelani, A.M. *Eur. J. Pharmacol.* **1983**, *90*, 251-255.
19. The data for IC_{50} are derived from 3 or 4 separate experiments and the values are expressed as the mean ; the 95% confidence limits are for 3 (6.2-12.8 10^{-5}M), for **4a** (5.5-6.6 10^{-5}M) and for **4b** (4.4-6.9 10^{-5}M).

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