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PREPARATION OF A DIKETOPIPERAZINE ANALOG OF LEUKOTRIENE D_4 (LTD_4)

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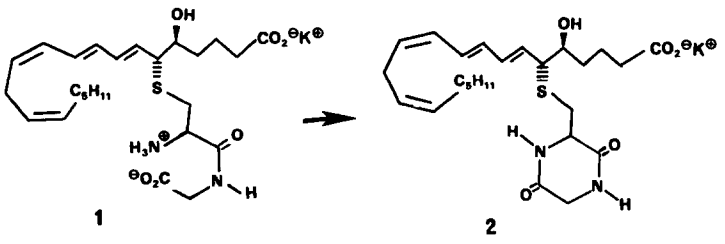
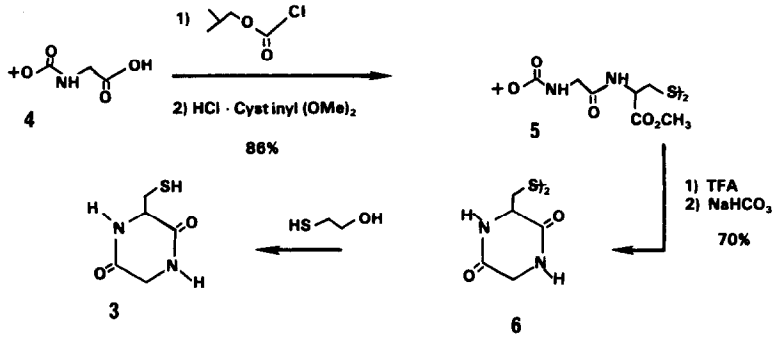
Summary: A LTD_4 analog has been synthesized in which the peptidyl fragment has been replaced by a nonionic diketopiperazine. Biological evaluation showed agonist activity, giving key information on LTD_4 geometry to the receptor.

The peptido-leukotrienes are a class of molecules which are extremely potent biologically.[1] Drugs which directly antagonize their effects and/or block their biosynthesis could potentially play a role in the treatment of several major diseases, e.g. asthma.[2] As part of our studies[3,4] directed towards the design of such drug candidates we were interested in what conformation the cysteinylglycyl portion of LTD_4 (1) would prefer while occupying a LTD_4 receptor.[5,6] Related to this question is the large body of work exploring the effect of changes in the peptide region on biological activity.[7] Studies, primarily by Lewis, Corey, et al[7b], revealed that a wide variety of peptide analogs are recognized as weaker agonists. However, in no case has the question of the geometry at the cysteinylglycyl amide link been probed. In order to explore this question we chose to prepare diketopiperazine (2) a cyclic analog of LTD_4. In this analog the amide linkage is restricted to the 'unnatural' cis form.



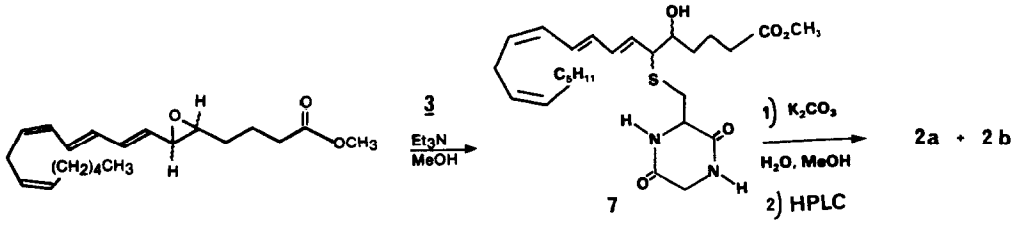
The required sulfhydryl precursor (3) was prepared in three steps from t-BOC protected glycine (4), as outlined in Scheme I.[8] Coupling of (4) with L-cystine dimethyl ester via the mixed carbonic anhydride afforded the cystinylglycyl derivative (5) in 85% yield.[10] Deprotection of (5) with trifluroacetic acid followed by cyclization with $NaHCO_3$, pH 8.5 in water, afforded the diketopiperazine disulfide (6) in 70% yield. Reduction of this disulfide with ß-mercaptoethanol produced the desired mercaptodiketopiperazine, (3).

Scheme I



At the time we did these studies we had access only to (+/-) LTA_4 methylester which was a 60/40 mixture of trans and cis epoxides.[3,9] Addition of sulfide (3) to this LTA_4 as outlined in Scheme II quantitatively afforded the diketopiperazine adduct (7) as an inseparable mixture of 5,6 diastereomers. Hydrolysis of the methyl esters with K_2CO_3/MeOH-H_2O afforded the corresponding diketopiperazine acids. These were readily separable during routine purification into two pairs of diastereomers (2a) and (2b) (HPLC: 25cm x 9.2mm ODS C_{18} 80/20 methanol/water).

Scheme II



These two pairs of compounds were evaluated in our guinea-pig tracheal spiral assay system.[3] We found that pair (2a) retained about 1/10 the agonist activity of LTD_4 whereas (2b) retained only about 1/1000 the activity of LTD_4.[11] Initially it was not possible to determine whether this response was due to an intrinsic activity of this molecule or if the compound was being hydrolytically cleaved to LTD_4.

Attempts to cleave open the diketopiperazine ring of either (2a) or (2b) by basic hydrolysis led only to general decomposition of the molecules (either excess K_2CO_3 or LiOH/MeOH-H_2O). Analysis of the reaction mixtures by HPLC revealed no LTD_4, or LTE_4. We believe that in preference to hydrolytic ring opening these conditions cause the diketopiperazine ring to eliminate the ß-sulfide linkage via a retro-Michael reaction. In addition recent reports from these laboratories have revealed that the apparent potency of LTD_4 in this assay system is increased approximately 10 fold if the amino-peptidase inhibitor L-cysteine (3 mM) is added to the assay system.[12] Addition of this inhibitor to the experiments in which either (2a) or (2b) were tested revealed no potentiation of the biological response. This suggested that LTD_4 was not being formed in vitro from (2a) or (2b). Furthermore the known leukotriene antagonist FPL-55712 specifically blocked the action of these compounds on guinea-pig trachea. These combined findings provide confidence that the biological effects induced by the diketopiperazines are direct and specific.

Previous studies had shown that when either the amine or (glycine) acid of LTD_4 where converted into amides the residual biological activity was less than 1/10 that of LTD_4.[7b] When both functionalities were blocked as amides the compounds were basically inactive. These diketopiperazines are essentially cyclic versions of the compound in which both the amine and acid position were blocked by amides. Because of the significant biological activity of (2a) we feel that cisoid geometry could be preferred at the cys-gly amide bond. This contrasts with the geometry normally ascribed to peptide amide bonds. Recent theoretical analyses of glycine containing dipeptides have shown that the energy difference between the cis and trans forms can be very small.[13] Indeed in some cases these calculations have shown that the zwitterionic form may prefer to be cis. Our discovery that diketopiperazines make good analogs of the peptide portion of the leukotrienes could provide an alternative approach to the preparation of stable nonpeptidyl leukotriene antagonists.

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REFERENCES

1. L. W. Chakrin and D. M. Bailey, "The Leukotrienes: Chemistry and Biology," Academic Press, 1984, New York
2. S. E. Dahlen, Acta Physiologica Scandinavica, Supp. 512, 1 (1983).
3. B. S. Tsai, P. Bernstein, R. A. Macia, J. Conaty, and R. D. Krell, Prostaglandins 23, 489(1982).
4. P. Loftus and P. R. Bernstein, J. Org. Chem., 48, 40(1983).
5. Both our published NMR studies (ref. 4) and additional unpublished ^{1}H NMR and ^{13}C NMR studies by Dr. C. Lerman (these laboratories) have not succeeded in defining a preferred solution conformation for this region of leukotriene D_4.
6. G. K. Hogaboom, S. Mong, H. L. Wu, and S. T. Crooke, Biochem. Biophys. Res. Comm. 116, 1136(1983).
7. a. R. D. Krell, F. J. Brown, A. K. Willard, and R. E. Giles, 'Pharmacological Antagonism of the Leukotrienes', pp 271-295 in ref. 1.
   b. R. A. Lewis, J. M. Drazen, K. F. Austen, M. Toda, F. Brion, A. Marfat, and E. J. Corey, Proc. Nat. Acad. Sci. 78, 4579(1981).
8. For an alternate approach to (3) see: F. Schneider and H. Wenek, Z. Physiol. Chem. 350, 1521(1968).
9. a. E. J. Corey, D. A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Samuelsson and S. Hammarstrom, J. Am. Chem. Soc., 102, 1436(1980).
   b. J. Rokach, Y. Girard, Y. Guindon, J. G. Atkinson, M. Larue, R. N. Young, P. Masson and G. Holme, Tet. Lett. 21, 1485(1980)
10. All compounds gave satisfactory spectral characterization, 250MHz ^{1}H NMR, IR, and where appropriate HPLC, UV, and/or MS. The leukotriene analogs were in general too unstable for elemental analysis, and/or MS.
11. The ED_{40} values were respectively: LTD_4=3.0 x 10^{-9}; isomers (2a)=3.7 x 10^{-8}; isomers (2b)=4.0 x 10^{-6}. Since analog (2a) is a mixture of two diastereomers and since both we and others (see ref. 3 and 7a) have shown that diastereomers other than the natural 5S,6R lack activity the relative potency of the 5S,6R diastereomer component of (2a) is probably 1/5 that of LTD_4.
12. D. W. Snyder, D. Aharony, P. Dobson, B. S. Tsai, and R. D. Krell, J. Pharm. Expt. Ther., 231, in press(1984).
13. L. R. Wright, R. F. Borkman, J. Phys. Chem., 86, 3956(1982).

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