Tetrahedron Letters, Vol.32, No.10, pp 1375-1378, 1991
Printed in Great Britain

0040-4039/91 $3.00 + .00
Pergamon Press plc

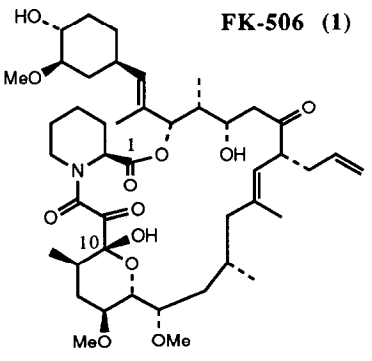
C10 N-ACYL MODIFIED FK-506: A POSSIBLE HYBRID ANALOGUE OF THE TRANSITION STATE OF PEPTIDYL-PROLYL *CIS-TRANS* ISOMERIZATION.

D.K. Donald,*[a] M. E. Cooper [a], M. Furber [a], E. Wells [b], R. Hutchinson [b] and F.M. Black [b]

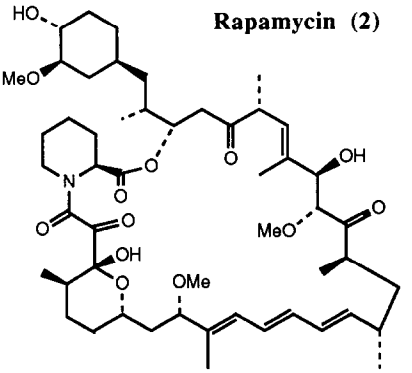
a) Department of Medicinal Chemistry and b) Department of Biochemistry,
Fisons PLC Pharmaceutical Division,
Bakewell Road, Loughborough, LE11 ORH, UK.

Abstract: Three C 10 N-Acyl derivatives of FK-506 have been prepared. The reduced receptor binding of these analogues is not consistent with a direct overlap of FK-506 and a peptide substrate.

The macrolide immunosuppressant FK-506 (1) has been the focus of considerable research effort since its structure was published in 1987 by Tanaka *et al* of the Fujisawa Pharmaceutical Company.[1] Its exceptional potency *in vitro*[2] and potential utility in human organ transplantation *in vivo*[3] have now been well documented. The masked 1,2,3 tricarbonyl structure of FK-506 (which is known in only one other natural product - the structurally related Rapamycin (2)[4]), has attracted considerable synthetic attention. Two total syntheses of FK-506 have been reported[5,6] as well as numerous syntheses of part structures of the molecule[7].



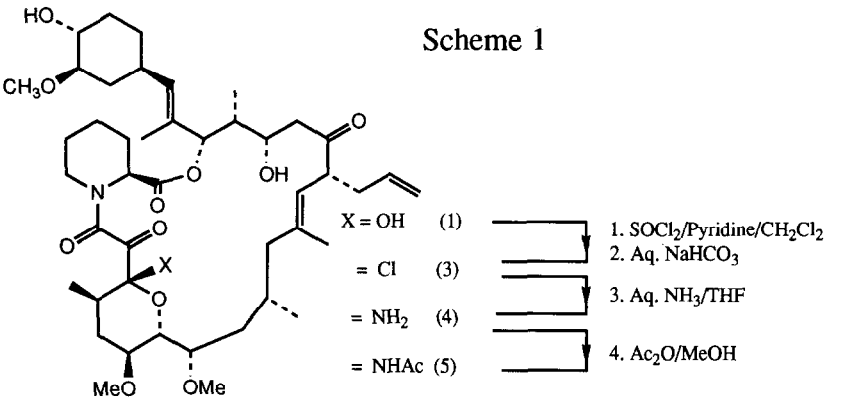
FK-506 (1)



Rapamycin (2)

A major aim of these studies is to identify what the structural features are in FK-506 which determine its biological activity and to understand the mechanism by which these lead to control of signal transduction in the T-lymphocyte. To this end the major cytosolic receptor for FK-506 has been identified,[8,9] sequenced, cloned[10] and overexpressed in E.

Scheme 1



Scheme 2

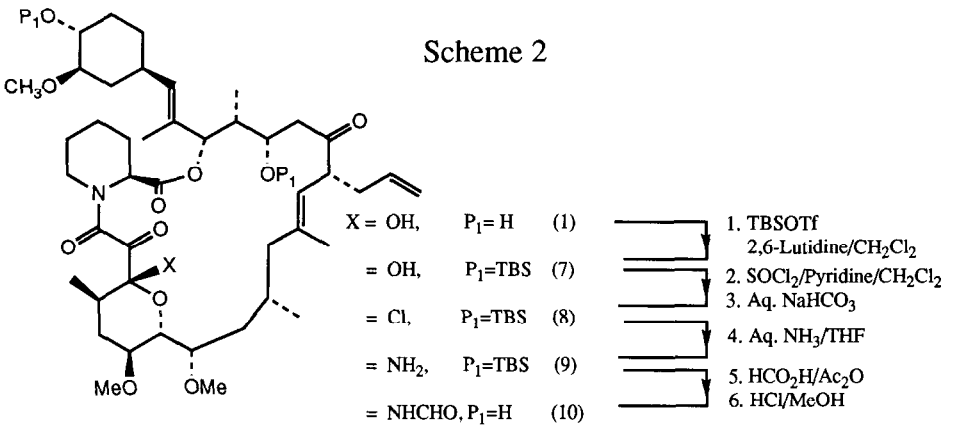


TABLE 1

COMPOUND	IC_{50} (M)
(1)	5×10^{-10}
(2)	5×10^{-10}
(4)	1.6×10^{-9}
(5)	3×10^{-7}
(6)	1.8×10^{-6}
(10)	5×10^{-7}

coli.[11] Like cyclophilin, the putative receptor for the immunosuppressant Cyclosporin,[12,13] the receptor has peptidyl-prolyl *cis-trans* isomerase activity.[8,9] That is it can catalyse *cis-trans* isomerization about tertiary amides such as those derived from proline. This could occur by nucleophilic addition of a group on the receptor onto the amide carbonyl of the substrate facilitating bond rotation.[14] Kinetic studies have indicated however that distortion of the amide bound substrate is the principal mechanism.[15] This has prompted ourselves and others[16] to speculate that the C1 to C10 region of FK-506, which is intimately involved in binding of FK-506 to its receptor, may be functioning as a transition state analogue of peptidyl *cis-trans* isomerization.

A possible correspondence between this section of FK-506 and a peptide substrate is shown in diagram 1 for the trans rotamer of FK-506.[17] In this the NH of the bound peptide was considered to be mimicked by the hydroxyl at C10 of FK-506 - an interaction that we have proved to be important from other studies.[18]

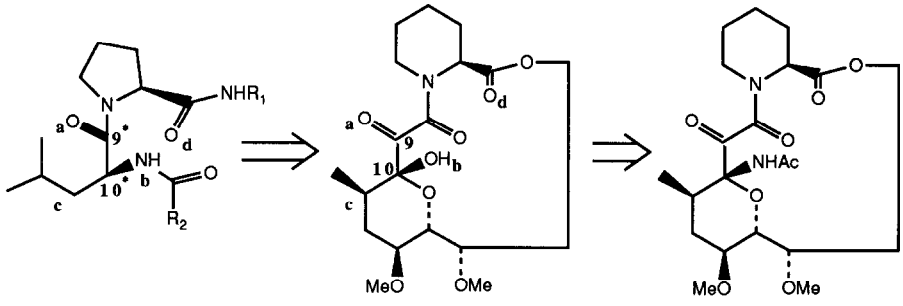


DIAGRAM 1

HYBRID STRUCTURE

To further explore this model we wished to make the hybrid structures (5) and (10) in which the N acyl group could mimic the extended peptide chain. The synthesis of compound (5) was accomplished in 4 steps starting from FK-506 as shown in scheme 1. Addition of a solution of FK-506 in dry dichloromethane to thionyl chloride and pyridine in dry dichloromethane under nitrogen at room temperature and subsequent aqueous hydrolysis and work-up gave crude C10 chloro FK-506 (3) in good yield. This material could be further purified by chromatography on silica but in the present case was used directly without further purification. Treatment of crude C10 chloro FK-506 with concentrated aqueous ammonia in THF followed by acetic anhydride in methanol then gave the desired compound (5) and its C10 epimer (6) in 13 and 9% overall yield from FK-506 after extensive chromatography. The N formyl compound (10) was prepared in a similar manner. Treatment of the C10 amino C24,32 bis TBS protected FK-506 (9) with formic acid/acetic anhydride followed by deprotection with dilute hydrochloric acid in methanol gave (10) in 31% overall yield from (9), (Scheme 2).

Neither (5) , (6) nor (10) showed appreciable binding in the rat cytosolic receptor assay, (Table 1).[19] The amino compound (4) however still bound with high affinity to the receptor indicating that the reduction in binding in (5), (6) and (10) was unlikely to be due to the difference in hydrogen bonding ability between an amide and an N-acylated aminal. A more plausible explanation for the reduction in binding in (5), (6) and (10) is that there is insufficient space at this position in the receptor for an extended chain. The results therefore suggest the absence of a direct overlap between FK-506 and a peptide substrate. A peptide substrate could in principle however bind to the same region of the receptor but in a different orientation from that which is available in hybrid structures such as (5) and (10). Thus rotation of a peptide relative to FK-506 in the binding site whilst still maintaining the same principal interactions could position the C-terminal section of a peptide into an allowable space on the receptor - for example that space which is normally occupied by other parts of the FK-506 molecule. Further work is in progress in our laboratories to try and clarify this issue.

References

1. Tanaka, H., Kuroda, A., Marusawa, H., Hatanaka, H., Kino, T., Goto, T., Hashimoto, M. and Taga, T., J. Amer. Chem. Soc., **109**, 5031-5033, (1987).
2. Kino, T., Inamura, N., Sakai, F., Nakahara, K., Goto, T., Okuhara, M., Kohsaka, M., Aoki, H. and Ochiai, T., Transplantation Proceedings, **19**, (5, Suppl. 6), 36-39, (1987).
3. Starzl, T.E., Todo, S., Fung, J., Demetris, A.J., Venkataraman, R. and Jain, A.,The Lancet, **2**, 1000-1004, (1989).
4. Vezina, C., Kudelski, A. and Sehgal, S.N., J. Antibiot. (Tokyo), **28**, 721-726, (1975).
5. Jones, T.K., Mills, S.G., Reamer, R.A., Askin, D., Desmond, R., Volante, R.P. and Shinkai, I., J. Amer. Chem. Soc., **111**, 1157-1159, (1989).
6. Nakatsuka, M., Ragan, J.A., Samakia,T., Smith, D.B., Uehling, D.E. and Schreiber, S.L., J. Amer. Chem. Soc., **112**, 5583-5601, (1990).
7. Of most relevance to the present discussion are syntheses of the C1 to C10 section of FK-506. These include
   a) Williams, D.R. and Benbow, J.W., J. Org. Chem., **53**, 4643-4644, (1988).
   b) Kocienski, P., Stocks, M., Donald, D., Cooper, M. and Manners, A., Tetrahedron Lett., **29**, 4481-4484, (1988).
   c) Egbertson, M. and Danishefsky, S.J., J. Org. Chem., **54**, 11-12, (1989).
   d) Wasserman, H.H., Rotello, V.M., Williams, D.R. and Benbow, J.W., J. Org. Chem., **54**, 2785-2786, (1989).
   e) Ireland, R.E. and Wipf, P., Tetrahedron Lett., **30**, 919-922, (1989).
   f) Rama Rao, A.V., Chakraborty, T.K. and Laxma Reddy, K., Tetrahedron Lett., **31**, 1439-1442, (1990).
8. Harding, M.W., Galat, A., Uehling, D.E. and Schreiber, S.L., Nature , **341**, 758-760, (1989).
9. Siekierka, J.J., Hung, S.H.Y., Poe, M., Lin, C.S. and Sigal, N.H., Nature, **341**, 755-757, (1989).
10. Maki, N., Sekiguchi, F., Nishimaki, J., Miwa, K., Hayano, T., Takahashi, N. and Suzuki, M., Proc. Natl. Acad. Sci. (U.S.A.), **87**, 5440-5443, (1990).
11. Standaert, R.F., Galat, A., Verdine, G.L. and Schreiber, S.L., Nature, **346**, 671-674, (1990).
12. Takahasi, N., Hayano, T. and Suzuki, M., Nature, **337**, 473-475, (1989).
13. Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T. and Schmid, F.X., Nature, **337**, 476-478, (1989).
14. Fischer, G., Berger, F. and Bang, H., FEBS Lett., **250**, 267-270, (1989).
15. Harrison, R.K. and Stein, R.L., Biochemistry, **29**, 1684-1689, (1990).
16. Rosen, M.K., Standaert, R.F., Galat, A., Nakatsuka, M. and Schreiber, S.L., Science, **248**, 863-866, (1990).
17. FK-506 exists as a mixture of rotamers about the amide bond in solution the composition of which is solvent dependent. NMR analysis in $CDCl_3$ gives a 3:2 mixture of *cis:trans* rotamers whilst crystallization from acetonitrile gave exclusively the *cis* rotamer - the X-ray structure of which has been published.[20] Binding of C13 doubly labelled FK-506 to its receptor has also shown that only one of the rotamers is bound, consistent with a specific interaction.[16] Although an overlap with the *trans* rotamer has been shown here an equally good overlap of regions a,b and c can be made for the *cis* rotamer. Studies are in progress in our laboratory to unambiguously define which rotamer is the bound one.
18. Donald, D.K., Hardern, D.N., Furber, M., Cooper, M.E., Hutchinson, R. and Wells, E., Unpublished results.
19. Siekierka, J.J., Staruch, M.J., Hung, S.H.Y. and Sigal, N.H., J.Immunol., **143**, 1580-1583, (1989).
20. Taga, T., Tanaka, H., Goto, T. and Tada, S., Acta. Crystallogr., Sect. C. : Cryst. Struct. Commun., **C43**, 751-753, (1987).

(Received in UK 30 November 1990)