Pure & Appl. Chem., Vol. 68, No. 3, pp. 565–568, 1996.
Printed in Great Britain.

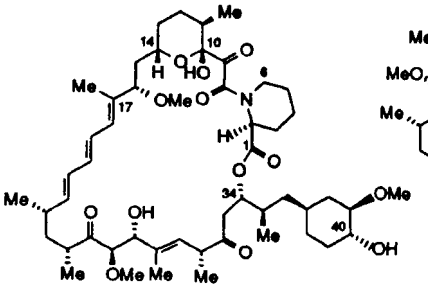
Studies directed towards the development of cyclic peptide-based analogs of macrolide immunosuppressants†

Tushar K Chakraborty

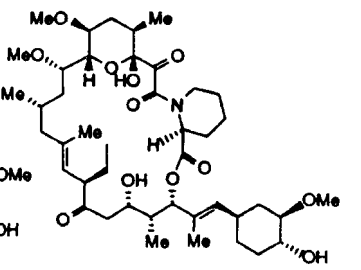
Indian Institute of Chemical Technology, Hyderabad 500 007, India

Abstract : A simple strategy for the facile synthesis of cyclic rapamycin-peptide hybrids is developed and the affinities of these synthetic hybrids for FKBP12 are evaluated. The results prompted to undertake a systematic study on the optimum length of the peptide cassette required for locking the binding domain of rapamycin in the specific conformation as present in its FKBP12-bound crystal structure. This led to the development of a high affinity FKBP12 ligand.

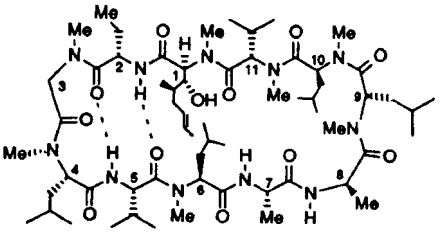
All macrocyclic immunosuppressants, like rapamycin (1) ascomycin (2), cyclosporin (3) etc., are of dual-domain nature which poses significant challenge to structure-based design of analogous ligands[1]. These immunosuppressants are possibly mimicking some endogenous peptidal substances which made us interested in designing and synthesizing cyclic peptide-based analogs of these natural products. These cyclic analogs will possess the binding domain



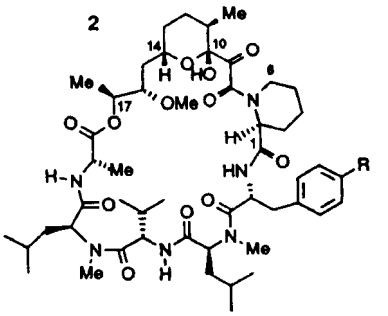
1



2



3

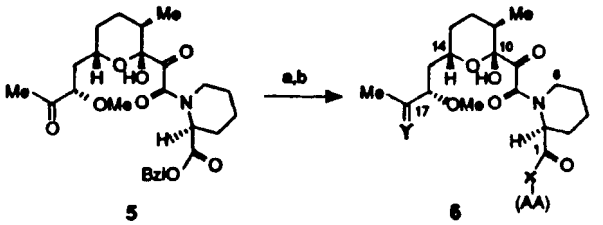


4a : R = H
b : R = OH

of rapamycin, C1-C17 segment whose two ends will be stitched to a suitable peptide-based effector domain. To start with we planned to use the effector domain of cyclosporin MeLeu-Val-MeLeu-Ala (residues 4-7)[2] in order to construct a bridging ligand **4** to bring FKBP12 and calcineurin together. Though it was understood that neither the binding domain of rapamycin nor the effector domain of cyclosporin would possibly retain their parent geometry

† The work presented was carried out at Sandoz Pharma Ltd., Basel

Scheme I. Synthesis of acyclic analog 6[a]



5 6

a : AA = L-Phe-OMe, X=NH, Y=O; b : AA=D-Phe-OMe, X=NH, Y=O;
c : AA=D-Tyr-OMe, X=NH, Y=O; d : AA=D-Phe-OBzl, X=NH, Y=O;
e : AA=L- -PheLac-MeLeu-Val-MeLeu-Ala-OBzl, X=O, Y=OH,H;
f : AA=L-Phe-MeLeu-Val-MeLeu-Ala-OBzl, X=NH, Y=OH,H.

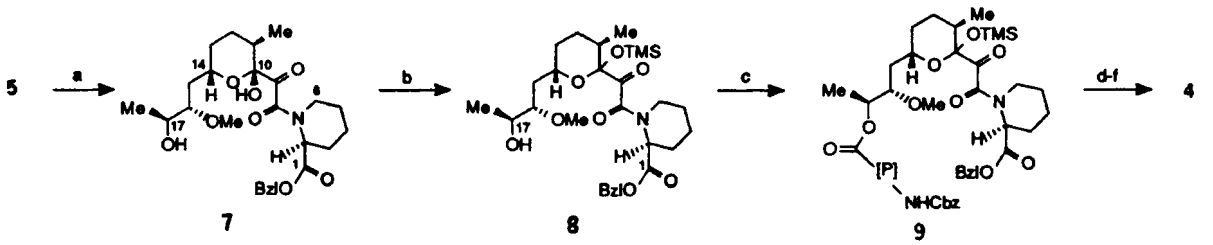
[a]Reagents and conditions : (a) H_2, Pd-C, MeOH, 20°C, quantitative yield; (b) HOBt (1.1 equiv.), EDCI.HCl(1.0 equiv.), DMF, 0°C, 15 min. and then C-end-protected amino acid (1.5 equiv.), 0°C, 2 h, 90-95%.

in this hybrid structure, the effort was thought worthwhile because the chemistry developed in the process could eventually lead to designing more efficient analogs.

A number of acyclic ligands (**6a-f**) were synthesized[3] first to search for the most suitable amino acid to replace the cyclohexyl moiety (Scheme I). The starting material for the syntheses of all these ligands was the benzyl ester **5**, itself synthesized in two simple steps from rapamycin[4]. Hydrogenation of **5** followed by coupling with respective C-terminal-protected amino acids gave the desired analogs **6a-d**[5a]. The binding studies of these ligands with FKBP12[6] (Table I) showed very weak binding for all of them. This was probably due to the greater entropic cost of binding such conformationally flexible acyclic molecules. Anyway, from the IC50 values it was decided to have a D-amino acid to replace the cyclohexyl moiety.

The syntheses of the cyclic analogs also started with **5** (Scheme II). Diastereoselective reduction of the methyl keto in **5** with lithium selectride at -78° gave a single product **7**[3], in 96% yield. Silylation of **7** with TMS-triflate followed by treatment with dilute acid gave the hemiketal-protected methyl carbinol **8** as a mixture (ca. 1:1) of two diastereomers

Scheme II. Synthesis of the cyclic analog 4[a]



7 8 9

a : -NH-[P]-CO- = -NH-[D-Phe-MeLeu-Val-MeLeu-Ala]-CO-
b : -NH-[P]-CO- = -NH-[D-Tyr(Bzl)-MeLeu-Val-MeLeu-Ala]-CO-

[a]Reagents and conditions : (a) L-Selectride (3 equiv.), THF, -78°C, 15 min, 96%; (b) TMS-triflate (2.2 equiv.), 2,6-lutidine (4 equiv.), CH_2Cl_2, 0°C, 15 min., followed by work-up and treatment with 0.1N HCl:THF (1:25), 15 min., 92%; (c) CbzNH-P-COOH (2 equiv.), DCC (2 equiv.), DMAP (0.2 equiv.), CH_2Cl_2, -10°C, 48 h, 85% (based on recovered starting material, ca. 60% conversion); (d) H_2, Pd-C, MeOH, 20°C, 1 h; (e) BOP-reagent (3 equiv.), diisopropylethylamine (7 equiv.), $CHCl_3$ (0.002M), 20°C, 4 h, 45% (from 9); (f) HF-Py, THF, 20°C, 24 h, 92%.

Table I. FKBP binding assays of the ligands[6]

Compound	IC_{50}(μM)	Compound	IC_{50}(μM)
5	50.0	**6e**	2.4
6a	16.0	**6f**	3.5
6b	2.4	**4a**	3.4
6c	7.9	**4b**	2.2
6d	5.5	**10**	0.0096

(92% yield). The mixture was then coupled with the peptide chain Cbz-D-Phe-MeLeu-Val-MeLeu-Ala-OH[5b] using DCC and catalytic amount of DMAP in CH_2Cl_2 at -10°C to avoid any possible epimerization[7] (82% yield based on recovered starting material). The resulting ester **9**, as a mixture of diastereomers, was hydrogenated and subjected to macrolactamization using BOP-reagent to get 45% yield of the desired cyclic product. Finally facile desilylation of the hemiketal hydroxyl furnished the cyclic analog **4a** in 92% yield as a mixture of rotamers (ca. 1:1 in DMSO). Following the same chemistry the D-Tyr-bearing analog **4b**[3] was also synthesized. The peptide fragment used in this case was Cbz-D-Tyr(Bzl)-MeLeu-Val-MeLeu-Ala-OH[5b].

The binding of these cyclic analogs **4a** and **4b** (Table I) did not show any dramatic improvement in their binding with FKBP12. In order to find out the reason for such weak binding, a detailed theoretical study was undertaken. The energy minimized structure of **4a** was superimposed on rapamycin in its FKBP12 bound X-ray structure[8]. MD simulation[9] was then carried out on the assembly comprising of **4a**, FKBP12 and 53 water molecules present in the X-ray structure for 20 ps during which time 20 frames were sampled at equal intervals and each frame was minimized using conjugate gradients. The resulting twenty minimized structures were then superimposed as shown in Figure I. This shows considerable deviations in the binding domain geometry during the simulation process which was due to the increased flexibility associated with the larger ring size. This large distortion in the binding domain conformation of **4a** explains why it showed a weak affinity for FKBP12.

When the same exercise was carried out with rapamycin bound to FKBP12 (Figure I), the binding domain conformation did not change at all during the whole simulation process, which was not surprising since rapamycin-FKBP12 X-ray structure was taken as the starting geometry which did not change much during a 20 ps MD simulation.

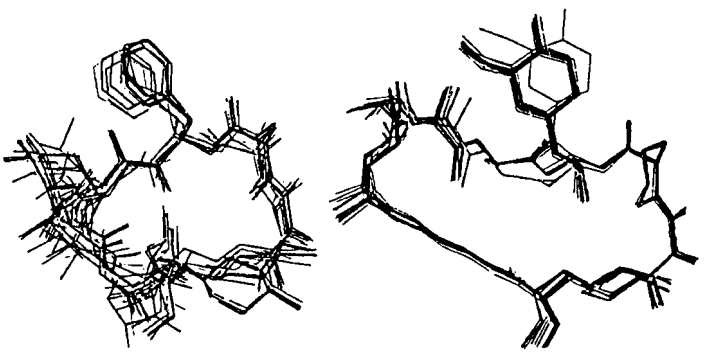
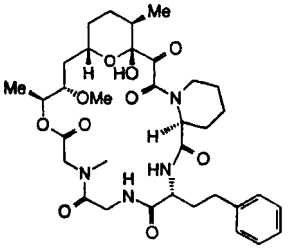


Figure I : Superimposition of 20 energy minimized structures of **4a** (left) and rapamycin (right) during 20 ps MD simulations on an assembly comprising of FKBP12, **4a** (or rapamycin) and 53 water molecules present in the FKBP12-rapamycin crystal structure. For clarity FKBP12 and water molecules are not shown.

This led us to believe that the conformation of the binding domain of rapamycin as present in its FKBP12 found X-ray structure should be targeted as an ideal conformation in order to achieve maximum binding with FKBP12. A systematic search was thus undertaken to find out the optimum chain length of the peptide cassette required to lock the binding domain in the desired conformation which was believed to be dependent on the ring size of these cyclic peptide-based analogs.



10

This finally led to the development of an excellent ligand **10** whose affinity for FKBP12 (IC50=9.6 nM) was almost comparable to those of natural products. The details of that study will be reported elsewhere.

Acknowledgments : I wish to express my sincere thanks to Prof. K.C. Nicolaou, Drs. R. Sedrani, S. Cottens, H. Fliri, M.D. Walkinshaw, H.P. Weber and R.M. Wenger for kind supports, useful discussions, and valuable suggestions; M. Ponelle for NMR: C. Quiquerez for MS; Drs G. Zenke and W. Schuler for binding assays.

References

1. a) P.J. Belshaw, S.D. Meyer, D.D. Johnson, D. Romo, Y. Ikeda, M. Andrus, D.G. Albery, L.W. Schultz, J. Clardy and S.L. Schreiber. Syn. Lett. 381 (1994) and references cited therein; b) K.K. Maggon. DN & P **7**, 389 (1994) and references cited therein.
2. The peptide residue MeLeu-Val-MeLeu-Ala-OBzl (residues 4 to 7 of cyclosporin) was kindly provided by Dr.R.M. Wenger of Sandoz Pharma AG; see also R.M. Wenger. Helv. Chim. Acta. **67**, 502 (1994).
3. All new compounds were characterized by spectroscopic methods. Yields refer to spectroscopically and chromatographically homogeneous materials.
4. M.T. Goulet and J. Boger. Tetrahedron Lett. **31**, 4845 (1990). The reported procedure was modified slightly: after the ozonolysis step, the product was isolated by chromatography, treated first with DBU in DMF for 4h to ensure complete retro-aldol reaction, followed by treatment with benzyl bromide to get the benzyl ester **5**.
5. a) Ester **6e** was synthesized from **7** by (i) disilylation (t-butyldimethylsilyl); (ii) hydrogenation, (iii) coupling with HO-L-β-PheLac-MeLaeu-Val-MeLeu-Ala-OBzl, prepared by reacting L-β-phenyllactic acid with MeLeu-Val-MeLeu-Ala-OBzl (ref.2); and finally (iv) desilylation. The same procedure was followed for **6f** except the peptide used was Phe-MeLeu-Val-MeLeu-Ala-OBzl. All peptide couplings were done using EDCI/HOBt in DMF and ester couplings with DCC/DMAP (cat) in CH_2Cl_2; b) MeLeu-Val-MeLeu-Ala-OBzl (ref.2) was coupled as described above (ref.5a) with suitably protected amino acid and benzyl ester removed by basic hydrolysis.
6. The binding capabilities of the ligands with FKBP12 were measured by a competitive binding assay developed in Sandoz which determined their affinities for FKBP12 compared to that of ascomycin. The IC50 values for ascomycin and rapamycin by the same assay were 0.5 nM and 0.45 nM respectively.
7. Little epimerization could not be avoided. The minor isomers could be separated easily by chromatography after macrocyclization step.
8. G.D. Van Duyne, R.F. Standaert, S.L. Schreiber and J. Clardy. J. Am. Chem. Soc. **113**, 7433 (1991).
9. All calculations were carried out using Insight II (2.2.0)/Discover (2.9) program in Silicon Graphics (4D-80) and Alliant concentrix 2800 (2.2.00, Mode FX28).