Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 9, pp. 1161-1166, 1994

0960-894X/94 $6.00+0.00

0960-894X(94)E0120-4

SYNTHESIS AND FKBP BINDING OF SMALL MOLECULE MIMICS OF THE TRICARBONYL REGION OF FK506

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Abstract: Small acyclic model compounds were synthesized to examine the importance of the C(9) carbonyl group of FK506 for FKBP binding. 4-(4'-Methoxyphenyl)-1-butyl (3", 4", 5"-trimethoxyphenylglyoxyl) pipecolate (compound 2d), containing the N-(glyoxyl)pipecolate motif of FK506, was found to bind to FKBP with IC_{50} of 16 μM. Replacement of the carbonyl group at the position corresponding to C(9) of FK506 with small nonpolar groups (hydrogen, methylene or methyl group, compounds 2a-2c) significantly reduced the binding affinity.

FK506 (Fig. 1) is a macrolide of bacterial origin with potent immunosuppressive activity.[1] Extensive mechanistic studies in the past several years have demonstrated that FK506 acts as an inhibitor of T-cell signal transduction by initially binding to a family of cytoplasmic proteins known as FK506 binding proteins, or FKBP.[2-3] The FKBP-FK506 complex is a potent inhibitor of calcineurin, a protein phosphatase necessary for the expression of many genes requiring Ca^{2+}-dependent transcriptional activation.[4-5] NMR and X-ray studies have revealed that the region of FK506 centered around the N-(glyoxyl)pipecolate moiety including the pyranose and substituted cyclohexyl moieties (the tricarbonyl region) is primarily responsible for FKBP binding, with the other portion of the molecule protruding and available for calcineurin interaction.[6-9]

It has been postulated that small acyclic molecules bearing the N-(glyoxyl)pipecolate moiety may mimic FK506 in terms of FKBP binding.[10-11] This type of compound is potentially useful for FK506 antagonism, for inhibition of the *cis-trans* prolyl isomerase activity of FKBP, or, to the extent that the FKBP-bound analogue inhibits calcineurin, for immunosuppresion. We have undertaken a study designed to explore the structural requirements of this type of molecule for FKBP binding. This report describes a study aimed at examining the importance of FK506 C(9) carbonyl group for FKBP binding, using model compounds 2a-2d. Compound 2d contains a N-(glyoxyl)-pipecolate

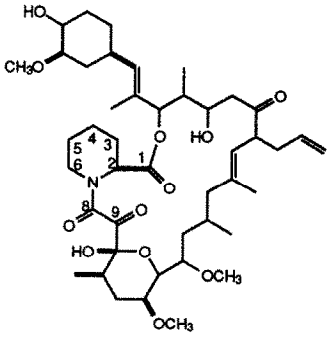
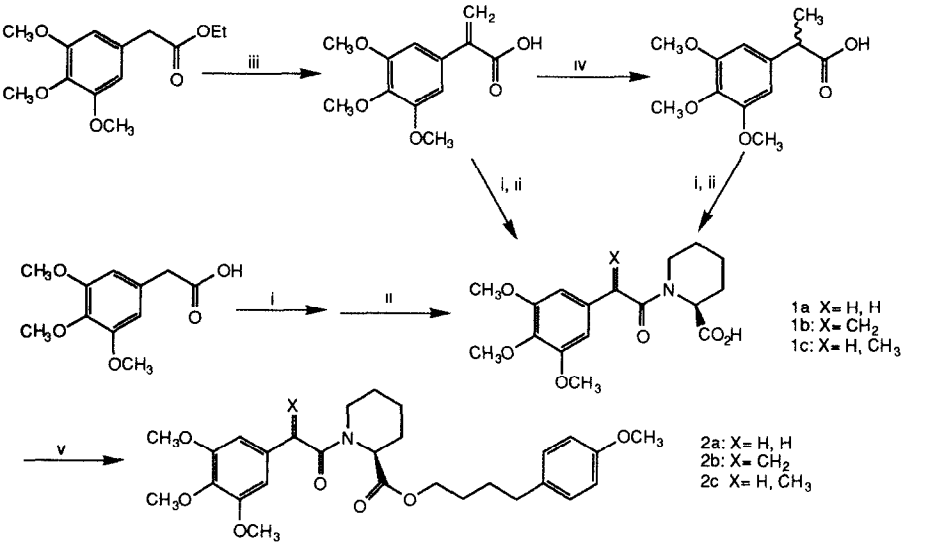


Figure 1. Structure of FK506

moiety, directly mimicking the FKBP binding domain of FK506. The synthesis and FKBP binding of this compound was reported recently.[6] In compounds 2a-2c the carbonyl oxygen at the position corresponding to C(9) of FK506 was replaced with two H's, a methylene, and a H and CH_3, respectively. It has been reported that the C(9) carbonyl of FK506 is not directly involved in any hydrogen bonding but interacts with a hydrophobic pocket formed by aromatic side chains of Tyr(26), Phe(36) and Phe(99) of FKBP.[7,13] Thus, it is conceivable that compounds in which this carbonyl is replaced by other groups with similar steric parameters will bind to FKBP. Compounds 2a-2c were synthesized to test this hypothesis.

The synthesis of compounds 2a-2c is summarized in Scheme I. (3, 4, 5-trimethoxyphenyl)acetic acid was converted to its acid chloride, which then reacted with trimethylsilyl *L*-pipecolate formed *in situ* to give intermediate product N-(3', 4', 5'-trimethoxyphenylacetyl)pipecolic acid (1a) in good yield. Phase transfer catalyzed condensation of ethyl (3, 4, 5-trimethoxyphenyl)acetate with formaldehyde according to the procedure of Werner gave α-(3, 4, 5-trimethoxyphenyl)acrylic acid,[14] which was converted to N-[α-(3', 4', 5'-trimethoxyphenyl)acryl]pipecolic acid (1b) via the acid chloride. N-[α-(3, 4, 5-trimethoxyphenyl)propionyl] pipecolic acid (1c) was obtained either by direct hydrogenation of 1b or via α-(3, 4, 5-trimethoxyphenyl) propionic acid. The intermediate products 1a-1c were then coupled with 4-(4'-methoxyphenyl)-1-butanol either through acid fluoride or carbodiimide catalyzed esterification to give the desired products 2a-2c in good yield.

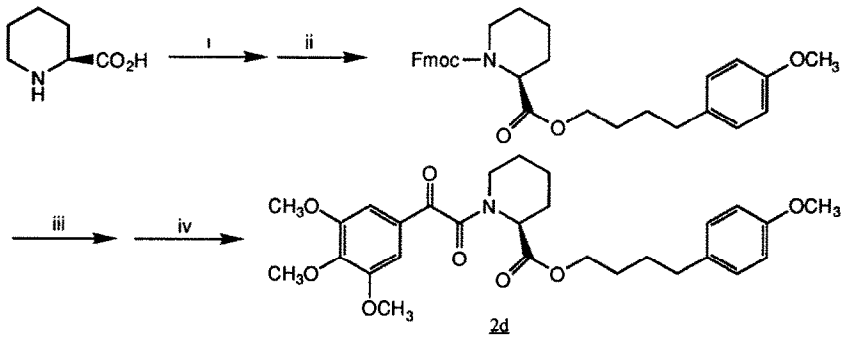
Scheme I



Conditions: i) oxalyl chloride, CH_2Cl_2, DMF (catalytic), 0 °C. ii) *L*-pipecolic acid, CH_2Cl_2, DIEA (2.5X), TMS-Cl (1.3 X). Combined yield for i and ii was 80-85%. iii) formaldehyde, K_2CO_3, Bu_4NI, toluene, then NaOH, 87%. iv) H_2, 10% Pd/C, 100%. v) 4-(4'-methoxyphenyl)-1-butanol, EDC, DMAP, CH_2Cl_2; or cyanauric fluoride, pyridine, CH_2Cl_2, then 4-(4'-methoxyphenyl)-1-butanol. 60-65%.

Attempted synthesis of 2d following the procedure similar to the synthesis of 2a-2c gave poor results. Although N-(3, 4, 5-trimethoxyphenylglyoxyl)pipecolic acid (1d, X=O) can be prepared efficiently, esterification using either acid fluoride or carbodiimide chemistry afforded the desired product only in very low yield (<10%). The difficulty associated with esterification of N-(glyoxyl)-pipecolic acid has been encountered by others, and was attributed to nucleophilic attack of the activated carboxylic group by the β-carbonyl oxygen.[15-16] Consequently, compound 2d was prepared following an alternative procedure shown in Scheme II.

Scheme II



2d

Conditions: i) CH_2Cl_2, DIEA (2.5X), TMS-Cl (1.3 X), then Fmoc-Cl, 98%. ii) oxalyl chloride, CH_2Cl_2, DMF (catalytic), 0 °C, then 4-(4'-methoxyphenyl)-1-butanol, 98%. iii) piperidine, CH_2Cl_2, 0 °C. iv) 3,4,5-trimethoxyphenylglyoxyl chloride, CH_2Cl_2. The combined yield for iii and iv was 64%.

A competition binding assay employing a recombinant FKBP-12 fusion protein was used to determine the FKBP binding properties of compounds 2a-2d.[17] The results, as shown in Table I, are expressed as IC_{50} of inhibition of a ligand (ascomycin-alkaline phosphatase) binding. As a reference, FK506 gave a IC_{50} of about 1 nM as determined by this assay.

The data in Table I indicate that compound 2d retains significant FKBP binding affinity, although it is approximately four orders of magnitude less potent than FK506. This result is in agreement with data reported for structurally similar acyclic N-(glyoxyl)-pipecolates.[11-12] For comparison, the binding affinity of 506BD, a cyclic compound containing the entire FKBP binding

Table I. FKBP Binding Affinity of Compounds 2a-2d

Entry	X	IC_{50} (μM)	% inhibition at 100 μM
2d	O	16	90
2a	H, H	nd	52
2b	CH_2	nd	39
2c	H, CH_3	nd	47

nd: activity was insufficient to calculate the IC_{50}.

domain of FK506 (the pipecolate as well as the pyranose and the substituted cyclohexyl moieties) constrained with a simplified bridge, is reduced by 20-fold in comparison with FK506.[19] An acyclic analog of 506BD, also containing the pipecolate, pyranose and the substituted cyclohexyl moieties, was reported to be only 50-fold less potent than FK506.[20]

We have carried out a NMR study of the complex between compound 2d and FKBP. The chemical shift and NOE data indicate that the pipecolate core of compound 2d sits at exactly the same location as for FK506 and interacts with the same amino acid residues of FKBP.

Substitution of the β-carbonyl of compound 2d with hydrogens, methylene or methyl groups (compound 2a-c) resulted in significant reduction in binding affinity. This result is somewhat surprising in light of a study by Emmer and Weber-Roth which showed that 9-deoxo-FK506 (replacement of the carbonyl with two hydrogens) led to only 4-fold reduction in binding affinity relative to FK506.[13] It is known that the C(9) carbonyl of FK506 contributes to FKBP binding through aromatic C-H-O interaction (electrostatic or dipole-dipole interaction) with the side-chain hydrogens of Tyr(26), Phe(36) and Phe(99) of FKBP.[6,12] The diminished binding affinities of compounds 2a-c indicate that hydrogen (2a), methylene (2b) or methyl group are not as effective for this interaction. It is also possible that the conformation of compounds 2a-c is sufficiently different from that of compound 2d to reduce the overall binding affinity. The results of the present study also indicate that the contribution of this interaction to the overall binding affinity is relatively insignificant for FK506, but substantial for a small acyclic compound such as compound 2d. This can be attributed to the interaction of the trimethoxyphenyl and *p*-methoxyphenyl moieties of compound 2d with other parts of the binding pocket being less favorable than the corresponding pyranose and the substituted cyclohexyl moieties of FK506.

From the standpoint of drug development, the ultimate goal in this area is to design simpler molecules that mimic the immunosuppressive activity of FK506 but with reduced toxicity. This requires molecules which not only bind to FKBP with high affinity, but also inhibit calcineurin.

Recently, modest success has been achieved by Andrus and Schreiber who reported several small acyclic compounds which inhibit calcineurin with low micromolar potency when bound to FKBP.[21] Despite this progress, obtaining simple molecules that mimic the immunosuppressive activity of FK506 with improved pharmacological properties remains a very challenging goal. A potentially rewarding approach is to construct and screen targeted synthetic chemical diversity libraries containing the pipecolate core structure. It is conceivable that this approach would allow rapid survey of a wide variety of analogs of compounds 2a-d in order to uncover compounds with the desired properties.

REFERENCES AND NOTES

1. For reviews, see: Schreiber, S. L. *Science* **1991**, *251*, 283. Schreiber, S. L. *Cell* **1992**, *70*, 365.
2. Siekierka, J. J.; Hung, S. H. Y.; Poe, M.; Lin, C. S.; Sigal, N. H. *Nature* **1989**, *341*, 758.
3. Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber, S. L. *Nature*, **1989**, *341*, 758.
4. Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807.
5. Liu, J.; Albers, M. W.; Wandless, T. J.; Luan, S.; Alberg, D. G.; Belshaw, P. J.; Cohen, P.; MacIntosh, C.; Klee, C. B.; Schreiber, S. L. *Biochemistry*, **1992**, *31*, 3896.
6. Armistead,D.M.; Boger, J.S.; Myers,H. V.; Saunders, J. O.; Tung, R.D. WO 92/00278, 1992.
7. Van Duyne D. G.; Standaert, R. F.; Karplus, P. A.; Schrieber, S. L.; Clardy, J. *Science*, **1991**, *252*, 839.
8. Michnick, S. W.; Rosen, M. K.; Wandless, T. J.; Schreiber, S. L. *Science*, **1991**, *252*, 836.
9. Petros, A. M.; Gampe Jr, R. T.; Germmecker, P. N.; Holzman, T. F.; Edalji, R.; Hochlowski, J.; Jackson, M.; McAlpine, J.; Luly, J. R.; Pilot-Matias, T.; Pratt, S.; Fesik, S. W. *J. Med. Chem.* **1991**, *34*, 2925.
10. Egan, D. A.; Logan, T. M.; Liang, H.; Matayoshi, E.; Fesik, S. W.; Holzman, T. F. *Biochemistry*, **1993**, *32*, 1920.
11. Hauske, J. R.; Dorff, P.; Julin, S.; DiBrino, J.; Spencer, R.; Williams, R. *J. Med. Chem.* **1992**, *35*, 4284.
12. Holt, D. A.; Luengo, J.; Yamashita, D.; Oh, H.-J.; Konialian, A. L.; Yen, H.-K.; Rozamus, L. W.; Brandt, M.; Bossard, M. J.; Levy, M. A.; Eggleston, D. S.; Liang, J.; Schultz, L. W.; Stout, T. J.; Clardy, J. *J. Am. Chem. Soc.* **1993**, *115*, 9925.
13. Emmer, G.; Weber-Roth, S. *Tetrahedron*, **1992**, *28*, 5861.
14. Werner, S.; Alfred, M. German Patent P33 17 356.7
15. Yohannes, D.; Myers, C. D.; Danishefsky, S. J. *Tetrahedron Lett.* **1993**, *34*, 2075.
16. Holt, D. A.; Konialian, a. L.; Brandt, M.; Levy, M. A.; Bossard, M. J.; Luengo, J. I.; Newman-Tarr, T.; Badger, A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1977.
17. FK-506 and ascomycin were produced at Abbott Laboratories (Abbott Park, IL) by methods to be described elsewhere. The fusion protein composed of FKBP and CMP-KDO synthetase (FKBP/CKS) was prepared as previously described.[18] An ascomycin conjugate of alkaline phosphatase was prepared by reaction of a C22 active ester of ascomycin with alkaline phosphatase (ascomycin-AP).

    In the assay, FKBP-CKS fusion protein was dissolved at 10-35 μg/ml in 20 mM sodium phosphate buffer, pH 7.4, and adsorbed to the wells of an Immuno Plate Maxisorp™

(Nunc, Naperville, IL) by incubation at ambient temperature for 2 hr. A solution of phosphate buffered saline (PBS), pH 7.4, containing 2 % bovine serum albumin (BSA) and 0.2 % Tween 20 was added to the wells to reduce nonspecific binding of the ascomycin-AP. After rinsing the wells with 0.2 % Tween 20 in PBS, the test compound in the PBS/BSA/Tween 20 buffer, or buffer alone, was added to the wells. An equal volume of ascomycin-AP ligand at 1 μg/ml in PBS/BSA/Tween 20 was added to the wells and incubated 2 hr at ambient temperature. After rinsing with 0.2 % Tween 20 in PBS, para-nitrophenyl phosphate at 1 mg/ml in 0.1 M aminomethyl propanol was added to the wells and the temporal change in 405 nm absorbance recorded.

18. Edalji, R.; Pilot-Matias, T.J .; Pratt, S. D.; Egan, D. A.; Severin, J. M.; Gubbins, E. G.; Petros, A. M.; Fesik, S. W.; Burres, N. S.; and Holzman, T. F. *J. Protein Chem.* **1992**, *11*: 213.
19. Somers, P. K.; Wandless, T. J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 8045.
20. Teague, S.; Stocks, M. J. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1947.
21. Andrus, M. B.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 10420.

(*Received in USA* 25 *February* 1994; *accepted* 29 *March* 1994)