FKBP12−Ligand−Calcineurin Interactions: Analogues of SBL506

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The macrocyclic lactone FK506 (**1**) potently suppresses T cell-mediated immune responses.[1] This is achieved through binding (K_d = 0.8 nM)[2] to an FK506-binding protein (FKBP12)[2,3] and inhibition by this drug−immunophilin complex of the calcium-dependent phosphatase calcineurin.[4] While atomic coordinates of the FKBP12−FK506 complex are available,[5] information concerning the interaction with calcineurin is limited[6] since the structures of immunophilin−ligand−calcineurin complexes have not yet been determined. Indeed, determination of the contact sites between FK506 and the calcineurin A and B subunits will be an important step toward the design of alternative immunosuppressants. In this context, the recently reported[7] acyclic (seco) variants of FK506 (**1**), particularly SBL506 (**2**), are of great interest. SBL506 lacks the constraints of the macrocyclic ring present in FK506 itself and, perhaps not surprisingly, displays reduced affinity for FKBP12. However, once bound to FKBP12, the ligand−immunophilin complex retains significant affinity for calcineurin (K_i = 0.33 μM), raising the possibility that simplified nonmacrocyclic immunosuppressants can be designed. This intriguing activity of SBL506 encouraged us to prepare closely related analogues **3** and **4** (Chart 1) to determine the specificity of the interaction with calcineurin.

SBL506 (**2**) possesses elements common to both FK506 (**1**) and the structurally related immunosuppressant rapamycin within its structure. In particular, the C27−C28 saturated side chain is reminiscent of rapamycin. SBL506 presumably binds to FKBP12 in a rapamycin-like fashion through interaction of its cyclohexyl hydroxyl with Gln-53 (Figure 1), but it has been pointed out previously that if the reduced side chain of SBL506 indeed adopts the same conformation as found in the FKBP12−rapamycin complex, steric interactions would exist between carbons C24 and C28.[7] To investigate the importance of such interactions, compounds **3** and **4** were chosen as synthetic targets. In compound **3**, the steric interaction between C24 and C28 is removed by reintroduction of the C27−C28 olefin unit found in FK506. Compound **3** should bind to FKBP12 in a manner analogous to FK506 because their binding regions are identical. In compound **4**, the saturated side chain is retained, but the functionalized cyclohexyl ring is replaced with a simple pyridine ring. This replacement was chosen for its ability to impart high affinity for FKBP12 in the absence of a hydrogen bonding interaction with Gln-53.[8]

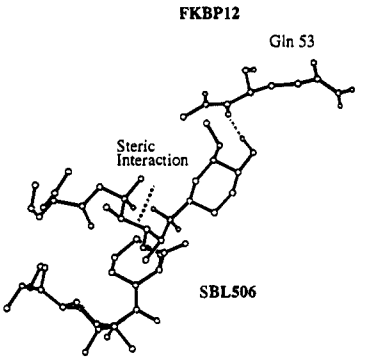
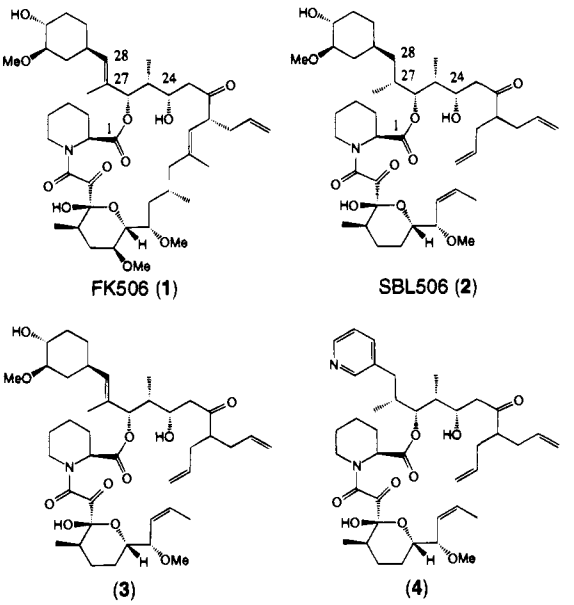


Figure 1. Hypothetical model of the FKBP12-bound conformation of SBL506.

Chart 1



Compound **3** was prepared as shown in Schemes 1 and 2. Stereoselective addition of diisopinocampheyl-(*Z*)-crotylborane (Ipc_2B-(*Z*)-crotyl), derived from (+)-α-pinene,[9] to aldehyde **5**[10] provided homoallylic alcohol **6**, which was acylated with BOC-(*S*)-pipecolinic acid and oxidatively cleaved to aldehyde **7**.[11] Precomplexation of aldehyde **7** with 10 equiv of boron trifluoride etherate, followed by the addition of 4 equiv of silyl enol ether **8**, gave the desired β-hydroxy ketone **9**,[12] which was converted in a one-pot protection−deprotection sequence[6] to amine **10**.

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(11) Aldehyde **7** is extremely prone to elimination and was used directly in the aldol reaction without purification. The low yield of the aldol reaction with this particular aldehyde is undoubtedly due to this instability. The analogous step in the preparation of compound **4** proceeded in 54% yield.

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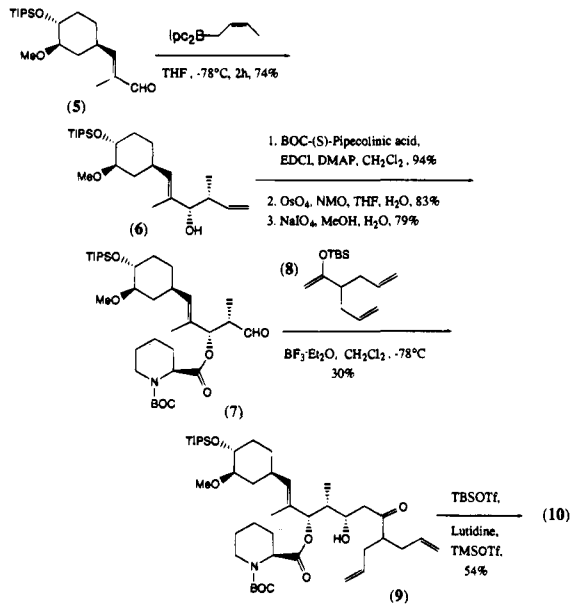
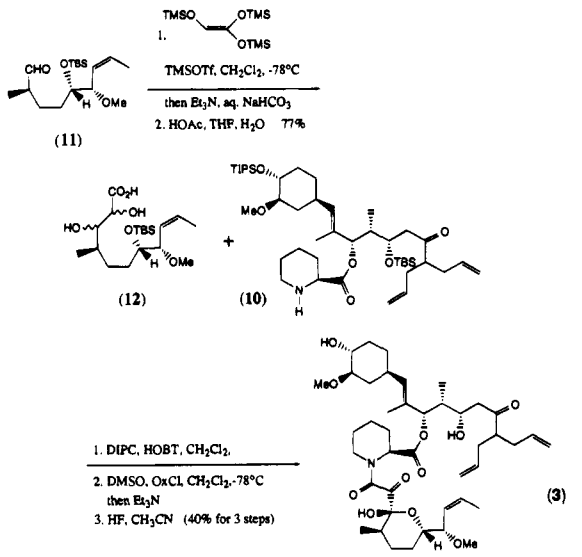
Scheme 1



Scheme 2



The masked tricarbonyl unit of compound **3**, which is common to both FK506 and rapamycin, is important to immunophilin binding, and a number of synthetic methods have been developed for incorporation of this functionality.[13] Frequently these methods involve several functional group protection–deprotection steps and result in low overall yields. Described in Scheme 2 is a novel and efficient three-step procedure for incorporating this unit without recourse into protection–deprotection steps.[14] The key step is the three-carbon homologation of aldehyde **11**[7] into diol acid **12** by reaction with tris(trimethylsiloxy)ethene in the presence of TMSOTf.[15] A mild aqueous workup, followed by coupling with amine **10**,[16] oxidation of both hydroxyl functions, and removal of all three silyl protecting groups with HF in acetonitrile, gave compound **3** as a 1:1 mixture of rotamers and a ~5:1 mixture of six-membered and seven-membered hemiketals.[7] Compound **4** was prepared by an analogous series of reactions.[17]

The binding constants for **3** (K_d 0.014 μM) and **4** (K_d 0.3 μM) were determined by a radioligand displacement assay using FKBP12 and [^{3}H]dihydro-FK506 and are comparable to the value determined for SBL506 (K_i 0.21 μM) employing a rotamase inhibition assay.[7] Surprisingly, however, neither compound **3** nor **4** showed any activity in the calcineurin inhibition assay.[18] To explain the difference in activity between SBL506 (**2**) and compound **3**, it might be suggested that the proposed steric interaction between C24 and C28 in the two side chains of SBL506 (Chart 1) force the diallyl ketone "arm" into an active conformation. The inactivity of compound **4**, however, argues against this explanation,[19] unless it is also assumed that there is an interaction between calcineurin and the functionalized cyclohexyl/pyridine ring system (a favorable interaction in the case of the cyclohexyl ring of SBL506, or an unfavorable interaction with the pyridine ring of compound **4**).

The discrepancy in activity between SBL506 (**2**) on the one hand and compounds **3** and **4** on the other is intriguing and suggests that the activity of SBL506 is highly specific. It is hoped that high-resolution structures of FKBP12 complexes of these compounds will shed light on this problem.

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Supporting Information Available: Spectral data for compounds **3**, **4**, **10**, **13** and **14** and full experimental data for the preparation of compound **3** from aldehyde **11** (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(12) The isomeric *anti* aldol diastereomer of **12** is also formed as a minor product. Interestingly, this isomer is isolated as the TBS ether (silyl transfer from the reagent).

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(17) The precursor aldehyde (*R*)-2-methyl-3-(3-pyridyl)propanal was prepared from 3-(3-pyridyl)propanol (Aldrich) by oxidation with DMSO/oxalyl chloride, formation of the SAMP-hydrazone, methylation (LDA, MeI, THF, −85 °C), and ozonolysis.

(18) Although we were unable to obtain a sample of SBL506 itself to confirm its activity in our own calcineurin assay, the procedure we use is identical to that used by Schreiber and Andrus, and as described by Liu et al. in ref 4. FK506 was used as a control to verify the assay.

(19) The reported activity of a truncated analogue of SBL506[7] also argues against this explanation. Compounds **13** and **14**, prepared by the author utilizing a procedure similar to that descirbed, are inactive in the calcineurin assay. Compound **14** is a close analogue of the truncated version of SBL506.