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# DESIGN, SYNTHESIS AND EVALUATION OF DUAL DOMAIN FKBP LIGANDS

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**Abstract:** A number of dual domain acyclic and macrocyclic pipecolyl α-ketoamide derivatives were prepared which possess the elements of previously described high-affinity FKBP binding domains as well as simplified mimics of the FK506 effector domain, a critical feature for immunosuppressive activity of the FKBP12-FK506 complex. Compounds of this study exhibited a range of FKBP *cis-trans* peptidyl-prolyl isomerase inhibitory activities but no activity in a splenocyte mitogenesis assay for immunosuppression.

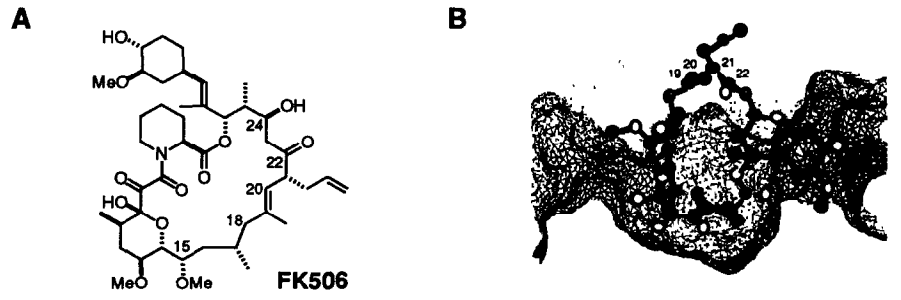
The immunosuppressants FK506, rapamycin, and cyclosporin A (CsA) exert their biological activities at the level of the T-cell through the intermediacy of intracellular drug-immunophilin complexes which interact with downstream targets involved in cytoplasmic signal transduction processes.[1] In the cases of FK506 and CsA, their complexes with immunophilin partners (FKBP12 and cyclophilin, respectively) have been shown to bind to and inhibit the calcium-dependent protein phosphatase calcineurin (PP2B).[2] The structurally related natural products FK506 and rapamycin, both ligands for FKBP12, possess two distinct molecular domains; one which is responsible for high-affinity interaction with FKBP12 and the second which participates in concert with a region of FKBP12 in the interaction with the downstream target protein. These motifs have been appropriately termed the binding domain (common to FK506 and rapamycin) and the effector domains, respectively.

One approach to the discovery of immunosuppressants functionally analogous to FK506 or rapamycin (that is, which act via initial complexation to FKBP12) consists of first identifying high-affinity binding domains followed by the fusion of an appropriate effector domain to produce a "dual domain" compound. We have recently described the design, synthesis, and characterization of high-affinity FKBP12 ligands based on a consideration of the natural binding domains;[3] the preceding two Letters describe more extensive structure-activity studies of both macrocyclic and non-macrocyclic analogs.[4] Herein we describe initial attempts to prepare biologically active dual domain compounds based on an analysis of the FK506 effector domain topography and its SAR.

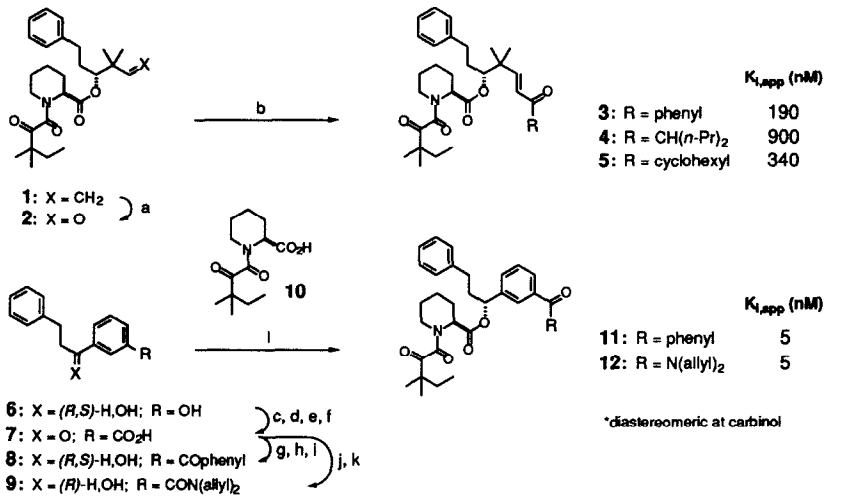
Examination of the atomic structure of the FKBP12-FK506 complex[5] reveals that the C-22 ketone, the C-21 allyl group, and the C-19/C-20 trisubstituted olefin are the most exposed elements of the FK506 effector domain (Figure 1B). Reduction of the C-22 ketone to the (*S*)-alcohol results in a loss of biological activity but only a minor change in FKBP12 binding.[6] Likewise, oxidation of C-18 to (*R*) or (*S*) alcohols or to the ketone results in total loss of biological activity accompanied by a 2 to 20 fold loss of FKBP12 affinity.[7] Replacement of the allyl group with ethyl or methyl results in a measureable but modest loss in calcineurin inhibitory activity[2b] as well as biological activity in cell proliferation assays,[8] but not a great loss in FKBP12 affinity.[2b] On the other hand, replacement of the allyl group with substantially larger hydrocarbon groups or appendages bearing polar substitution results in appreciable loss of biological activity.[8] Finally, O-demethylation of the C-15 methoxyl results in a dramatic loss of calcineurin inhibitory activity, again without a corresponding reduction of FKBP12 binding.[2b] Together these observations imply that the critical features of the FK506 effector may be minimally described as a carbonyl within a size-limited hydrophobic environment (allyl group and trisubstituted olefin). Incorporation of polar groups into the hydrophobic region is detrimental to calcineurin recognition.

As phenylpropyl esters of pipecolate derivatives have been demonstrated to be good ligands for FKBP12, especially analogs with aryl and cycloalkyl substitution on the ester carbinol center,[3,4] our first approach to synthetic dual domain compounds was to attach alkyl carbonyl moieties to these carbinol substituents. Thus, compounds **3-5, 11** and **12** were prepared as depicted in Scheme I.

*Figure 1. FK506 structure (A) and bound conformation[6] with a cross section of the FKBP12 surface (B).*




*Scheme I.*

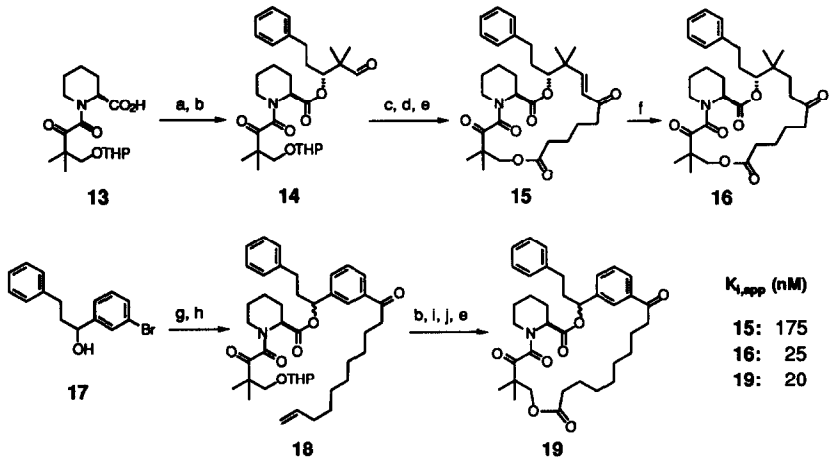



a) $O_3$, $Me_2S$; b) $(MeO)_2POCH_2COR$, DBU; c) nBuLi, $Tf_2NPh$; d) $Pd(OAc)_2$, dppp, MeOH, $Et_3N$, CO, $ClCH_2CH_2Cl$; e) Jones reagent; f) $K_2CO_3$, MeOH, $H_2O$, reflux; g) $ClCO_2Me$, HNMeOMe; h) $NaBH_4$; I) PhMgBr; j) $SOCl_2$, $HN(allyl)_2$; k) $(+)Ipc_2BCl$; l) **10**, DCC, DMAP

Compounds **3-5**, possessing α,β-unsaturated ketones and gem-dimethyl substitution (at what would be the C-25 position in FK506), exhibited relatively poor affinity for FKBP12 as evidenced by apparent rotamase inhibition constants in the range of 190-900 nM.[9] This level of inhibition is not inconsistent, however, with that observed for the parent binding domain **1** ($K_{i,app}$ = 250 nM).[4a] In contrast, compounds **11** and **12** exhibited very potent inhibition of FKBP12 ($K_{i,app}$ = 5 nM), again consistent with the inhibition observed for the parent diphenylpropyl ester ($K_{i,app}$ = 10 nM)[4a] and the notion that the alkyl carbonyl moieties (incorporated as effector motifs apart from the binding domain) play little role in the binding interactions with FKBP12. Unfortunately, none of the compounds (**3-5**, **11**, or **12**) displayed any detectable immunosuppressant-like activity in an *in vitro* splenocyte mitogenesis assay[10] at drug concentrations up to 10 μM ($IC_{50}$ for FK506 = 1 nM).

Extending the strategy of incorporating only the minimal elements of the perceived effector domain to macrocyclic analogs, compounds **15**, **16**, and **19** were prepared by methods analogous to those described in the preceding Letter and depicted in Scheme II. The phenyl ketone analog **19** as well as the saturated ketone **16** exhibited very respectable affinity for FKBP12 ($K_{i,app}$ = 20 nM and 25 nM, respectively). Unfortunately, as was the case with the non-macrocyclic analogs, compounds **15**, **16**, and **19** showed no measureable immunosuppressive properties at concentrations up to 10 μM.

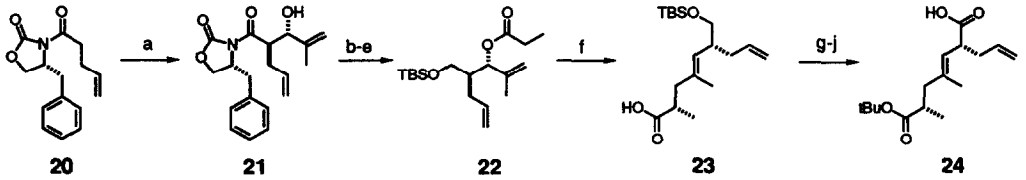
*Scheme II.*




a) (*R*)-$PhCH_2CH_2CH(OH)C(CH_3)_2CH{=}CH_2$,[3] DCC, DMAP; b) $O_3$, $Me_2S$; c) $(MeO)_2POCH_2CO(CH_2)_4CO_2tBu$, DBU; d) TFA, $CH_2Cl_2$; $Et_3N$, MeOH; e) $Cl_3H_3C_6COCl$, $Et_3N$, DMAP, toluene; f) $H_2$, Pd/C, EtOH; g) NaH; tBuLi, THF; $MeONMeCO(CH_2)_8CH{=}CH_2$; h) **13**, $Cl_3H_3C_6COCl$, $Et_3N$, DMAP, toluene; I) $NaClO_2$; J) $TsOH{\cdot}H_2O$, THF

Thus, the lack of observed biological activity in the highly simplified systems prompted the synthesis of dual domain compounds with a more fully elaborated effector domain that more closely resembled the FK506 domain. The synthesis of the C16-C22 fragment of FK506 began with the known oxazolidinone **20**[11] using the anti-selective Evans aldol conditions reported by Heathcock[12] to provide aldol **21** (Scheme III). Removal of the auxiliary,[13] LAH reduction, protection of the alcohol as its TBS ether, and esterification with propionic anhydride and DMAP gave ester **22**. Ireland-Claisen rearrangement (as reported by Shirahama[14]) gave carboxylic acid **23**. Four step functional group adjustment then gave the desired effector subunit **24**. Four variations of the binding domain (**27-30**) were prepared which differed in (1) the tether length between the α-ketoamide portion of the binding domain and the effector domain, and (2) the absence or presence of the phenethyl side chain and a C-25 (FK506 numbering) methyl group (Scheme IV). Coupling of each binding domain to the common effector domain **24**, followed by double deprotection of the THP and *tert*-butyl ester protective groups (TFA then $Et_3N$ in MeOH) and final macrolactonization[4b] gave the four dual domain analogs **31-34** (Scheme IV).

*Scheme III.*

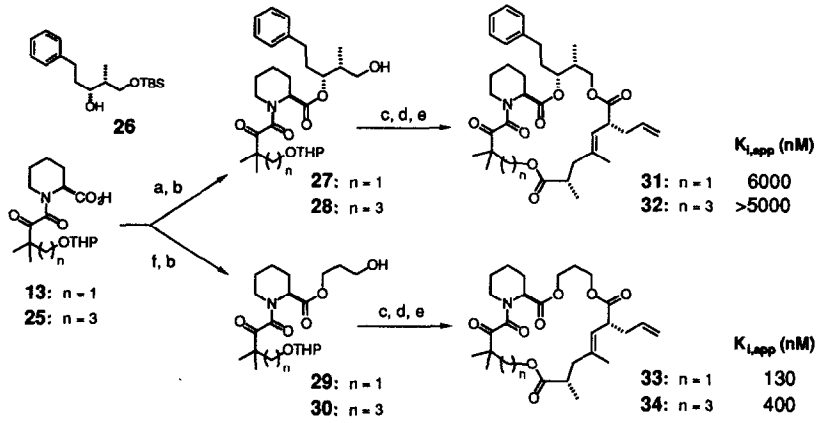



a) $Et_2AlCl$, $Bu_2BOTf$, $Et_3N$, methacrolein; b) LiOH, $H_2O_2$, THF; c) LAH, THF; d) TBSCl, $Et_3N$, $CH_2Cl_2$; e) $(EtCO)_2O$, $Et_3N$, DMAP, $CH_2Cl_2$; f) LDA, HMPA, THF, TMSCl; g) $Cl_3H_3C_6COCl$, tBuOH, $Et_3N$, DMAP; h) $Bu_4NF$, THF; I) TPAP, NMO, $CH_2Cl_2$, 4Å sieves; J) $NaClO_2$

Remarkably, the most highly functionalized analogs, **31** and **32**, exhibited at best only micromolar affinity for FKBP12 despite possessing what was perceived to be the optimal elements of the simplified binding domain. Compounds **33** and **34**, which lacked the phenylethyl binding domain appendage, actually showed improved FKBP12 inhibitory activity, but still one or two orders of magnitude weaker than expected based on the less complex macrocyclic analogs. Apparently the introduction of the rigidifying elements of the FK506 effector domain into these macrocycles resulted in an unproductive conformational preorganization for FKBP12 binding.[15] Compounds **31-34** showed no activity in the mitogenesis assay. Whether these compounds lack measurable biological activity as a consequence of a relatively poor

ability to form the FKBP12 binary complex, or as a result of not possessing an appropriately oriented effector domain to interact with calcineurin in the weak complex that is formed, is as yet an unanswered question.

***Scheme IV.***




a) 26, $Cl_3H_3C_6COCl$, $Et_3N$, DMAP; b) $Bu_4NF$, THF; c) 24, $Cl_3H_3C_6COCl$, $Et_3N$, DMAP; d) TFA, $CH_2Cl_2$; $Et_3N$, MeOH; e) $Cl_3H_3C_6COCl$, $Et_3N$, DMAP, toluene; f) $TBSO(CH_2)_3OH$, $Cl_3H_3C_6COCl$, $Et_3N$, DMAP

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