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NOVEL DERIVATIVES AT THE C21 POSITION OF THE FK-506 MACROCYCLE

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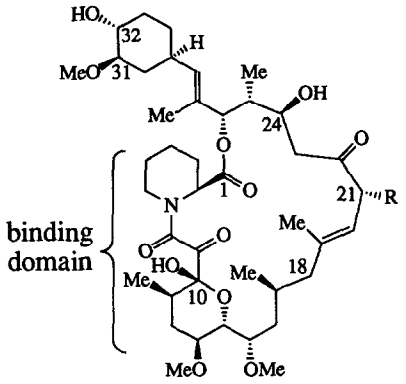
Abstract: *A number of C21 derivatives of FK-506 were prepared and their immunosuppressant properties evaluated in a T-cell proliferation assay. Some of these compounds are surprisingly potent antagonists of FK-506-mediated immunosuppression.*

First reported in 1987 by Fujisawa, the macrolide FK-506 (**1**) has subsequently been of great interest to medicinal chemists since it was found to be 100-fold more active *in vitro* as an immunosuppressant than cyclosporin A, the current therapy for transplant recipients.[1,2] FK-506 is the most potent of a group of structurally similar natural products with immunosuppressant properties [(**1**),(**2**) and (**3**)], which differ only in the nature of the C21 side-chain.[3]

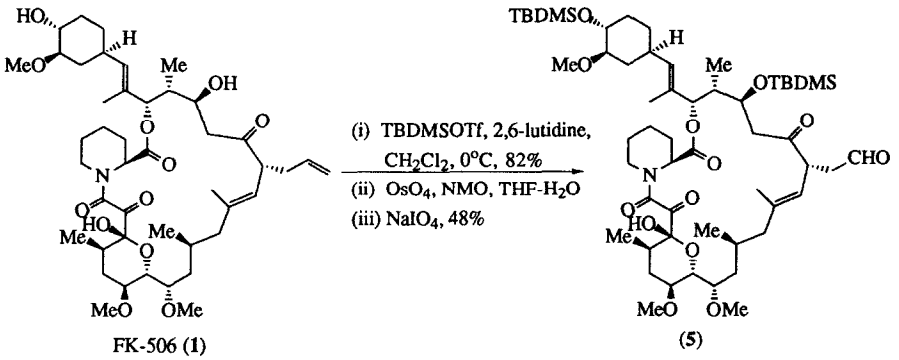


	T-cell proliferation[4] IC_{50}(nM)	
(**1**) R = allyl	0.18	**FK-506**
(**2**) R =Me	2.72	
(**3**) R = Et	0.69	**Ascomycin**
(**4**) R = Pr	0.93	

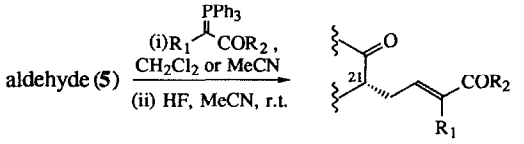
The drive to develop an 'FK-506-like' compound that exhibits fewer side-effects and better bioavailability than either cyclosporin A or FK-506, but retains the immunosuppressive activity, has led to the preparation of many structurally diverse derivatives.

The structure-activity relationship established by macrolides (**1**) - (**4**) clearly indicates that the C21 appendage is critical to the potency of this class of immunosuppressants; merely reducing the allyl group of FK-506 to yield (**4**) led to a five-fold decrease in potency. The compounds described in this communication were therefore designed to probe the available area sterically and determine whether polar groups could be tolerated. All the derivatives were prepared from aldehyde (**5**) which was easily obtained from FK-506 by a Johnson-Lemieux

type oxidation of the allyl side-chain.[5]

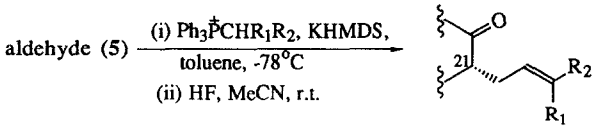


Compounds (**6**) through (**10**) were prepared by the reaction of aldehyde (**5**) with the appropriate stabilized ylids and subsequent desilylation. Esters (**6**), (**7**) and (**8**) were prepared at ambient temperature in dichloromethane solvent in good yield (40-70%) while ketone (**9**) and aldehyde (**10**) were prepared in acetonitrile at 60°C.



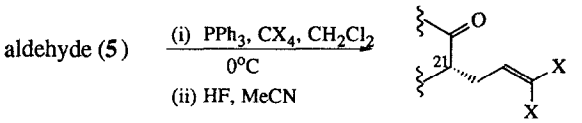
(**6**) R_1 = H, R_2 = OEt
(**7**) R_1 = H, R_2 = OMe
(**8**) R_1 = Me, R_2 = OEt
(**9**) R_1 = H, R_2 = Me
(**10**) R_1 = H, R_2 = H

Olefins (**11**) through (**17**) were prepared by addition of (**5**) to a solution of the appropriate unstabilized ylid at -78°C (20-50%) followed by desilylation.



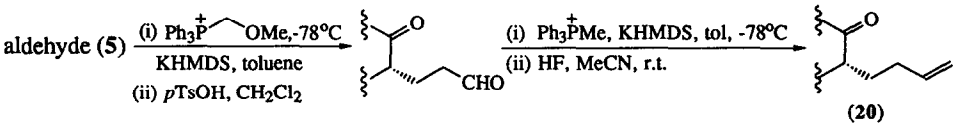
(**11**) R_1 = H, R_2 = Ph
(**12**) R_1 = H, R_2 = vinyl
(**13**) R_1 = Me, R_2 = H
(**14**) R_1 = Et, R_2 = H
(**15**) R_1 = *n*Pr, R_2 = H
(**16**) R_1 = *n*pentyl, R_2 = H
(**17**) R_1 = R_2 = Me

Dihaloolefins (**18**) and (**19**) were prepared by the Corey-Fuchs procedure.[6] Dichloroolefin (**19**) was prepared using bromotrichloromethane as the reagent since the recommended use of dibromodichloromethane led to the formation of a regioisomeric mixture of bromochloroolefins.

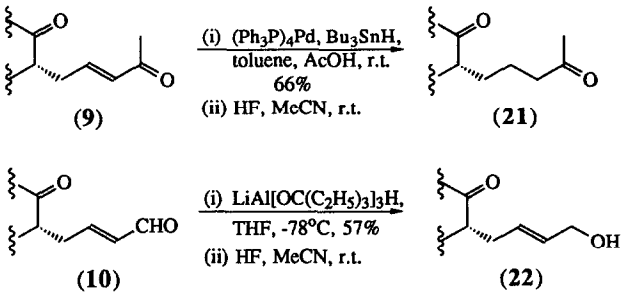


(**18**) X = Br
(**19**) X = Cl

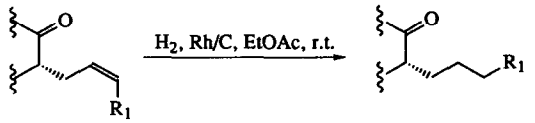
Compound (**20**), the higher homologue of FK-506, was prepared by a two step homologation of aldehyde (**5**) using the successive Wittig procedures shown. The first Wittig results in the formation of an enol ether which is hydrolysed to the intermediate aldehyde shown; this was then methylenated in the standard way and deprotected to give (**20**).



Saturated aldehyde (**21**) was prepared from unsaturated ketone (**9**) simply by 1,4-reduction while allylic alcohol (**22**) was prepared from unsaturated aldehyde (**10**) by 1,2-reduction as shown.



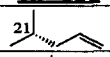
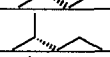
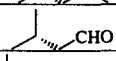
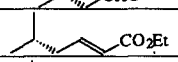
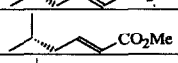
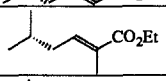
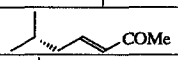
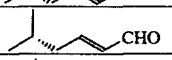
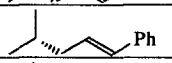
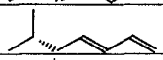
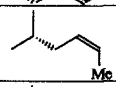
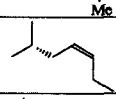
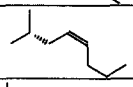
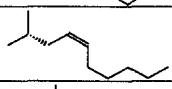
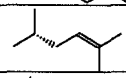
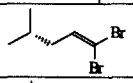
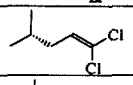
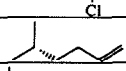
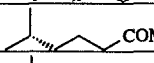
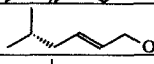
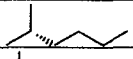
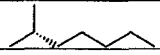
Compounds (**23**) and (**24**), with saturated side-chains were prepared from their unsaturated counterparts, (**13**) and (**14**), by catalytic hydrogenation.



(**13**) R_1 = Me
(**14**) R_1 = Et

(**23**) R_1 = Me
(**24**) R_1 = Et

It is known that the anti-proliferative effects of FK-506 arise by suppressing the production of the growth-promoting lymphokines e.g. IL-2. In fact, these effects can be reversed *in vitro* by the addition of exogenous IL-2 to inhibited cell cultures.[4] To assess the biological activity of the synthetic derivatives prepared above each compound was evaluated in a T-cell proliferation assay using murine splenic T-cells. Cells were stimulated to proliferate by the addition of phorbol myristate acetate (PMA) and ionomycin, a mode of T-cell activation which by-passes the T-cell receptor or other surface molecules, and has previously been shown to be highly sensitive to the inhibitory effects of FK-506.[7] The derivative under study was then added at known concentrations and the cultures incubated at 37°C for 48hrs before harvesting. IC_{50} values were calculated by measurement of ^3H thymidine uptake relative to a control, where no drug had been added to the proliferating cell cultures, and

Compound #	Side-chain substituent on C21	Agonist IC_{50} (nM)	Antagonist EC_{50} (nM)
(1)		0.18	
(2)		2.72	
(3)		0.69	
(4)		0.93	
(5)		20.5	
(6)		11800	88
(7)		>10000	51
(8)		10000	228
(9)		1327	11.8
(10)		1182	34.1
(11)		1677	partial
(12)		2087	partial
(13)		2.14	
(14)		100	992
(15)		3600	1780
(16)		3400	partial
(17)		16.9	partial
(18)		17.0	
(19)		7.12	
(20)		4.98	
(21)		inactive	64
(22)		11400	212
(23)		2.62	
(24)		4248	3522

the results are shown in the table. Compounds (**6**), (**7**), (**8**), (**15**), (**16**) and (**22**) exhibit weak anti-proliferative activity which is not reversible by addition of IL-2 to the cultures and therefore these derivatives are assumed not to be operating by the same mechanism as FK-506. Since the highest concentration of drug tested was 10μM the IC_{50} for compounds (**6**) and (**22**) was obtained by extrapolation and is shown in parentheses. Compound (**21**) is classed as inactive since it exhibited no effect upon the cells up to 10μM. The compounds that showed weak inhibitory activity in the T-cell proliferation assay were further tested for antagonist activity.[8] In this assay proliferating T-cells were treated with 1.2nM FK-506, an amount known to cause >90% inhibition of proliferation. Derivatives of interest were then added at known concentrations and their ability to reverse FK-506-derived inhibition of proliferation was again measured by ^3H thymidine uptake relative to a control. Partial antagonists are defined as compounds that were unable to completely reverse FK-506-derived inhibition of proliferation, up to a concentration of 10μM.

The agonist vs. antagonist property of FK-506 analogues appears to be determined by only minor structural differences. This is exemplified by comparing compounds (**13**), (**14**) and (**17**) where the insertion of a single methylene or addition of a methyl group changes the compound from a potent agonist to a weak agonist/antagonist or a partial antagonist. Generally it was found that side-chains containing unsaturation with alkyl substituents larger than methyl attached to the double bond yielded weak antagonists or partial antagonists. For alkyl side-chains with no unsaturation (compounds (**2**), (**3**), (**4**), (**23**) and (**24**)) there is only a small steric area within which to maintain activity; this reaches a maximum for the ethyl derivative, (**3**), but rapidly falls off as the chain is extended to the *n*-pentyl derivative (**24**).

The most dramatic effect, however, was noted in the preparation of side-chain derivatives containing a carbonyl group four carbon atoms removed from the macrolide. These compounds were all very potent antagonists of FK-506-mediated immunosuppression, the most potent being the unsaturated methyl ketone (**9**) and aldehyde (**10**). In contrast to this result the starting aldehyde (**5**), which has its carbonyl two carbon atoms from the macrolide, is an agonist.

Recent discoveries have shown that upon addition to the cell FK-506 binds to an ubiquitous, 12kDa protein known as FK-506 binding protein (FKBP-12).[9] It is this FK-506/FKBP-12 binary complex which constitutes the 'drug' that elicits the observed effects upon the immune system. The target of this complex has recently been found to be the calcium- and calmodulin-dependent serine-threonine protein phosphatase known as calcineurin with which FKBP-12 alone does not appear to interact under normal cellular conditions.[10]

FKBP-12 is present in the cytosol of T-cells in relatively high concentration (~5-10 μM) as estimated from saturation binding experiments with ^3H-37,38-dihydro-FK-506.[11] Maximal suppression of T-cell proliferation (>90%) occurs at an extracellular FK-506 concentration of ~1.2 nM. Under these circumstances only a small fraction of the total pool of cytosolic FKBP-12 is bound by the drug. To antagonize this effect it might reasonably be predicted that sufficient antagonist to displace bound FK-506 and sequester free FKBP-12 would be required.[12] However the situation is not so straightforward since the most potent antagonist prepared, (**9**), has an EC_{50} = 11.8 nM; thus even after the addition of antagonist there should still be a significant concentration of unbound FKBP-12 remaining.[13] While it is unlikely that all the FKBP-12 present in the cell is available for FK-506 binding the precise mechanism of antagonism remains unclear, although our data suggests that it may not be due to simple competition for FKBP-12 binding. It is apparent from these results, however, that the size and nature of the side-chain substituent is critical to maintaining the immunosuppressive activity. This area obviously

plays a vital role in the interaction of the FKBP-drug complex with its target (e.g. calcineurin) in order to yield some of the most potent agonists and antagonists prepared so far in our programme.

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