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INVESTIGATION OF THE EFFECTS OF SYNTHETIC, NON-CYTOTOXIC IMMUNOPHILIN INHIBITORS ON MDR

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Recently, we reported the synthesis and biological evaluation of a series of novel immunophilin inhibitors,[1] that are selective for FKBP and demonstrate no inhibition of cyclophilin. FKBP is the binding protein for FK506 and it is the FKBP-FK506 complex (immunophilin-drug complex), which suppresses signal transduction in T-cells through disruption of a complex series of events.[2] Although consideration of the proposed solution conformations as well as the bound conformations of the FKBP inhibitors FK506 and rapamycin (Figure 1) influenced the design elements utilized to construct these novel inhibitors,[3] the uniqueness of this series derives from the successful incorporation of an *amino-ketone* moiety as a recognition element for FKBP. Thus, these small molecule FK506 mimetics more closely resemble the transition state for FKBP binding than do their corresponding *amido-ketone* analogs.[4] However, these synthetic targets do not demonstrate potent functional immunosuppressive activity, since they lack the effector region necessary for complementary binding to afford the functionally active complex with the serine/threonine phosphatase calcineurin.[5]

Since we have clearly separated the recognition region from the functional subunit for these selective, non-cytotoxic FKBP inhibitors, we were intrigued to find that FK506 and rapamycin comprise a novel chemical class of multidrug resistance (MDR) modulators.[6] These compounds modulated P-glycoprotein (P-gp) mediated drug transport comparably to cyclosporin A (CsA).[7,8] CsA is presently being used as a MDR modulator in the clinic and is showing encouraging responses.[9] FK506 and rapamycin were also effective in potentiating the chemosensitivity of KBV1 cells to several chemotherapeutic agents. For example, Adriamycin, vinblastine, etoposide and colchicine in cell growth assays, inhibited the binding of photoactivatable drug analogs with P-gp, but were not systemically efficacious in *in vivo* tumor models of MDR.[7] Furthermore, FK506 and FK520 were recently shown to be substrates for P-gp-mediated transport.[10] This suggests that these compounds exert their inhibitory effect not only by blocking the initial binding of the anticancer cytotoxic drug, but also throughout the course of the drug efflux process. Unfortunately, the available data do not support clinical use of FK506 or rapamycin to reverse MDR of tumors, since these compounds are potent

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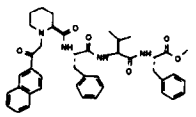
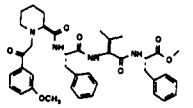
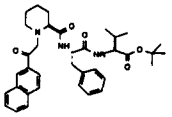
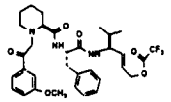
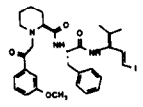
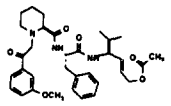
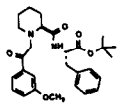
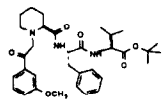
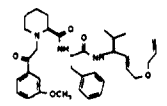
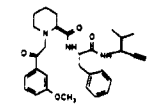
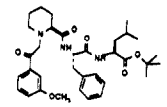
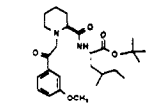
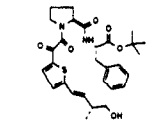
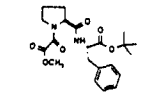
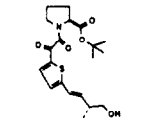
immunosuppressive agents and the clinically achieved plasma concentrations are too low for effectively inhibiting P-gp function.[11]

There are several groups seeking improved FK506 derivatives, which would be more potent and better-tolerated MDR modulators.[12] We have evaluated a series of synthetic, non-cytotoxic, FKBP selective inhibitors (IC_{50}'s ranging from 0.3 μM to >100 μM) for their capacity to modulate P-gp transport. These compounds lack potent functional immunosuppressive activity. Table 1 lists *in vitro* potencies for a series of amino-ketones and amido-ketones versus FKBP inhibition and MDR reversal. For example, these targets display inhibitory activity versus FKBP that ranges from 300 nM to >100 μM and their inhibitory activity versus MDR ranges from 5 μM to >50 μM. Although FK506 is more potent than the synthetic materials versus FKBP (1 nM vs. >100 μM), FK506 inhibits MDR activity at the *same* level as some of the synthetic molecules. Furthermore, within the series of synthetic targets there is no correlation between FKBP inhibitory activity and reversal of MDR suggesting that potent FKBP inhibition is irrelevant for useful MDR reversal *in vitro*. For example, the most potent FKBP inhibitor (Table 1, entry 1) displays no useful P-pg inhibition, whereas the synthetic target that is least potent versus FKBP (10^5 less potent than FK506, entry 16) is only about three fold less potent than FK506 versus MDR reversal. Interestingly, entry 3 in Table 1 is the most potent synthetic MDR modulator, which is equivalent in potency to FK506, however, it is a thousand fold less potent than FK506 versus FKBP inhibition.

The series of non-cytotoxic, synthetic targets listed in Table 1 define an interesting SAR versus MDR. For example, it is apparent that the structural component of the FK506 molecule that imparts functional immunosuppressive activity is not required for useful P-gp inhibition, since the synthetic FKBP inhibitors lack the structural element which imparts functional activity. A similar lack of correlation between immunosuppression and modulation of MDR has been previously reported for CsA.[13] Furthermore, since these molecules are selective for the immunophilin FKBP, it is unlikely that their capacity to modulate MDR is a result of non-specific binding to cyclophilin, the immunophilin which is selective for CsA.

Table 1[1,8]

ENTRY	COMPOUND	FKBP (μM)	MDR (μM)
1		0.3	>50
2		1.0	>50
3		1.0	5.0
4		1.0	49
5		2.0	30
6		2.0	>50
7		3.0	>50

8		3.0	6.0
9		3.0	6.0
10		5.0	11.0
11		6.0	7.0
12		8.0	>50
13		100	>50
14		>50	>50
15		>100	>50

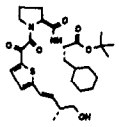
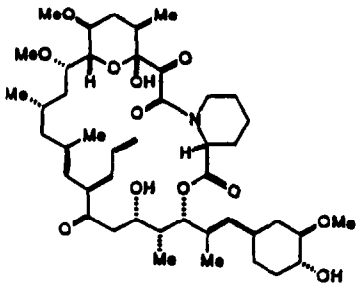
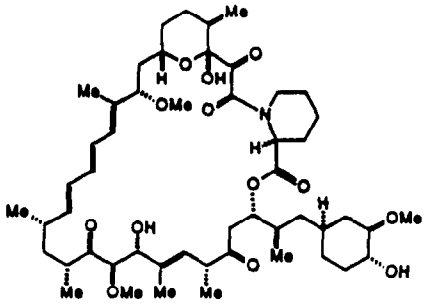
16		>100	13
17	FK506	0.001	5.0

Figure 1. FKBP Selective Inhibitors.



FK506



Rapamycin

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