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STRUCTURE-ACTIVITY STUDIES OF SYNTHETIC FKBP LIGANDS AS PEPTIDYL-PROLYL ISOMERASE INHIBITORS

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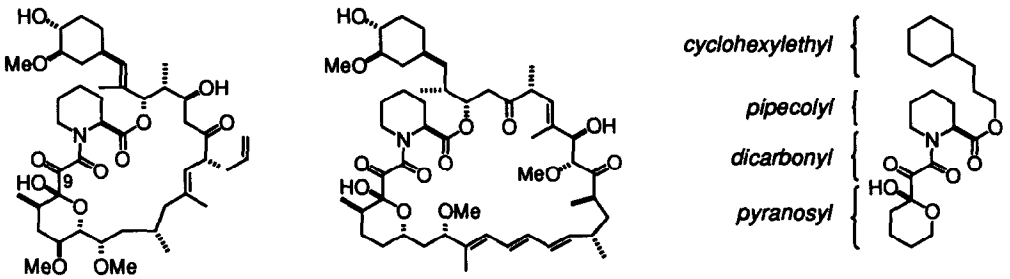
Abstract: A series of non-macrocyclic pipecolyl α-ketoamides were prepared and evaluated as FKBP *cis-trans* peptidyl-prolyl isomerase inhibitors. These compounds exhibited inhibition constants as low as 2 nM. Their design was based on a consideration of the common FKBP-binding elements of FK506 and rapamycin. Structure-activity relationships are discussed.

The FK506-binding proteins (FKBPs) and cyclophilins (CyPs) are ubiquitous families of immunosuppressant binding proteins collectively termed immunophilins.[1] While these two families have no significant sequence homology or readily apparent tertiary structural similarities, both possess *cis-trans* peptidyl-prolyl isomerase (PPIase or rotamase) activities which are inhibited by their cognate immunosuppressant ligands: FK506 (**1**) and rapamycin (**2**) for FKBP and cyclosporin A for CyP. Although inhibition of rotamase activity was an inviting hypothesis for the mechanism of immunosuppression, studies have now demonstrated that inhibition of this catalytic activity alone is insufficient to effect immunosuppression. Instead, the immunophilin-immunosuppressant complexes appear to be the pharmacological mediators through their interaction with downstream targets.[1] The calcium/calmodulin-dependent protein phosphatase calcineurin has been shown to be the common target for the FKBP-FK506 and CyP-CsA complexes.[2] The biomolecular target for the FKBP-rapamycin complex (which does not inhibit calcineurin) remains elusive.

Figure 1.



FK506 (1) rapamycin (2) minimal binding domain

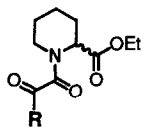
Protein-ligand X-ray crystallographic structural studies[3] coupled with biochemical studies[4] suggest that FK506 and rapamycin possess two molecular domains: 1) an FKBP-recognition or binding domain -- common to the natural products and essential for high-affinity to FKBP, and 2) an effector domain -- different in each natural product and essential for imparting immunosuppressant activity. The effector domains protrude from

the FKBP binding pocket to form part of the composite protein-ligand surfaces from which elements of both protein and ligand contribute to the immunosuppressant pharmacophores.

Recently we reported on a series of totally synthetic, high-affinity FKBP ligands which possess elements of the FK506/rapamycin binding domain only and which exhibit potent inhibition of FKBP rotamase activity.[5] The binding of these molecules to FKBP was shown by X-ray crystallography to be identical to that of FK506 and rapamycin. These structurally simplified FKBP inhibitors possess utility as potential molecular platforms for construction of immunosuppressant-effector domains and may also serve as tools to study the effects of rotamase inhibition on cell biochemistry in the absence of immunosuppression. As part of an effort to assess the relative contributions of binding domain components to FKBP recognition, modifications were explored in the four regions of the binding domain, as depicted in Figure 1, and the results of this structure-activity study are summarized below.[6]

Pyranose. Hemiketal **3**, prepared from racemic ethyl pipecolate, exhibited 7 μM inhibitory activity (Table 1). While hydrogen-bonding of the pyranose hydroxyl to protein is apparent in the X-ray crystal structure of the FKBP-FK506 complex,[3a] methylation of this hydroxyl (**4** and **6**), removal of the pyran oxygen (**5** and **6**), or removal of both oxygens (compound **7**) had no detrimental effect on the inhibitory activity in this series. The cyclohexyl analog **7** was equipotent to **3**, exhibiting a $K_{i,app}$ of 2 μM.[7] Indeed, replacement of the pyranose with bulky alkyl groups led to compounds of slightly higher affinity (e.g. **17**, $K_{i,app}$ = 660 nM).

Table 1.

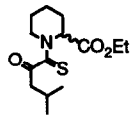
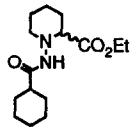
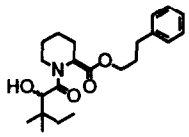
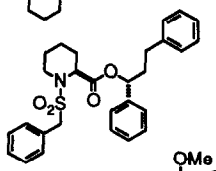
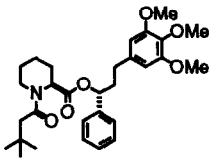
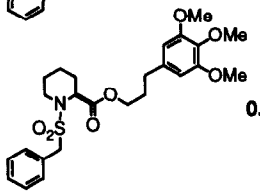


compd no.	R	$K_{i,app}$ (μM)	compd no.	R	$K_{i,app}$ (μM)
3		**7**	**11**		**9**
4		**4**	**12**		**170**
5 [a]		**1.6**	**13**		**50**
6 [a]		**1**	**14**		**6**
7 [a]		**2**	**15**		**2**
8		**33**	**16**		**2**
9		**20**	**17** [a]		**0.66**
10		**8**	**18**		**1**

[a] (*S*)-Pipecolic acid derivative.

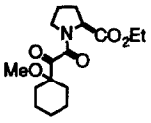
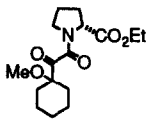
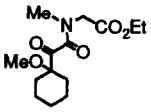
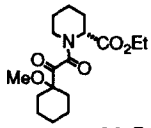
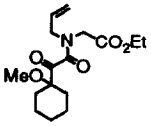
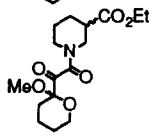
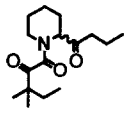
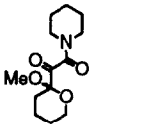
Dicarbonyl. The α-ketoamide motif has been proposed as a twisted-amide rotamase-transition state mimic.[8] The amide carbonyl is involved in hydrogen-bonding with protein in all FKBP-ligand crystal structures reported to date. The ketone lies approximately orthogonal to the plane of the amide in all structures and engages a putative carbonyl binding pocket formed by three aromatic residue sidechains of FKBP. Nevertheless, thioamide **19** (Table 2) exhibited activity comparable to its oxo analog **15** (Table 1). However, the reduction of the ketone to the alcohol (**20**) or methylene (**21**) did result in a diminution of binding (relative to their dicarbonyl analogs: **36** and **65**, Table 4) -- consistent with that observed for C-9 reduced FK506 analogs.[9] Replacement of the ketoamide with acyl hydrazides (**22**) resulted in a near total loss of activity. On the other hand, alkyl sulfonamides in this series (**23** and **24**) exhibited notable inhibitory activity in the sub-micromolar range.

Table 2.

compd no.	structure	$K_{i,app}$ (μM)	compd no.	structure	$K_{i,app}$ (μM)
19		**4.3**	**22**		**730**
20		**2.3**	**23**		**0.16**
21		**0.2**	**24**		**0.23**

Pipecolate. From these studies L-pipecolic acid appeared to be the optimal amino acid in this non-macrocyclic series of compounds (Table 3). L-Proline analogs (e.g. **25**) were slightly less potent inhibitors[10] while the N-methyl and N-allyl glycine analogs (**26** and **27**) showed a greater loss of activity (cf. **4**, Table 1, $K_{i,app}$ = 4 μM). While the X-ray crystal structures of pipecolyl ester derivatives complexed to FKBP reveal no hydrogen bonding involving the ester alkoxyl oxygen,[5] the propyl ketone analog (**28**) exhibited significantly reduced inhibitory activity relative to the ethyl esters. The D-enantiomers of both prolyl and pipecolyl analogs (**29** and **30**) were also relatively inactive as were the nipecotyl and piperidyl derivatives **31** and **32**.

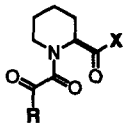
Table 3.

compd no.	structure	$K_{i,app}$ (μM)	compd no.	structure	$K_{i,app}$ (μM)
25		**8**	**29**		**600**
26		**23**	**30**		**22**
27		**260**	**31**		**180**
28		**30**	**32**		**160**

Cyclohexylethyl. Mimicry of the cyclohexyl domain of the natural products with cyclohexylpropyl or phenylpropyl esters afforded a 4 to 6-fold enhancement in affinity over the corresponding ethyl esters (Table 4, **34** and **36** vs. **17**).[11] Amide replacements of the ester (**69-73**), while tolerated, were less potent than the analogous esters.[12] Oxidation of the phenyl ring increased activity -- the 3,4,5-trimethoxyphenylpropyl ester **47** demonstrating the highest affinity for compounds examined possessing a single stereocenter ($K_{i,app}$ = 12 nM). Substitution of the carbinol center with phenyl or cyclohexyl groups also led to a significant improvement (up to 10-fold), although the stereochemistry of the carbinol is crucial in each case (**58** and **61** vs. **60** and **62**). Naphthyl substitution of the carbinol also led to increased activity (**67**; $K_{i,app}$ = 4-10 nM), although substitution with tertiary-pentenyl (**63**) or gem-dimethyl (**55**) had little effect. Compound **64**, possessing both phenethyl oxidation and phenyl substitution of the carbinol, displayed the greatest activity of compounds reported here ($K_{i,app}$ = 2-6 nM).

In summary, consideration of the FK506/rapamycin binding domain has led to a series of simplified FKBP ligands with low nanomolar rotamase inhibitory activity. The SAR studies outlined herein suggest the importance of the pipecolate α-ketoamide bearing an ester appendage to provide suitable mimicry of the natural product cyclohexylethyl domain. The substituted pyranose domain of the natural products can be effectively supplanted with bulky alkyl groups. The compounds described may serve as useful tools in the study of the intracellular roles of immunophilins as rotamases and also represent a foundation for the design and synthesis of dual domain immunosuppressants acting through the same biochemical mechanisms as FK506 and/or rapamycin.

Table 4.



compd no.	X	R[b]	$K_{i,app}$ (μM)
17	OCH_2CH_3	A	0.660
2		B	1.00
33	$OCH_2C(CH_3)_3$	A	0.410
34	$OCH_2CH_2CH_2$-cyclohexyl	A	0.186
35		B	0.250
36	$OCH_2CH_2CH_2Ph$	A	0.110
37	OCH_2Ph	B	0.40
38	OCH_2-1-naphthyl	A	0.250
39	OCH_2-2-naphthyl	A	0.200
40	OCH_2CH_2Ph	A	0.340
41	$OCH_2CH_2CH_2CH_2Ph$	A	0.100
42	OCH_2-(*E*)-$C(CH_3)$=CHPh	B	0.300
43	OCH_2-(*E*)-CH=CH-3,4-$(OCH_3)_2$Ph	A	0.035
44 [a]		B	0.200
45	$OCH_2CH_2CH_2$-4-(OCH_3)Ph	A	0.070
46	$OCH_2CH_2CH_2$-3,4-$(OCH_3)_2$Ph	A	0.020
47	$OCH_2CH_2CH_2$-3,4,5-$(OCH_3)_3$Ph	A	0.012
48	$OCH_2CH_2CH_2$-2-(OCH_3)Ph	A	0.060
49	$OCH_2CH_2CH_2$-4-(OH)Ph	A	0.095
50	$OCH_2CH_2CH_2$-4-(OBz)Ph	A	0.060
51	$OCH_2CH_2CHPh_2$	A	0.027
52	$OCH_2CH_2CH_2$-3-pyridyl	A	0.165
53	$OCH_2CH_2CH_2$-3-indolyl	A	0.023
54	OCH_2CH_2-3-indolyl	A	0.330
55 [a]	$OC(CH_3)_2CH_2CH_2$-cyclohexyl	B	0.200
56	(*R*)-OCH(Ph)CH_2CH_2Ph	A	0.010
57	(*S*)-OCH(Ph)CH_2CH_2Ph	A	0.3-0.6
58	(*R*)-OCH(Ph)CH_2CH_2-cyclohexyl	A	0.010
59		B	0.020
60	(*S*)-OCH(Ph)CH_2CH_2-cyclohexyl	B	16.0
61	(*R*)-OCH(cyclohexyl)CH_2CH_2Ph	A	0.007
62	(*S*)-OCH(cyclohexyl)CH_2CH_2Ph	A	0.300
63	(*R*)-OCH[$C(CH_3)_2CH$=CH_2]CH_2CH_2Ph	A	0.250
64	(*R*)-OCH(Ph)CH_2CH_2-3,4-$(OCH_3)_2$Ph	A	0.002-0.006
65	(*R*)-OCH(Ph)CH_2CH_2-3,4,5-$(OCH_3)_3$Ph	A	0.009
66	(*R*)-OCH(Ph)CH_2CH_2-3,4-(OCH_2O)Ph	A	0.009
67	(*R*)-OCH(2-naphthyl)CH_2CH_2Ph	A	0.004-0.010
68	(*R,S*)-OCH(CH_2Ph)CH_2CH_2Ph	A	0.055
69 [a]	$NHCH_2CH_2CH_2Ph$	B	1.0
70 [a]	$NHCH_2CH_2Ph$	B	3.0
71	(*R*)-NHCH(CO_2Et)CH_2CH_2Ph	B	1.9
72	(*R*)-NHCH(CH_2OH)CH_2Ph	B	60.0
73 [a]	$N(CH_2Ph)CH_2CH_2CH_2Ph$	B	20.0

[a] Mixture of (*R,S*)-pipecolyl derivatives. [b] A = $C(CH_3)_2CH_2CH_3$; B = cyclohexyl.

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