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Synthesis and Evaluation of Chiral Bicyclic Proline FKBP12 Ligands

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Abstract—As part of our ongoing program to explore novel structural classes of FKBP12 ligands, we herein wish to report a new class of FKBP12 ligands containing chiral bicyclic proline analogues. Details of the synthetic routes, together with preliminary biological activity, will be presented.

The discovery that small molecule ligands for the peptidyl-prolyl isomerase (PPIase) FKBP12 possess powerful neuroprotective and neuroregenerative properties in vitro and in vivo suggests that they may have therapeutic utility in treating neurodegenerative diseases such as Parkinson's Disease.[1,2] The neurotrophic effects of these compounds are independent of the immunosuppressive pathways by which drugs such as FK506 and rapamycin operate.[3,4] Immunosuppressant FK506 possesses two distinct binding domains: an immunophilin-binding domain that binds to FKBP12 and an 'effector domain' that mediates the interaction of the drug–immunophilin complex with the secondary protein target (Fig. 1). Recently, we reported that small molecules that mimic only the FKBP-binding portion of FK506, such as GPI 1046 (Fig. 1), lack immunosuppressant activity but are extraordinarily potent neurotrophic agents in vitro and promote neuroregeneration in vivo.[5]

Previous work by ourselves and other groups exploring the SAR of small molecules that mimic only the FKBP-binding domain portion of FK506 have focused largely on esters, thioesters, amides and ketones of proline or pipecolic acid.[6–8] As indicated by the bound conformation of FK506, additional steric volume may be tolerated above the plane of the pipecolic or prolyl ring. The observation that the adamantane ring system fits well

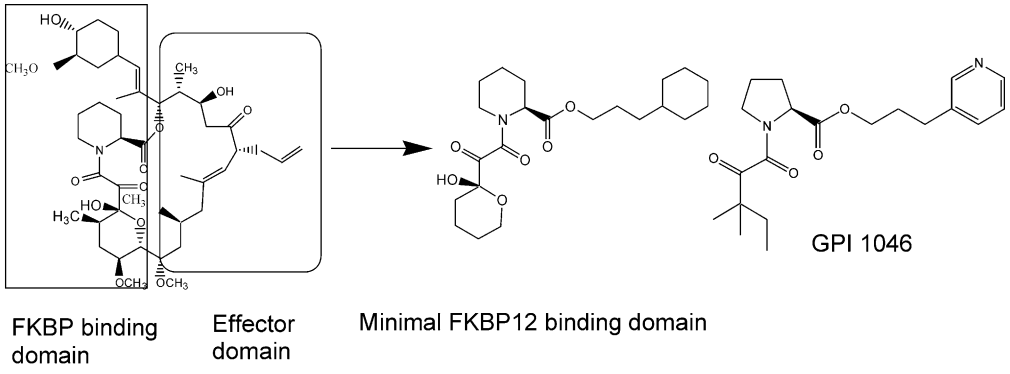

Figure 1. FK506 and small molecule mimetics of the FKBP12 binding domain.

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into the proline-binding pocket of FKBP12[9] suggests that bridged bicyclic structures can serve as effective ligands. Workers at Agouron reported that bicyclic structures such as **1** and **2** are effective FKBP inhibitors with 9.2 and 0.28 μM inhibition constants, respectively (Fig. 2).[9,10] We now report the synthesis and preliminary biological evaluation of bicyclic, bridged analogues of our previously described prolyl FKBP ligands, exemplified by **8a**.

One of the most widely used synthetic tools in the formation of asymmetric bicyclic ring structures has been the Diels–Alder reaction due to its high stereospecificity and regioselectivity.[11] Recently, there have been numerous reports in literature describing the synthesis of cyclic α-amino acid derivatives, in particular, aza-Diels–Alder reactions performed with chiral imines resulting in optically active α-amino acids.[12]

Scheme 1 shows the synthesis used to prepare the key intermediates **3**. Ethyl glyoxylate and (*R*)-1-phenylethyl amine were condensed with a 5 Å molecular sieves in methylene chloride to form (*R*)-1-phenylethyl imine in situ. The chiral imine was treated with trifluoroacetic acid and BF_3·etherate followed by cyclopentadiene at −78 °C, yielding the cyclo addition adduct **3** in a highly *exo* and diastereoselective manner.[12] The observed *exo*/*endo* ratio of 94:6 was consistent with reports in literature.[13,14] The major adduct **3**(*exo*) was easily separated from the minor adduct **3**(*endo*) by a silica gel column as a single diastereoisomer. Both structures were confirmed using 1D/2D NMR and NOE spectral techniques.

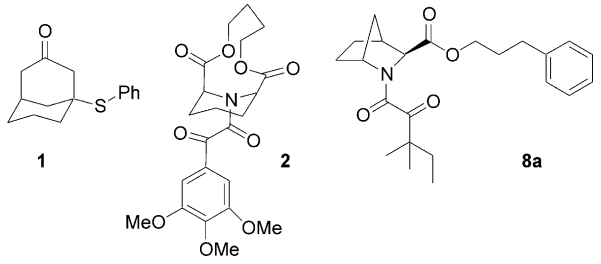
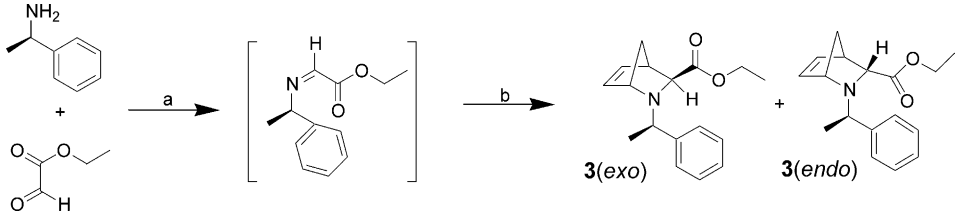


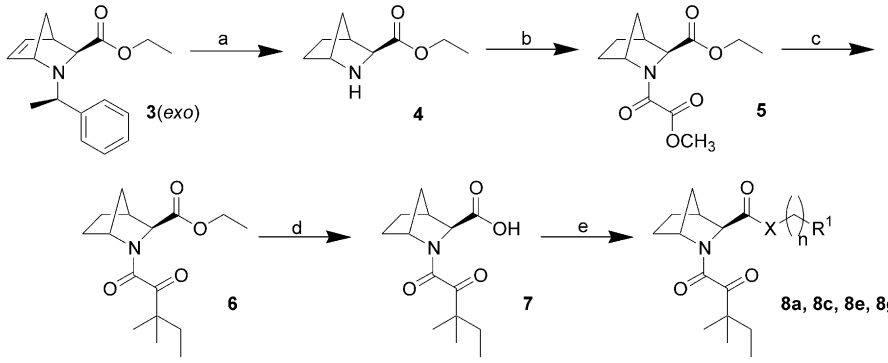
Figure 2. FKBP12 ligands.

Scheme 2 illustrates the synthesis of the desired (*N*-glyoxyl)prolyl esters and thioesters from the bicyclic intermediates. Hydrogenation of benzylamine **3**(*exo*) using Pearlman's catalyst resulted in **4**. Moderate pressure (70 psi) was required to achieve both deprotection of the benzyl amine and saturation of the double bond. Acylation of **4** with methyl chlorooxoacetate provided the oxamate **5** in good yield. Reaction of the oxamate with the appropriate Grignard reagents at −78 °C resulted in a selective and high-yielding reaction at the more electrophilic keto carbonyl of the keto amide moiety, furnishing the glyoxylate **6**. Basic hydrolysis of the ethyl ester using 2 N LiOH gave the free acid **7**. The final ester and thioester compounds (*exo*-analogues **8a**, **8c**, **8e**, **8g**, **8i**) were obtained by coupling the free acid **7** with various alcohols and thiols using the DCC esterification method. Similarly, synthesis from **3**(*endo*) led to the final *endo*-compounds (**8b**, **8d**, **8f**, **8h**, **8j**). Table 1 presents the structures of the compounds prepared.

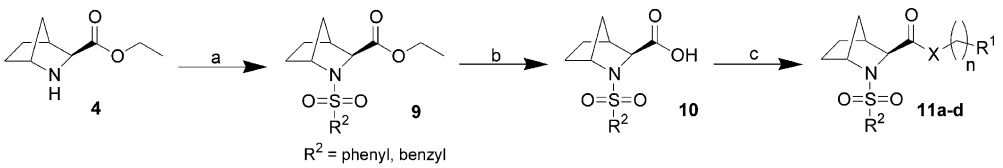
Similar to the aforementioned synthesis of the (*N*-glyoxyl)ester and thioester series, several (*N*-sulfonyl)-ester and thioester analogues were prepared as seen in Scheme 3. Reaction of aminoester **4** with varying sulfonyl chlorides afforded sulfonamides **9**. Basic hydrolysis of the ester yielded the free acid **10**, which was followed by esterification, giving the final ester and thioester



Scheme 1. Reagents and conditions: (a) 5 Å sieves, CH_2Cl_2, 0 °C; (b) TFA, BF_3 etherate, cyclopentadiene.



Scheme 2. Reagents and conditions: (a) 20% $Pd(OH)_2$, EtOAc, 70 psi; (b) chlorooxoacetate, TEA, CH_2Cl_2, 0 °C; (c) 1.25 equiv 1,1-dimethylpropyl magnesium chloride, THF, −78 °C, 3 h; (d) 2 N LiOH, MeOH, 3 h; (e) HO-R^1 or HS-R^1, 1,3-dicyclohexylcarbodiimide, 4-dimethylaminopyridine, CH_2Cl_2, overnight.



Scheme 3. Reagents and conditions: (a) $ClSO_2R^2$, TEA, CH_2Cl_2, 0 °C; (b) 2 N LiOH, MeOH, 3 h; (c) HO-R^1 or HS-R^1, 1,3-dicyclohexylcarbodiimide, 4-dimethylaminopyridine, CH_2Cl_2, overnight.

Table 1. (*N*-Glyoxyl)prolyl esters and thioesters

Compd	Adduct	X	*n*	R^1	IC_{50} (nM)
8a	*exo*	O	3	C_6H_5	670
8b	*endo*	O	3	C_6H_5	>25,000
8c	*exo*	O	3	Pyridine	15,000
8d	*endo*	O	3	Pyridine	>25,000
8e	*exo*	O	4	C_6H_5	710
8f	*endo*	O	4	C_6H_5	5100
8g	*exo*	S	3	C_6H_5	2300
8h	*endo*	S	3	C_6H_5	22,000
8i	*exo*	S	2	$CH(C_6H_5)_2$	980
8j	*endo*	S	2	$CH(C_6H_5)_2$	>25,000

Table 2. *N*-Sulfonyl esters and thioesters

Compd	Adduct	X	*n*	R^1	R^2	IC_{50} (nM)
11a	*exo*	O	3	C_6H_5	C_6H_5	650
11b	*exo*	S	3	C_6H_5	C_6H_5	910
11c	*exo*	O	3	C_6H_5	$CH_2C_6H_5$	1300
11d	*exo*	S	3	C_6H_5	$CH_2C_6H_5$	720

compounds (**11a–d**). Table 2 presents the structures of the compounds prepared.

Inhibition of the rotamase activity of FKBP12 by test compounds was assayed as described by Kofron,[15] using the peptide *N*-succinyl Ala-Leu-Pro-Phe-*p*-nitroanilide (Bachem) as the substrate. The IC_{50} values were obtained from three independent estimations with deviations generally lower than 15%, and used as measures of relative ligand binding affinities. The results of FKBP12 inhibitory activity are included in Tables 1 and 2. From the structure–activity data, it appears that the *exo*-analogues, which mimic the natural L-proline/L-pipecolic acid, show significant activity with IC_{50}s ranging from high nanomolar to low micromolar (**8c** is an exception). The IC_{50}s are reduced by approximately one order of magnitude when compared to their prolyl/pipecolyl ring counterparts.[4] In contrast, the *endo*-analogues lose significant inhibitory activity with most of *endo* compounds failing to show activity up to a 25 μM concentration. This is consistent with our previous observations of l and d stereoisomers of prolyl compounds.[7] As shown in Table 2, *N*-sulfonyl esters and thioesters are generally equipotent to their glyoxyl analogues.

Molecular modeling experiments were performed to better understand the SAR of the bicyclic compounds. The crystal structure of (1*R*)-1,3-diphenyl-1-propyl-(2*S*)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-piperdinecarboxylate (SB3) bound to FKBP12 (PDB code: 1FKG) was used for all experiments.[16] SB3 was used as a template to build both enantiomers of each bicyclic compound into the active site of FKBP12. Each compound was minimized in the active site with FKBP12 held rigid.[17]

All of the bicyclic compounds fit into the active site of FKBP12. The pharmacophore binding elements in the active site utilized by potent ligands are two hydrophobic pockets (one prolyl binding pocket and a proximal spherical binding pocket) and two hydrogen bond donors (Tyr82 side-chain OH and Ile56 backbone NH). The *exo*-analogues (l-enantiomer mimics) are predicted to bind similarly to SB3, filling both hydrophobic pockets and binding the Ile56 backbone NH and the Tyr82 OH. Figure 3 shows the predicted binding mode of compound **8a** complexed with FKBP12 as an example. Two less productive binding modes are predicted for the *endo*-analogues (d-enantiomer mimics). In both binding modes, the hydrophobic pockets are filled, though the orientation of the bicyclic group is different in each binding mode. In the first binding mode an optimal interaction with the Tyr82 OH is maintained but at the expense of a weaker interaction with the Ile56 backbone NH [2.0 Å (l) vs 2.5 Å (d)]. In the second binding mode an optimal interaction with the Ile56 backbone NH is retained, which results in the obliteration of the interaction with the Tyr82 OH. Since optimal interactions with the four pharmacophore binding elements can not be maintained in either of the predicted binding modes for the *endo*-analogues, it is predictable that they have lower IC_{50}s than the corresponding *exo*-analogues.

In order to evaluate the neuroprotective and neurotrophic properties of these constrained bicyclic compounds, we have tested representative compounds to an in vivo model of neurodegeneration. MPTP lesioning of dopaminergic neurons in mice was used as an animal model of Parkinson's disease as described previously.[5] Four-week-old male CD1 white mice were dosed ip with 30 mg/kg of MPTP for 5 days. In a 'post-MPTP' protocol, compounds **8a** and **11a**, or vehicle, were administered 10 mg/kg po, beginning on the third day following cessation of MPTP treatment, for 5 days. At 18 days following MPTP treatment, the animals were sacrificed and the striata were dissected and homogenized. Immunostaining was performed on sagital and coronal brain sections using anti-tyrosine hydoxylase Ig to quantitate survival and recovery of dopaminergic neurons. In animals treated with MPTP and vehicle, a substantial loss of 60–70% of functional dopaminergic terminals was observed as compared to non-lesioned

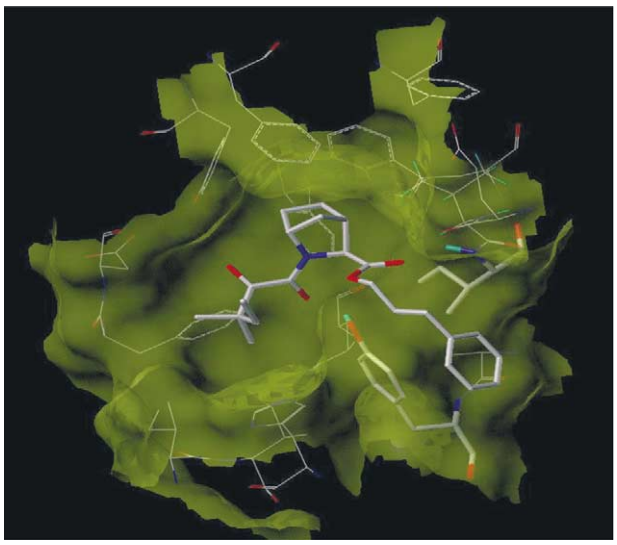


Figure 3. The predicted binding mode of **8a** complexed to FKBP12. **8a** and FKBP12 residues Ile56 and Tyr82 are shown in capped sticks. The surface of FKBP12 is shown in yellow.

animals. However, animals that received these compounds subsequent to lesioning with MPTP were effective in restoring striatal innervention. Administered orally, compounds **8a** and **11a** regenerated striated dopaminergic terminals to about 48 and 25% of control levels, respectively.

In conclusion, we have used the hetero Diels–Alder reaction to synthesize a novel series of chiral bicyclic proline ligands that are mimetics of the FKBP-binding domain of FK506. *exo* Analogues of these compounds were shown to retain good binding affinity toward FKBP12 while some representative compounds have shown efficacy in a mouse model of Parkinson's disease, suggesting their potential therapeutic utility in treating degenerative disorders of the nervous system.

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