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Solid-Phase Synthesis of FKBP12 Inhibitors: *N*-Sulfonyl and *N*-Carbamoylprolyl/pipecolyl Amides

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Abstract—In parallel with our work on solution-phase parallel synthesis of ligands for the rotamase enzyme FKBP12, we herein report a methodology for the solid-phase synthesis of two classes of inhibitor, *N*-sulfonyl and *N*-carbamoylprolyl and pipecolyl amides along with their in vitro/in vivo biological results. © 2002 Elsevier Science Ltd. All rights reserved.

Immunophilins are enzymes that possess peptidyl-prolyl isomerase (PPIase) activity, and bind the immunosuppressant drugs FK506, cyclosporin A, and rapamycin.[1] Small molecule ligands for the immunophilin FKBP12, such as GPI 1046 (**1**) and GPI 1337 (**2**) (Fig. 1) show great promise as a powerful new strategy for treating degenerative disorders of the nervous system.[2] These compounds possess potent neurotrophic actions in vitro and in vivo, and promote structural and functional recovery in animal models of neurodegenerative disease. Compounds such as GPI 1046 and GPI 1337 are devoid of immunosuppressive activity, are orally bioavailable, readily cross the blood–brain barrier, and may be useful therapeutic agents for treating disorders such as Parkinson's Disease, Alzheimer's Disease, peripheral neuropathies, and spinal cord injuries.

As part of our extensive program to explore the therapeutic utility of various classes of FKBP12 ligands, we applied combinatorial chemistry to the synthesis of structures such as **3** and **4** (Fig. 1), which possess sulfonamide and urea linkages, respectively, in replacement of the α-ketoamide moiety of **2**. Esters of these structures were prepared by solution-phase techniques, as described in the accompanying paper.[3] Here we describe the application of solid-phase synthetic methodology to the synthesis of amides of proline and pipecolic acid, to produce compounds exemplified by **5**–**8** (Fig. 1).

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Compounds were prepared according to Scheme 1. Kenner's acylsulfonamide 'safety-catch' linker is stable to basic or nucleophilic conditions, but upon conversion to the *N*-substituted acylsulfonamide can be cleaved by hydroxyl or amine nucleophiles to generate ester or amide products.[4–6] Treatment with trimethylsilyldiazomethane is a particularly convenient and useful procedure for activating the linker. Since the desired products were amide derivatives of amino acids, an attractive strategy was to couple the amino acid (proline or pipecolic acid) to the polymer, via the acylsulfonamide linker, at the carboxylate position. Following the cleavage of protection of the resin bound amino acid, the amino group was treated with various sulfonyl chlorides or isocyanates yielded the intermediate sulfonamides and ureas shown in Scheme 1. Activation of the linker with TMS–CHN_2, followed by treatment with amines, delivered the final products in the two series.[7]

In vitro characterization of the FKBP12 inhibitory effects and neurotrophic actions of FKBP12 ligands was performed using the following assays.

Inhibition of FKBP12 Rotamase Activity

Inhibition of the rotamase activity of FKBP12 by test compounds was assayed as described by Kofron,[8] using the peptide *N*-succinyl Ala-Leu-Pro-Phe *p*-nitroanilide (Bachem) as substrate. Apparent K_i's were obtained and used as measures of relative ligand binding affinities. Table 1 presents the results from screening compound libraries for FKBP12 inhibitory activity.

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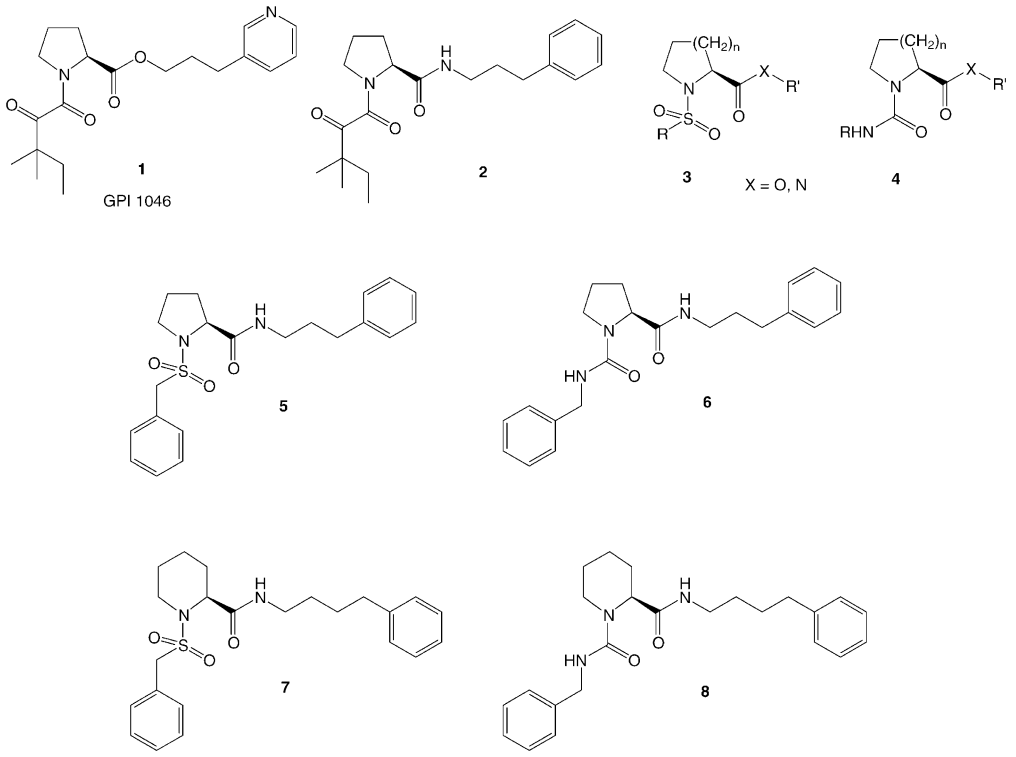
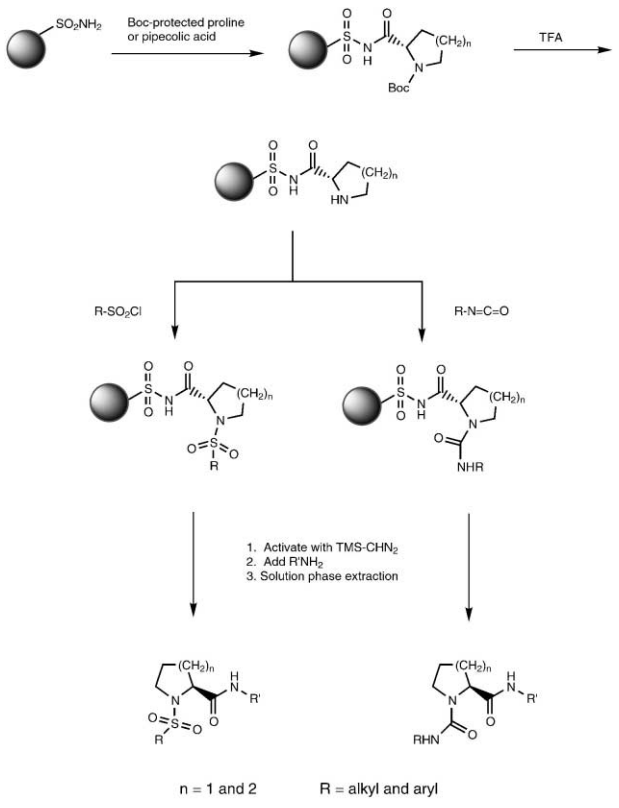


Figure 1. Neurotrophic FKB12 ligands.



Scheme 1. FKBP12 ligand library synthesis.

MPTP Model of Parkinson's Disease in Mice

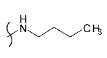
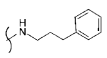
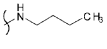
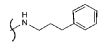
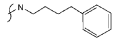
N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioning of dopaminergic neurons in mice was used as an animal model of Parkinson's Disease.[9] Four-week-old male CD1 white mice were dosed ip with 30 mg/kg of MPTP for 5 days. Test compounds (10 mg/kg), or vehicle, were administered po, beginning 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 saggital and coronal brain sections using anti-tyrosine hydoxylase Ig to quantitate survival and recovery of dopaminergic neurons. Table 2 presents the recovery of striatal dopaminergic innervation by compounds **5**–**8**.

Figure 2 shows the combinatorial building blocks used in the present study, together with the screening data generated for selected library members (Table 1). Data are inhibition constants (K_i's) for inhibiting the peptidylprolyl isomerase activity of FKBP12.

Compounds prepared by this solid-phase method were quite pure as obtained from solution-phase extractive workup. As indicated by representative HPLC traces, for example **40**, **44**, **48**, and **52** (Table 3), library members were typically obtained in >90% purity and the yields were 54–70%. The sulfonamide compounds were quite straightforward to prepare by this method, but urea-containing structures were problematic. Intramolecular cyclization upon cleavage from the linker to generate hydantoins was a predominant pathway for many of these compounds, hence the fewer number of ureas relative to sulfonamides in the tables. This problem was particularly prevalent in the pipecolic acid series.

Increasing hydrophobicity of the amide side chain generally correlated with increasing enzyme inhibitory activity, in line with previously reported results by our-

Table 1. Biological data from selected members of the libraries[a]

					
	**9** > 50,000 nM		**10** 37,000 nM	**11** 9500 nM	
	**12** > 50,000 nM	**13** > 50,000 nM	**14** 33,000 nM	**15** 7600 nM	
		**16** > 50,000 nM	**17** 22,000 nM	**18** 1300 nM	
				**5** 2800 nM	
	**19** 42,000 nM	**20** 22,000 nM	**25** 32,000 nM	**21** 13,000 nM	
		**22** > 50,000 nM	**23** > 50,000 nM	**24** 5400 nM	
		**25** 33,000 nM	**26** 30,000 nM	**27** 1400 nM	
	**28** > 50,000 nM	**29** > 50,000 nM	**30** > 50,000 nM	**31** > 50,000 nM	
	**32** > 50,000 nM	**33** > 50,000 nM	**34** > 30,000 nM	**6** 4200 nM	
	**35** > 50,000 nM	**36** > 50,000 nM	**37** > 50,000 nM	**38** > 33,000 nM	
					
	**39** 4700 nM	**40** 2500 nM	**41** 1500 nM	**42** 720 nM	
	**43** 1200 nM	**44** 1300 nM	**45** 1600 nM	**46** 640 nM	
	**47** 11,000 nM	**48** 3900 nM	**49** 2400 nM	**50** 1100 nM	
	**51** > 50,000 nM	**52** 39,000 nM	**53** 13,000 nM	**54** 2200 nM	**7** 110 nM
					**8** 1600 nM

[a]Inhibition constants (K_i's) determined.

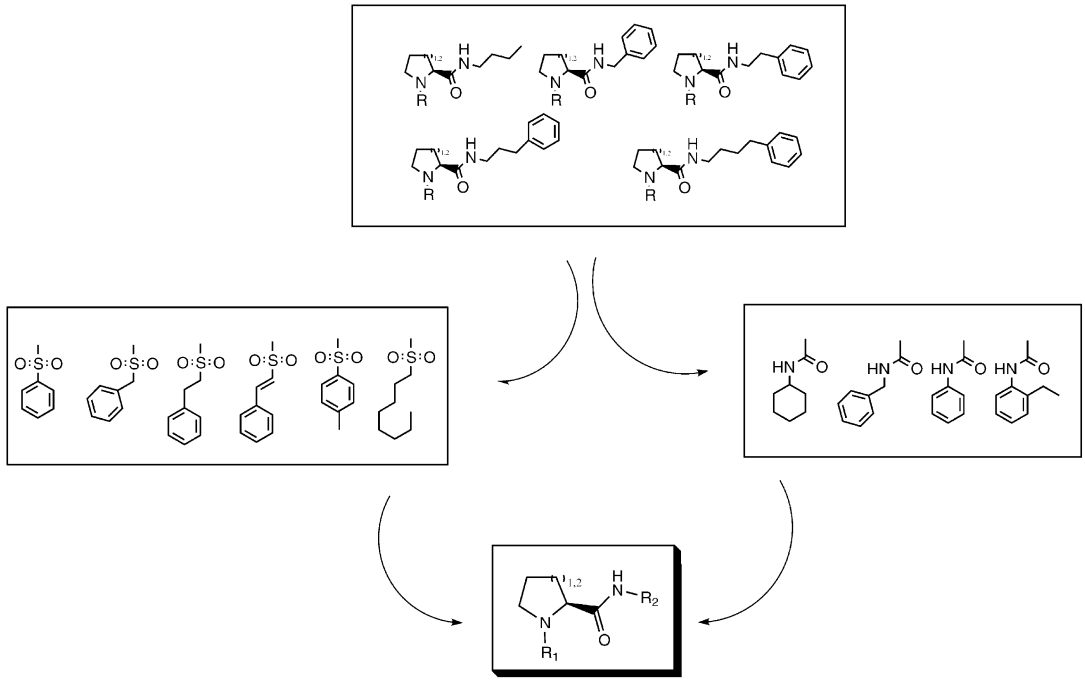


Figure 2.

selves and others, and consistent with the strongly hydrophobic nature of the FKBP binding pocket.[2] A steady decrease in K_i in a given series generally occurs as the amide R_2 group changes from butyl to benzyl, phenethyl and phenylpropyl (cf., **39**, **40**, **41** and **42**, and **51**, **52**, **53**, **54** and **7**, for example). These trends are consistent with those observed for the corresponding ester analogues (see accompanying paper[3]), although the amide compounds are generally at least an order of magnitude less potent (e.g., **1**, $K_i = 0.010$ μM, and **2**, $K_i = 1.0$ μM). For the six-membered ring (pipecolic acid) series, the four-carbon phenylbutyl ester side chain was optimal (**7**).

Varying the R group attached to the sulfonyl or carbamoyl linkage also affected activity. The *trans*-phenylethenyl group (**18**) was the best sulfonamide substituent in the proline series; increasing the flexibility by reducing the double bond to the phenethyl moiety resulted in a 10-fold loss of potency (**21**). Benzylsulfonamides were somewhat more potent than the phenyl or *p*-toluyl sulfonamides. The hydrophobic *n*-octyl group was also active. In the pipecolic acid series, the *para*-substituted phenyl sulfonamide was slightly more potent than other substituents. Replacing the phenyl substituent of urea **27** ($K_i = 1.4$ μM) with benzyl (**6**, $K_i = 4.2$ μM) or cyclohexyl (**38**, $K_i = 33$ μM) resulted in 3- and 24-fold loss of activity, respectively. Substituting the phenyl ring in the *ortho* position abolished activity (**31**).

Sulfonamides **5** and **7**, and ureas **6** and **8**, were synthesized in larger quantities by conventional solution-phase synthesis for in vivo evaluation. We have previously described in detail the ability of FKBP12 ligands such as GPI 1046 (**1**, Fig. 1) to promote structural and functional recovery in an animal model of Parkinson's Disease.[2,9] When given orally to mice subsequent to destruction of the dopaminergic pathway by the neurotoxin MPTP, compounds **5**–**8** produced regeneration of dopaminergic innervation in the striatum (Table 2). Pipecolic acids (**7** and **8**) were particularly effective, producing 37 and 42% recovery of striatal innervation. Both the sulfonamide and the urea were effective, and are comparable in potency to GPI 1046 (40%).

These results extend our earlier reports on the therapeutic utility of FKBP12 ligands in treating neurological disorders. Using solid-phase synthesis of combinatorial libraries, we have rapidly identified potent FKBP12 ligands for animal testing. Application of this methodology to new, structurally diverse FKBP12 ligands is ongoing in our laboratories.

Table 2. Recovery of TH-immunostaining in MPTP-treated mice by post-MPTP treatment with immunophilin ligands (10 mg/kg, po)

Compd	% Recovery
1	40.0
5	17.0
6	31.0
7	37.0
8	42.0

Table 3. HPLC results of four representatives

Compd	MW (MS+)	R_t (min)	Purity %	Yield %
40	358.39	19.5	>98	67
44	386.44	22.4	>98	56
48	384.42	21.5	>98	54
52	372.41	19.9	>98	70

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7. The compounds were made from Argogel-Polystyrene resin with acylsulfonamide linker as follows: **Coupling of amino acid**: ArgoGel-AS-SO_2NH_2 (4 g, 0.39 mmol/g) resin was swollen in DMF and added to the mixture of Boc-Pro (8 mmol); PyBOP (8 mmol) and *i*-Pr_2EtN (16 mmol) in 50 mL DMF. The slurry was agitated overnight and filtered, and the resin was washed with DMF (3×50 mL); 50% AcOH/DCM (2×50 mL); THF (3×50 mL) and MeOH (3×50 mL). The resin was dried under high vacuum for 4 h. **Deprotection**: The amino acid coupled resin were washed with DCM (3×50 mL) and Boc protecting group was removed in 50% TFA/DCM (50 mL) for 2 h. After filtration the resin was washed with DCM (3×50 mL); THF (3×50 mL) and DCM (3×50 mL). **Reaction with sulfonyl chlorides or isocyanates**: The resin was divided into four portions of 1 g each and added to four different chloride compounds (2 mmol) in DCM (10 mL) with Et_3N (4 mmol for sulfonyl chloride and 1 mmol for isocyanate). The mixture was agitated overnight and filtered. The resin was washed with DCM (3×20 mL); THF (3×20 mL) and MeOH (3×20 mL), and dried under a high vacuum for 4 h. **Compound activation and cleavage**: Each portion of the resin was swollen in THF and washed with 5% TFA/THF to neutralize the acylsulfonamide moiety. After agitation in 15 mL THF with $TMSCHN_2$ (2 mL, 2 M in hexane) for 2 h, the resin was drained and washed with THF (1×20 mL); MeOH (3×20 mL) and THF (1×20 mL). Each of the resin was divided into another four parts (total 16 reaction vessels) and suspended in 5 mL of THF. Four different amines (1 mmol each) were added and agitated overnight. The portions of the resin were washed with DCM (2×5 mL), THF (2×5 mL), and the combined filtrates were concentrated and dissolved in 10 mL of EtOAc, which was extracted with 1 N HCl (3×10 mL). The organic solvent was evaporated and the residue dried in vacuo for 4 h to afford the cleavage product. **HPLC conditions for product analysis**: **Column**: Vadac C18 4.6×250 mm **Wavelength**: 210 nm **Flow rate**: 2 mL/min **Solution A**: 0.1% TFA in 5% $MeCN/H_2O$.

Solution B: 0.1% TFA in MeCN. After 3 min elution with 100% A, a linear gradient of 0–25% B was developed over 5 min, then 25–75% B was continued over another 20 min. Finally the column was washed with 100% B for 5 min.
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