Synthesis of *N*-Glyoxyl Prolyl and Pipecolyl Amides and Thioesters and Evaluation of Their In Vitro and In Vivo Nerve Regenerative Effects

Gregory S. Hamilton, Yong-Qian Wu,* David C. Limburg, Douglas E. Wilkinson, Mark J. Vaal, Jia-He Li, Christine Thomas, Wei Huang, Hansjorg Sauer, Douglas T. Ross, Raj Soni, Yi Chen, Hongshi Guo, Pamela Howorth, Heather Valentine, Shi Liang, Dawn Spicer, Mike Fuller, and Joseph P. Steiner

Department of Research, Guilford Pharmaceuticals, Inc., Baltimore, Maryland 21224

Received December 7, 2001

The recent discovery that small molecule ligands for the peptidyl−prolyl isomerase (PPIase) FKBP12 possess powerful neuroprotective and neuroregenerative properties in vitro and in vivo suggests therapeutic utility for such compounds in neurodegenerative disease. The neurotrophic effects of these compounds are independent of the immunosuppressive pathways by which drugs such as FK506 and rapamycin operate. Previous work by ourselves and other groups exploring the structure−activity relationships (SAR) of small molecules that mimic only the FKBP binding domain portion of FK506 has focused on esters of proline and pipecolic acid. We have explored amide and thioester analogues of these earlier structures and found that they too are extremely potent in promoting recovery of lesioned dopaminergic pathways in a mouse model of Parkinson's disease. Several compounds were shown to be highly effective upon oral administration after lesioning of the dopaminergic pathway, providing further evidence of the potential clinical utility of a variety of structural classes of FKBP12 ligands.

Introduction

FKBP12 (FK506 binding protein) is a member of a large, ubiquitous, and highly conserved family of enzymes known collectively as the peptidylprolyl cis−trans isomerases (PPIases).[1] These proteins catalyze the interconversion of cis and trans amide bond rotamers in proline-containing substrates (PPIase or rotamase activity). PPIases have been found to play roles in a variety of cellular processes, including regulation of hetero-oligomeric complexes and protein trafficking.[2] Certain PPIases have also been characterized as the cellular binding proteins for the immunosuppressant drugs FK506, rapamycin, and cyclosporin A and are known as the immunophilins.[3] Members of the FKBP class of immunophilins bind the immunosuppressant drugs FK506 and rapamycin and mediate their interactions with the secondary protein targets calcineurin and RAFT, respectively.[2] Immunosuppresant drugs such as FK506 possess two distinct binding domains: an immunophilin binding domain, which binds to FKBP12, and an "effector domain", which mediates interaction of the drug−immunophilin complex with the secondary protein target (Figure 1). Inhibition of calcineurin and RAFT by the drug−immunophilin complexes is the mechanistic basis for the observed immunosuppressant effects of FK506 and rapamycin.

The discovery that FKBP12 is present in the brain at levels nearly 50 times higher than in the immune system suggests possible nervous system roles for the immunophilins.[4] It was subsequently found that FK506 mimicked the effects of trophic factors such as nerve growth factor in vitro, promoting neurite outgrowth in neuronal cultures at picomolar concentrations.[5] In vivo, FK506 promoted regeneration and repair of damaged facial[6] and sciatic nerves[7] in rats, suggesting therapeutic utility for immunophilin ligands in treating neurological disorders.[2,8,9]

We reported recently that small molecules that mimic only the FKBP binding portion of FK506, such as GPI 1046 (**1**, Figure 1), lack immunosuppressant activity but are extraordinarily potent neurotrophic agents in vitro and promote neuroregeneration in vivo.[10] Compound **1** binds to FKBP in a manner similar to FK506 but lacks the effector domain essential for calcineurin inhibition and immunosuppression. Compound **1** and related structures promote structural and functional recovery in a variety of animal models of neurodegenerative disease.[11] The compound is orally active, readily crosses the blood−brain barrier, and is capable of regenerating functional neuronal pathways following severe lesioning. Our studies suggest that FKBP12 ligands such as **1** may be effective in treating neurological disorders such as Parkinson's disease, Alzheimer's disease, spinal cord injury, peripheral neuropathies, and others.

While a number of ester-based FKBP12 ligands have been described in the literature,[9] very few examples of their amide or thioester analogues have appeared. As part of our program exploring various classes of FKBP12 ligands, we prepared a number of amide and thioester analogues of previously reported FKBP12 ligands. Here, we describe the potent in vivo nerve regenerative effect of these structures in an animal model of neurodegenerative disease.

Chemistry

Scheme 1 shows the general synthetic route for the compounds. Reaction of proline methyl ester or methyl pipecolinate with methyl chlorooxoacetate provided the oxamate as the key intermediate. Reaction of the oxamate with various Grignard reagents at −78 °C

* To whom correspondence should be addressed. Tel: +1-410-631-6823. Fax: +1-410-631-6848. E-mail: wuy@guilfordpharm.com.

10.1021/jm010556c CCC: $22.00
Published on Web 07/09/2002

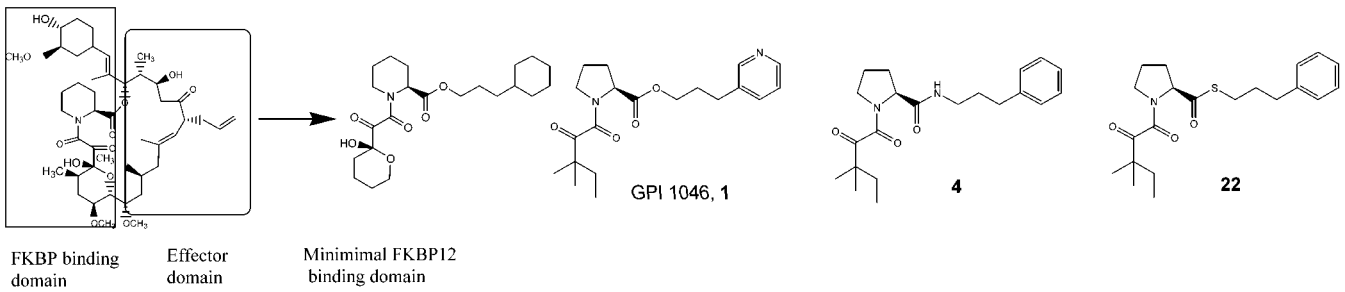
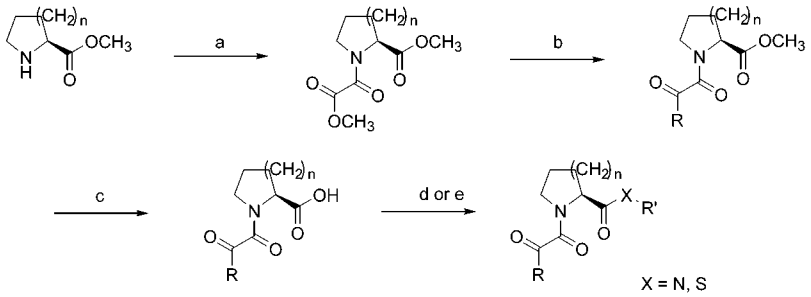


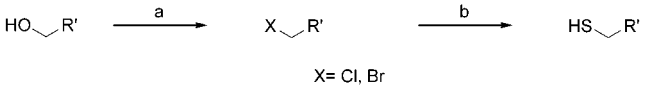
Figure 1. FK506 and small molecule mimetics of the FKBP12 binding dormain.

Scheme 1[a]



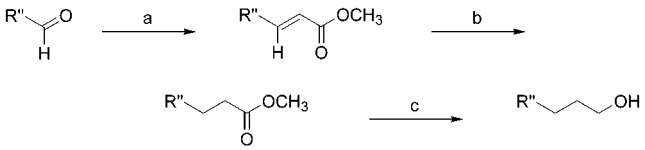
[a] Reagents and conditions: (a) Chlorooxoacetate, TEA, CH_2Cl_2, 0 °C. (b) RMgCl (1.25 equiv), THF, −78 °C, 3 h. (c) 2 N LiOH, MeOH, 3 h. (d) CH_2Cl_2, Et_3N, isobutyl chloroformate, −10 °C, and then R′-NH_2. (e) HS-R′, 1,3-dicyclohexylcarbodiimide, 4-(dimethylamino)pyridine, CH_2Cl_2, overnight.

Scheme 2[a]



[a] Reagents and conditions: (a) $SOCl_2$, MeOH or CBr_4, Ph_3P, CH_2Cl_2, 0 °C. (b) Thiourea, EtOH, NaOH, reflux.

Scheme 3[a]



[a] Reagents and conditions: (a) $Ph_3PCHCOOCH_3$, THF. (b) H_2, Pd/C, THF. (c) LAH, THF, reflux.

resulted in selective and high-yielding reaction at the more electrophilic keto carbonyl of the keto amide moiety, furnishing glyoxylates. Base hydrolysis of the methyl ester gave the free acids. Final amide compounds were obtained by coupling the carboxylic acids with various amines using the mixed anhydride method. Thioesters were synthesized by coupling the acids with various thiols using the DCC esterification method.

Thiols were synthesized as shown in Scheme 2. Alcohols were converted to halides using thionyl chloride or carbon tetrabromide. Reaction of the alkyl halide with thiourea provided the isothiuronium salt followed by alkaline hydrolysis, which afforded the thiol. Non-commercial alcohols were synthezized as shown in Scheme 3. Aryl aldehydes under Wittig conditions yielded unsaturated esters and upon hydrogenation using palladium on carbon afforded the saturated ester. The esters were then reduced using LAH to give the corresponding alcohols. Tables 1 and 2 present the structures of the amide and thioester analogues, respectively, prepared and evaluated in this study.

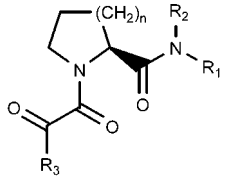
Results and Discussion

We have previously reported on the in vitro and in vivo neurotrophic effects of our first generation FKBP12 ligands, exemplified by the prototype compound **1**, that bind to FKBP12 and produce powerful nerve regeneration effects. In the process of seeking second generation compounds, a number of novel series of immunophilin ligands have been developed in our neuroimmunophilin program. In the present study, we synthesized *N*-glyoxyl prolyl and pipecolyl thioesters and amides and evaluated their neurotrophic effects.

Representative test compounds were evaluated for their in vitro inhibitory activity against the PPIase activity of recombinant purified human FKBP12. Apparent K_i values were obtained and used as measures of relative ligand binding affinities. The compounds described are mimetics of the FKBP binding domain of FK506 (Figure 1) and, as expected, are ligands for and inhibitors of FKBP12. As the in vitro data shown in Table 3 indicate, representative members of the two series bind to FKBP12 and inhibit its rotamase activity with K_i values ranging from low nanomolar to low micromolar concentration. The thioesters were particularly potent FKBP12 inhibitors, while the corresponding amide analogues were 10−100 times less potent in this regard. This is consistent with our observations in other compound series, such as urea- and sulfonamide-based FKBP12 ligands, that amides are generally an order of magnitude less potent than esters as FKBP12 PPIase inhibitors.[12,13]

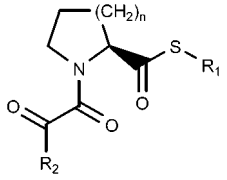
Promotion of fiber extension (neurite outgrowth) from cultured chick sensory neurons was used as an in vitro assay for nerve growth effects. Representative compounds were found to be remarkably potent, producing striking increases in process extension at picomolar concentrations (Table 3). Neurites elicited by the actions of these compounds were indistinguishable from those

Table 1. Structures and Analytical Data for *N*-Glyoxyl Prolyl and Pipecolyl Amides



compd	*n*	R_1	R_2	R_3	anal
2	1	CH_3	H	1,1-dimethylpropyl	C, H, N
3	1	2-phenethyl	H	1,1-dimethylpropyl	C, H, N
4	1	3-phenylpropyl	H	1,1-dimethylpropyl	C, H, N
5	1	3-phenylpropyl	H	*tert*-butyl	C, H, N
6	1	3-(3-pyridyl)propyl	H	1,1-dimethylpropyl	C, H, N
7	1	3-(*p*-hydroxyphenyl)propyl	H	1,1-dimethylpropyl	C, H, N
8	1	3-phenylpropyl	CH_3	1,1-dimethylpropyl	C, H, N
9	1	4-phenylbutyl	H	1,1-dimethylpropyl	C, H, N
10	1	1,5-diphenyl-3-pentyl	H	1,1-dimethylpropyl	C, H, N
11	2	CH_3	H	1,1-dimethylpropyl	C, H, N
12	2	3-phenylpropyl	H	1,1-dimethylpropyl	C, H, N
13	2	4-phenylbutyl	H	1,1-dimethylpropyl	C, H, N
14	2	4-phenylbutyl	H	*tert*-butyl	C, H, N
15	2	4-phenylbutyl	H	phenyl	C, H, N
16	2	4-(3-pyridyl)butyl	H	1,1-dimethylpropyl	C, H, N
17	2	4-(*p*-hydroxyphenyl)butyl	H	1,1-dimethylpropyl	C, H, N
18	2	5-phenylpentyl	H	1,1-dimethylpropyl	C, H, N
19	2	3,3-diphenyl-1-propyl	H	1,1-dimethylpropyl	C, H, N

Table 2. Structures and Analytical Data for *N*-Glyoxyl Prolyl and Pipecolyl Thioesters



compd	*n*	R_1	R_2	anal
20	1	2-phenethyl	1,1-dimethylpropyl	C, H, N
21	1	2-phenethyl	cyclopentyl	C, H, N
22	1	3-phenylpropyl	1,1-dimethylpropyl	C, H, N
23	1	3-phenylpropyl	cyclohexyl	C, H, N
24	1	3-(3-pyridyl)propyl	1,1-dimethylpropyl	C, H, N
25	1	3-(*o*-chlorophenyl)propyl	1,1-dimethylpropyl	nd
26	1	3-(*o*-fluorophenyl)propyl	1,1-dimethylpropyl	C, H, N, S
27	1	3-(*m*-chlorophenyl)propyl	1,1-dimethylpropyl	nd
28	1	3-(*m*-fluorophenyl)propyl	1,1-dimethylpropyl	C, H, N, S
29	1	3-(*p*-chlorophenyl)propyl	1,1-dimethylpropyl	C, H, N, S
30	1	3-(*p*-(trifluoromethyl)phenyl)propyl	1,1-dimethylpropyl	C, H, N, S
31	1	3-(*p*-methoxyphenyl)propyl	1,1-dimethylpropyl	C, H, N
32	1	3-(2-naphthyl) propyl	1,1-dimethylpropyl	nd
33	1	3,3-diphenyl-1-propyl	1,1-dimethylpropyl	C, H, N
34	1	4-phenylbutyl	1,1-dimethylpropyl	C, H, N
35	2	2-phenethyl	1,1-dimethylpropyl	C, H, N
36	2	3-phenylpropyl	1,1-dimethylpropyl	C, H, N, S
37	2	3,3-diphenyl-1-propyl	1,1-dimethylpropyl	C, H, N, S

produced by treatment with nerve growth factor: more processes and longer processes are both produced by increasing doses of the drugs, and the neurites form a dense, highly arborized network with a morphology comparable to that produced by NGF treatment. Photomicrographs of neurite outgrowth promoted by the action of compound **22** at various doses are shown in Figure 2.

The neurotrophic properties of the (*N*-glyoxyl)prolyl and pipecolyl esters were evaluated in an in vivo model of neurodegeneration. The nigrostriatal dopaminergic pathway in the brain is particularly enriched in FKBP12. This pathway is responsible for controlling motor movements. Parkinson's disease is a serious neurodegenerative disorder resulting from degeneration of this motor pathway and subsequent decrease in dopaminergic transmission.[14] *N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin that selectively destroys dopaminergic neurons.[15] Lesioning of the nigrostriatal pathway in animals with MPTP has been utilized extensively as an animal model of Parkinson's disease. These compounds were shown to possess potent neuroprotective and neuroregenerative properties in a mouse MPTP model of Parkinson's disease following either systemic or oral administration.

We investigated the therapeutic effects of the present compounds in two different dosing paradigms. In a study utilizing a "protective" or concurrent dosing

Table 3. In Vitro Data for Representative Examples

compd	inhibition[a] K_i (nM)	promotion of neurite outgrowth ED_{50} (nM)
1	7.5[b]	0.06[b]
4	1100	nd
6	820	nd
19	420	nd
20	12.2	0.77
22	11.0	<0.01
24	8.7	nd
31	8.9	0.01
32	6.0	nd
33	7.0	nd
37	86.0	nd

[a] Values of kinetic constants are the means of at least three independent estimations. Standard deviations were generally lower than 15%. [b] Ref 2.

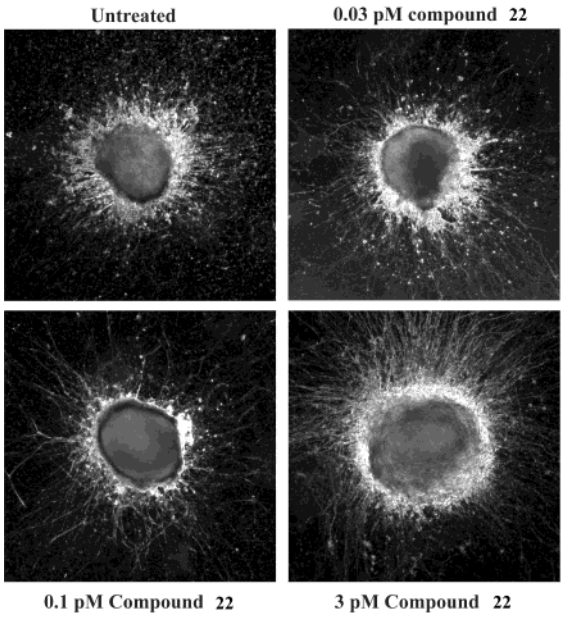


Figure 2. Photomicrographs of neurite outgrowth promoted by compound **22**.

protocol, mice were treated with MPTP and test compounds concurrently for 5 days.[11] Test compounds were given for an additional 5 days, and after 18 days, the animals were perfused and the brains were fixed, cryoprotected, and sectioned. Staining with an antibody against tyrosine hydroxylase (TH) was used to quantitate survival of dopaminergic neurons.

Table 4 presents the data from these experiments as percent recovery of striatal dopaminergic innervation relative to MPTP-treated animals not receiving test drugs. In animals treated with MPTP and vehicle, a substantial loss of 60−70% of functional dopaminergic terminals was observed as compared to nonlesioned animals. Lesioned animals receiving test compounds (4 mg/kg, sc, or 10 mg/kg po) concurrently with MPTP showed a striking recovery of TH-stained striatal dopaminergic terminals, as compared with controls, demonstrating the ability of the compounds to block the degeneration of dopaminergic neurons produced by MPTP. The powerful therapeutic potential of these compounds was further extended by the finding that the compounds were effective in the mouse MPTP model following oral administration. When administered concurrently with MPTP at a dose of 10 mg/kg po, compounds such as **22**, **30**, **35**, and **37** produced a remarkable recovery of striatal dopaminergic innervation to 70% of control levels.

Table 4

	% recovery TH immunostaining		
	dosing paradigm		
compd	concurrent (4 mg/kg sc)	concurrent (10 mg/kg po)	post-MPTP (10 mg/kg po)
1	39.0 ± 4.0	43.0 ± 3.0	35.0 ± 5.0
2	nd	nd	16.1 ± 4.0
3	nd	nd	44.1 ± 4.0
4	nd	nd	39.0 ± 4.0
5	nd	nd	28.1 ± 4.0
6	nd	nd	4.7 ± 5.0
7	nd	nd	1.8 ± 3.0
8	nd	nd	20.5 ± 4.0
9	nd	nd	50.1 ± 4.0
10	nd	nd	6.9 ± 4.0
11	nd	nd	31.6 ± 5.0
12	nd	nd	65.3 ± 4.0
13	nd	nd	54.1 ± 6.0
14	nd	nd	44.9 ± 5.0
15	nd	nd	18.7 ± 3.0
16	nd	nd	29.4 ± 4.0
17	nd	nd	60.4 ± 6.0
18	nd	nd	37.4 ± 3.0
19	nd	nd	41.9 ± 5.0
20	54.0 ± 5.3	66.0 ± 5.0	31.4 ± 4.0
21	41.0 ± 4.0	41.0 ± 4.0	nd
22	65.0 ± 4.0	72.0 ± 6.0	nd
23	70.0 ± 7.0	52.0 ± 5.0	nd
24	31.0 ± 3.0	31.0 ± 4.0	nd
25	24.0 ± 5.0	nd	nd
26	70.0 ± 7.0	nd	nd
27	16.0 ± 5.0	nd	nd
28	59.0 ± 5.0	nd	nd
29	35.0 ± 4.0	nd	nd
30	77.0 ± 8.0	71.0 ± 5.0	34.0 ± 4.0
31	37.0 ± 8.0	nd	nd
32	77.0 ± 6.0	36.0 ± 5.0	10.0 ± 4.0
33	56.0 ± 5.0	39.0 ± 9.0	37.0 ± 5.0
34	48.0 ± 4.0	48.0 ± 6.0	nd
35	61.0 6.5	73.0 ± 5.0	nd
36	nd	nd	30.0 ± 5.0
37	nd	81.0 ± 7.0	72.0 ± 5.0

Additional therapeutic utility of these compounds was revealed by the finding that they were effective in restoring striatal innervation when administered after lesioning with MPTP. In these "regenerative" or post-MPTP dosing experiments, mice received treatment with FKBP ligands beginning 3 days following cessation of MPTP treatment, at which time, in these MPTP-treated mice, the reduction in striatal TH positive fiber density is maximal. Animals were treated with drugs for a total of 5 days, and on the 18th day of the experiments, the animals were perfused and their brains were fixed, sectioned, and stained as in the protective protocol. As demonstrated by the data in Table 4, both amides and thioesters were efficacious in regenerating striatal dopaminergic terminals post-MPTP lesioning, suggesting a remarkable ability of the compounds to regenerate damaged central neuronal pathways. Compounds such as **12**, **17**, and **37** restored dopaminergic innervation in MPTP-treated animals to 65−72% of control levels postlesioning. The dose−response curve for **33** following oral administration in the protective and regenerative models is shown in Figure 3.

Many of the present compounds represent a significant advance over the earlier ester counterparts in

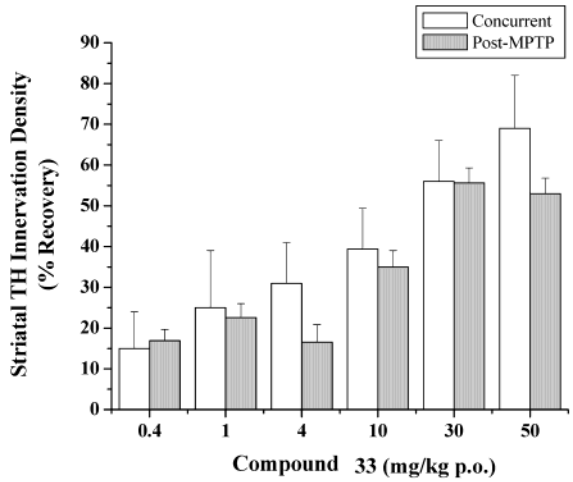


Figure 3. Dose−response curve of compound **33**.

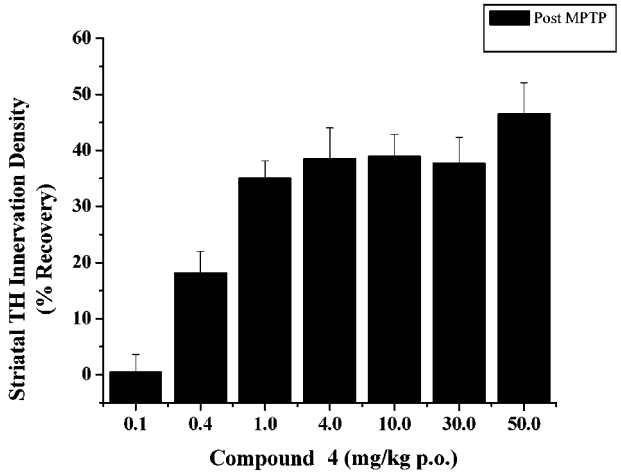


Figure 4. Dose−response curve of compound **4**.

terms of in vivo potency. For example, compounds **12** and **37** produced 65 and 72% recovery, respectively, of striatal innervation recovery in MPTP-lesioned rodents when administered orally at a dose of 10 mg/kg daily, as compared to 40% recovery by **1** in the parallel experiment.[2] Compound **4** regenerated striatal dopaminergic terminals in a well-behaved dose-dependent fashion (Figure 4) and produced significant effects at doses as low as 0.4 mg/kg, whereas significant effects for **1** in a similar dose−response study began at 4 mg/kg. Members of the present series thus achieve maximal effects in the Parkinson's disease model comparable to **1** but are as much as 10-fold more potent.

The mechanism whereby the neuroprotective/regenerative effects of small FKBP ligands are exerted is unknown. It does not appear that inhibition of FKBP12 rotamase activity has a linear correlation with potent neurotrophic effects in vitro and in vivo. It is possible that other FKBPs (e.g., FKBP52 as a possibility suggested in the literature[16]), present in lower concentrations in nerve cells, mediate the actions of these compounds. Another possibility is that interaction of the compounds with FKBP or an FKPB-like protein results in formation of an active complex, leading to a gain of function for the FKBP. We believe that the identification of the subsequent molecular targets of the protein/ligand complex responsible for mediating the nerve regenerative effects of these compounds will undoubtedly shed light on the mechanism of action.

Although many unanswered questions remain regarding the actions of these compounds, our in vivo data with these amide/thioesterstructures underscore our findings that small molecule FKBP ligands possess excellent oral bioavailability, readily cross the blood−brain barrier, and exert powerful neuroregenerative effects. Some members of the amide and thioester series described herein are fully as efficacious as previously reported immunophilin ligands in the MPTP model of Parkinson's disease but exert their regenerative effects at significantly lower doses. As such, they hold promise as a new class of therapeutic agents for the treatment of disorders such as Parkinson's, Alzheimer's, stroke, MS, and peripheral neuropathies.

Experimental Section

General Methods. All commercially available starting materials and solvents were reagent grade. Anhydrous tetrahydrofuran (THF) and diethyl ether were used as obtained from EM-Science. (*S*)-Pipecolic acid was obtained as the (*R*)-tartrate salt from Oxford Asymmetry. Analytical thin-layer chromatography (TLC) was carried out using Merck DC-F_{254} precoated silica gel plates. Flash chromatography was performed using kieselgel 60 (230−400 mesh) silica gel. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on either a Varian 300 MHz or a Bruker 400 MHz instrument. Chemical shifts are reported in parts per million (ppm). Mass spectral analyses were performed by Oneida Research Services, Whitesboro, NY. Elemental analyses were determined by Atlantic Microlab, Norcross, GA, and are within ±0.4% of the calculated values unless otherwise noted.

General Procedure for the Synthesis of Oxamates, Exemplified for (2*S*)-1-(1,2-Dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic Acid. A solution of L-proline methyl ester hydrochloride (3.08 g, 18.6 mmol) in dry methylene chloride was cooled to 0 °C and treated with triethylamine (3.92 g, 38.7 mmol; 2.1 equiv). After the formed slurry was stirred under a nitrogen atmosphere for 15 min, a solution of methyl oxalyl chloride (3.20 g; 26.1 mmol) in methylene chloride (45 mL) was added dropwise. The resulting mixture was stirred at 0 °C for 1.5 h. After it was filtered to remove solids, the organic phase was washed with water, dried over $MgSO_4$, and concentrated. The crude residue was purified on a silica gel column, eluting with 50% ethyl acetate in hexane, to obtain 3.52 g (88%) of methyl (2*S*)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate as a reddish oil. ^{1}H NMR ($CDCl_3$): δ 1.93 (m, 2H); 2.17 (m, 2H); 3.62 (m, 2H); 3.71 (s, 3H); 3.79, 3.84 (s, 3H total); 4.86 (dd, 1H, J = 8.4, 3.3 Hz).

A solution of methyl (2*S*)-1-(1,2-dioxo-2-methoxyethyl)-2-pyrrolidinecarboxylate (2.35 g, 10.9 mmol) in 30 mL of THF was cooled to −78 °C and treated with 14.2 mL of a 1.0 M solution of 1,1-dimethylpropylmagnesium chloride in THF. After the resulting homogeneous mixture was stirred at −78 °C for 3 h, the mixture was poured into saturated ammonium chloride (100 mL) and extracted into ethyl acetate. The organic phase was washed with water, dried over $MgSO_4$, and concentrated, and the crude material obtained upon removal of the solvent was purified on a silica gel column, eluting with 25% ethyl acetate in hexane, to obtain 2.10 g (75%) of the oxamate as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H); 1.22 (s, 3H); 1.26 (s, 3H); 1.75 (m, 2H); 1.87−2.10 (m, 3H); 2.23 (m, 1H); 3.54 (m, 2H); 3.76 (s, 3H); 4.52 (m, 1H, J = 8.4, 3.4 Hz).

A mixture of methyl (2*S*)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylate (2.10 g; 8.23 mmol), 1 N LiOH (15 mL), and methanol (50 mL) was stirred at 0 °C for 30 min and at room temperature overnight. The mixture was acidified to pH 1 with 1 N HCl, diluted with water, and extracted into 100 mL of methylene chloride. The organic extract was washed with brine and concentrated to deliver 1.73 g (87%) of (2*S*)-1-

(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid as a snow-white solid, which did not require further purification. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H); 1.22 (s, 3H); 1.25 (s, 3H); 1.77 (dm, 2H); 2.02 (m, 2H); 2.17 (m, 1H); 2.25 (m, 1H); 3.53 (dd, 2H, J = 10.4, 7.3 Hz); 4.55 (dd, 1H, J = 8.6, 4.1 Hz).

General Procedure for Coupling Amines with Carboxylic Acids, Exemplified for (2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-(3-phenylpropyl) Formamide (4). A solution of (2*S*)-1-(1,2-dioxo-3, 3-dimethylpentyl)-2-pyrrolidine-carboxylic acid (726 mg, 3 mmol) and triethylamine (0.425 mL, 3.3 mL) in 10 mL of methylene chloride was cooled to 0 °C, and isobutylchloroformate (0.39 mL, 3 mmol) was added dropwise. After it was stirred for 5 min, this mixture was treated with 0.43 mL (3 mmol) of phenylpropylamine, added dropwise. After 1 h, TLC indicated that the reaction was complete. The solvent was removed in vacuo, and the crude residue was purified on a silica gel column, eluting with 33% ethyl acetate in hexane, to obtain 1.08 g (93%) of **3** as an oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.81−0.87 (t, 3H); 1.19 (s, 6H); 1.68−2.59 (m, 8H): 2.64 (t, 2H); 3.21−3.25 (m, 2H); 3.25−3.40 (m, 2H); 4.51−4.54 (m, 1H); 7.14−7.28 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-methyl-formamide (2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (t, 3H, J = 7.5 Hz); 1.21 (s, 6H); 1.70 (m, 2H); 1.91 (m, 2H); 2.06 (m, 1H); 2.41 (m, 1H); 2.81 (s, 3H); 3.44 (m, 2H); 4.54 (m, 1H); 6.78 (br, 1H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-(2-phenethyl)formamide (3). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.83 (t, 3H, J = 7.5 Hz); 1.20 (s, 6H); 1.69 (m, 2H); 1.95 (m, 1H); 2.28 (m, 1H); 2.80 (m, 2H); 3.38 (m, 2H); 3.50 (m, 2H); 4.42 (m, 1H); 6.75 (br, 1H); 7.22−7.29 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxobutanoyl)pyrrolidin-2-yl]-*N*-(3-phenylpropyl)formamide (5). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (s, 9H); 1.83 (m, 2H); 1.93 (m, 2H); 2.08 (m, 1H); 2.45 (m, 1H); 2.66 (m, 2H); 3.28 (m, 2H); 3.42 (m, 2H); 4.54 (m, 1H); 5.82 (m, 1H); 7.26 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-(3-(3-pyridyl)propyl)formamide (6). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, J = 7.5 Hz); 1.22 (s, 6H); 1.71 (m, 2H); 1.83 (m, 2H); 1.94 (m, 2H); 2.02 (m, 1H); 2.35 (m, 1H); 2.62 (m, 2H); 3.28 (m, 2H); 3.46 (m, 2H); 4.56 (m, 1H); 7.10 (m, 1H); 7.50 (m, 1H); 8.44 (m, 2H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-[3-(4-hydroxyphenyl)propyl]formamide (7). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J = 7.5 Hz); 1.24 (s, 6H); 1.70 (m, 6H); 1.78 (m, 2H); 2.05 (m, 1H); 2.41 (m, 1H); 2.54 (t, 2H); 3.24 (m, 2H); 3.44 (m, 2H); 4.53 (m, 1H); 6.73 (d, 2H, J = 8.30); 6.75 (br, 1H); 6.98 (d, 2H, J = 8.30 Hz).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-(3-phenylpropyl)-*N*-methyl Formamide (8). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, 3H); 1.22 (d, 6H); 1.60−2.20 (m, 8H); 2.60 (m, 2H); 3.46 (m, 2H); 4.85 (m, 1H); 7.22 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-(4-phenylbutyl)formamide (9). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, J = 7.5 Hz); 1.21 (s, 6H); 1.53−1.71 (m, 6H); 1.90 (m, 2H); 2.05 (m, 1H); 2.41 (m, 1H); 2.60 (m, 2H); 3.26 (m, 2H); 3.43 (m, 2H); 4.54 (m, 1H); 6.85 (br, 1H); 7.25−7.28 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)pyrrolidin-2-yl]-*N*-[3-phenyl-1-(2-phenylethyl)propyl]formamide (10). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, J = 7.5 Hz); 1.23 (s, 6H); 1.72 (m, 4H); 1.82 (m, 2H); 1.95 (m, 2H); 2.10 (m, 1H); 2.42 (m, 1H); 2.63 (m, 4H); 3.47 (m, 2H); 4.02 (m, 1H); 4.56 (m, 1H); 6.58 (m, 1H); 7.21 (m, 10H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)(2-piperidyl)]-*N*-methylformamide (11). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H, J = 7.5 Hz); 1.22 (s, 6H); 1.45 (m, 2H); 1.72 (m, 4H); 2.47 (m, 2H); 2.83 (m, 3H); 3.25 (m, 2H); 5.08 (m, 1H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)(2-piperidyl)]-*N*-(3-phenylpropyl)formamide (12). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H, J = 7.5 Hz); 1.22 (s, 6H); 1.45 (m, 2H); 1.72 (m, 4H); 1.83 (m, 2H); 2.45 (m, 2H); 2.65 (m, 2H); 3.20 (m, 2H); 3.30 (m, 2H); 5.08 (m, 1H); 6.02 (bs,1H); 7.23 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)(2-piperidyl)]-*N*-(4-phenylbutyl)formamide (13). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H, J = 7.5 Hz); 1.22 (s, 6H); 1.54 (m, 4H); 1.71 (m, 6H); 2.45 (m, 2H); 2.63 (m, 2H); 3.20 (m, 2H); 3.30 (m, 2H); 5.04 (m, 1H); 6.00 (bs,1H); 7.23 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxobutanoyl)(2-piperidyl)]-*N*-(4-phenylbutyl)formamide (14). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.27 (s, 9H); 1.69−1.82 (m, 10H); 2.30−2.62 (m, 4H); 2.43 (m, 1H); 2.50 (m, 2H); 3.80 (m, 1H); 4.72 (m, 1H); 5.95 (br, 1H); 6.60 (br, 1H); 7.20−7.56 (m, 5H).

(2*S*)-[1-(2-Oxo-2-phenylacetyl)(2-piperidyl)]-*N*-(4-phenylbutyl)formamide (15). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.64−1.85 (m, 10H); 2.05 (m, 1H); 2.38 (m, 3H); 3.31 (m, 2H); 3.45 (m, 1H); 4.05 (m, 1H); 5.22 (m, 1H); 6.08 (br, 1H); 6.55 (br, 1H); 7.25−7.97 (m, 10H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)(2-piperidyl)]-*N*-(4-{3-pyridyl}butyl)formamide (16). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H, J = 7.5 Hz); 1.22 (m, 6H); 1.62 (m, 12H); 2.45 (m, 2H); 3.10 (m, 1H); 3.32 (m, 3H); 5.05 (d, 1H, J = 5.3); 6.05 (bs, 1H); 7.21 (m, 1H); 7.51 (m, 1H); 8.43 (m, 2H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)(2-piperidyl)]-*N*-(4-{*para*-hydroxyphenyl}butyl)formamide (17). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H, J = 7.5 Hz); 1.26 (m, 8H); 1.50 (m, 4H); 1.70 (m, 4H); 2.55 (m, 2H); 3.20 (m, 3H); 4.13 (m, 1H); 4.98 (m, 2H); 5.05 (m, 1H); 6.34 (bs, 1H); 6.90 (m, 4H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)(2-piperidyl)]-*N*-(5-phenylpentyl)formamide (18). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, 3H, J = 7.5 Hz); 1.23 (s, 6H); 1.40 (m, 2H); 1.52 (m, 4H); 1.71 (m, 6H); 2.45 (m, 2H); 2.61 (m, 2H); 3.15 (m, 2H); 3.28 (m, 2H); 5.05 (d, 1H, J = 5.4 Hz); 5.96 (bs, 1H); 7.21 (m, 5H).

(2*S*)-[1-(3,3-Dimethyl-2-oxopentanoyl)piperidin-2-yl]-*N*-(3,3-diphenylpropyl)formamide (19). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, J = 7.5 Hz); 1.23 (s, 6H); 1.72 (m, 6H); 2.28 (m, 3H); 3.20 (m, 3H); 4.00 (m, 3H); 5.02 (m, 1H); 5.98 (bs, 1H); 7.24 (m, 10H).

General Procedure for the Synthesis of Propyl Alcohols from Aldehydes, Exemplified for 3-(2-Naphthyl)-1-propanol. A solution of 2-naphthaldehyde (5.11 g, 32.7 mmol) and methyl (triphenylphosphoranylidene)acetate (11.0 g, 32.9 mmol) in THF (75 mL) was refluxed overnight under nitrogen atmosphere. After it was cooled, the solvent was evaporated and the residue was treated with 500 mL of ether. After it was stirred for an hour, the mixture was cooled to 0 °C and the undissolved solids were filtered off. The filter cake was washed with additional ether, and the combined organic portions were concentrated. The crude material was purified on silica gel, eluting with 10% ethyl acetate in hexane, to obtain 6.80 g (98%) of methyl 3-(2-naphthyl)prop-2-enoate as a white crystalline material. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.83 (s, 3H); 6.56 (d, 1H, J = 16 Hz); 7.52 (m, 2H); 7.68 (m, 1H); 7.83−7.93 (m, 5H).

Methyl 3-(2-naphthyl)prop-2-enoate (6.90 g, 32.5 mmol) in 50 mL of THF was treated with a catalytic amount of 10% Pd/C and hydrogenated at 40 psi overnight. The reaction mixture was filtered through Celite, concentrated, and purified on a silica gel column, eluting with 5% ether in hexane, to obtain 6.80 g (98%) of methyl 3-(2- naphthyl)propanoate as a clear oil, which crystallized on standing. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.71 (t, 2H, J = 8.10 Hz); 3.10 (t, 2H, J = 7.62 Hz); 3.82 (s, 3H); 7.33 (m, 1H); 7.43 (m, 2H); 7.62 (m, 1H); 7.74 (m, 3H).

Methyl 3-(2-naphthyl)propanoate (2.00 g, 9.30 mmol) was dissolved in 30 mL of THF and treated with 10 mL of a 1 M solution of lithium aluminum hydride in THF, with stirring and under a nitrogen atmosphere. After it was refluxed for 3 h, the reaction was allowed to cool to room temperature. After it was cooled in an ice bath, it was carefully treated with 2 N HCl until all excess hydride had been consumed and the gelatinous lithium salts formed had gone into solution. The product was extracted into 2 × 100 mL of ethyl acetate; the combined organic fractions were washed with 2 N HCl, water, and brine. The crude residue obtained by evaporating the

solvent was purified on silica gel, eluting with 20% ethyl acetate in hexane, to obtain 1.60 g of 3-(2-naphthyl)propan-1-ol as a clear oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.98 (m, 2H); 2.87 (t, 2H, J = 7.38 Hz); 3.70 (t, 3H, J = 6.42 Hz); 7.23−7.45 (m, 3H); 7.62 (m, 1H); 7.76 (m, 3H).

General Procedure for Synthesis of Thiols from Alcohols, Exemplified by 3,3-Diphenyl-1-propylthiol. A solution of 3,3-diphenyl-1-propanol (35.1 g, 165 mmol) and carbon tetrabromide (60.3 g, 182 mmol) in methylene chloride (500 mL) was cooled to 0 °C, and triphenylphosphine (47.7 g, 129 mmol) was added. The resulting mixture was stirred for 24 h and then concentrated in vacuo. The crude material was passed through a flash column, eluting with 10% methylene chloride in hexane, to yield 39.9 g (88%) of 3,3-diphenyl-1-bromopropane as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03−2.55 (m, 4H); 4.13 (m, 1H); 7.17−7.28 (m, 10H).

A solution of 3,3-diphenyl-1-bromopropane (35.0 g, 127 mmol) in 95% ethanol (25 mL) was added to a refluxing solution of thiourea (10.6 g, 140 mmol) in 125 mL of 95% ethanol. After the resulting mixture was refluxed for 24 h, 76.3 mL of 10% (w/v) NaOH solution was added, and refluxing continued for 2 h. After it was cooled to room temperature, the reaction mixture was diluted with water and extracted with 3 × 400 mL of ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated. The crude yellow oil was purified on a silica gel column (5% ethyl acetate/hexane) to obtain 21.8 g (75%) of the thiol as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.03−2.55 (m, 4H); 4.13 (m, 1H); 7.17−7.28 (m, 10H).

General Procedure for Coupling of Thiols with Carboxylic Acids, Exemplified for 3,3-Diphenyl-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (33). A solution of (2*S*)-1-(1,2-dioxo-3,3-dimethylpentyl)-2-pyrrolidinecarboxylic acid (2.99 g, 12.4 mmol) and dicyclohexylcarbodiimide (3.84 g, 18.6 mmol) in methylene chloride (150 mL) was treated with 3,3-diphenyl-1-propylthiol (3.40 g, 14.8 mmol) and dimethylamino pyridine (0.450 g, 3.72 mmol). After it was stirred at room temperature for 24 h, the reaction mixture was filtered through Celite, concentrated, redissolved in ether, filtered once more through Celite, and concentrated in vacuo to a pale yellow oil. This was purified on a flash column to obtain 4.19 g (75%) of the product, as a pale yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.86 (t, 3H, J = 6.6 Hz); 1.22 (s, 3H); 1.27 (s, 3H); 1.60−2.20 (m, 6H); 2.31 (q, 2H, J = 7.4 Hz); 2.77−2.85 (m, 2H); 3.52 (t, 2H, J = 7.3 Hz); 4.00 (t, 1H, J = 7.7 Hz); 4.66 (dd, 1H, J = 8.5, 2.8 Hz); 7.14−7.29 (m, 10H). TLC: R_f = 0.65 (25% EtOAc/hexane).

2-Phenethyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarbothioate (20). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85 (t, 3H, J = 7.5 Hz); 1.29 (s, 3H); 1.31 (s, 3H); 1.70−2.32 (m, 6H); 2.92 (t, 2H, J = 7.4 Hz); 3.22 (t, 2H, J = 7.4 Hz); 3.58 (m, 2H); 4.72 (m, 1H); 7.23−7.34 (m, 5H).

2-Phenethyl (2*S*)-1-(3-Cyclopentyl-1,2-dioxopentyl)-2-pyrrolidinecarbothioate (21). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.57−1.61 (m, 6H); 1.75−2.03 (m, 6H); 2.10−2.30 (m, 2H); 2.82−2.88 (m, 2H); 3.08−3.15 (m, 2H); 3.58−3.74 (m, 3H); 4.80−4.82 (m, 1H); 7.19−7.28 (m, 5H).

3-Phenyl-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarbothioate (22). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.86 (t, 3H, J = 7.2 Hz); 1.22 (s, 3H); 1.25 (s, 3H); 1.55−1.80 (m, 4H); 1.87 (tt, 2H, J = 6.9, 6.9 Hz); 2.05−2.28 (m, 2H); 2.65 (t, 2H, J = 6.9 Hz); 2.90 (t, 2H, J = 6.9 Hz); 3.50 (m, 2H); 4.68 (m, 1H); 7.13−7.29 (m, 5H).

3-Phenyl-1-propyl (2*S*)-1-(3-Cyclohexyl-1,2-dioxopentyl)-2-pyrrolidinecarbothioate (23). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.10−1.35 (m, 6H); 1.50−2.30 (m, 10H); 2.60−2.70 (t, 2H, J = 7.3 Hz); 2.82−2.94 (m, 2H); 3.08−3.15 (m, 1H); 3.63−3.73 (dd, 2H, J = 12.8, 7.0 Hz); 4.71 (m, 1H); 7.13−7.28 (m, 5H).

3-(3-Pyridyl)-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarbo-thioate (24). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.78−0.88 (m, 3H); 1.15−1.25 (m, 6H); 1.70−1.75 (m, 3H); 1.88−1.93 (m, 2H); 1.98−2.25 (m, 3H); 2.65−2.70 (m, 2H); 2.89−2.92 (m, 2H); 3.51−3.67 (m, 2H); 4.66−4.75 (m, 1H); 7.17−7.25 (m, 1H); 7.47−7.49 (m, 1H); 8.42−8.44 (m, 2H).

3-(*o*-Chlorophenyl)-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (25). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, 3H, J = 7.42 Hz); 1.23 (s, 3H); 1.27 (s, 3H); 1.59−2.27 (m, 7H); 2.80 (t, 2H, J = 7.53 Hz); 2.96 (t, 2H, J = 7.1 Hz); 3.12−3.23 (m, 1H); 3.55 (t, 2H, J = 5.13 Hz); 4.69−4.72 (m, 1H); 7.12−7.34 (m, 4H). MS: M^+ = 410.

3-(*o*-Fluorophenyl)-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (26). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, 3H, J = 7.5 Hz); 1.223(s, 3H); 1.26 (s, 3H); 1.70−2.09 (m, 7H); 2.19−2.23 (m, 1H); 2.71 (t, 2H, J = 7.4 Hz); 2.93 (t, 2H, J = 7.12 Hz); 3.55 (t, 2H, J = 6.74 Hz); 4.70 (dd, 1H, J = 8.54, 3.03 Hz); 6.99−7.07 (m, 2H); 7.14−7.19 (m, 1H); 7.20−7.26 (m, 1H).

3-(*m*-Chlorophenyl)-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (27). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, 3H, J = 7.4 Hz); 1.23 (s, 3H); 1.26 (s, 3H); 1.66−2.09 (m, 7H); 2.16−2.26 (m, 1H); 2.66 (t, 2H, J = 7.45); 2.87−2.94 (m, 2H); 3.53−3.57 (m, 2H); 4.69 (dd, 1H, J = 8.44, 2.97 Hz); 7.04 (d, 1H, J = 7.00 Hz); 7.15−7.27 (m, 3H).

3-(*m*-Fluorophenyl)-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (28). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, 3H, J = 7.5 Hz); 1.23(s, 3H); 1.26 (s, 3H); 1.73−2.09 (m, 7H); 2.19−2.23 (m, 1H); 2.68 (t, 2H, J = 7.4 Hz); 2.91 (t, 2H, J = 7.24 Hz); 3.55 (t, 2H, J = 6.9 Hz); 4.70 (d, 1H, J = 8.54 Hz); 6.85−6.95 (m, 3H); 7.22−7.27 (m, 1H).

3-(*p*-Chlorophenyl)-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (29). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, 3H, J = 7.50 Hz); 1.23 (s, 3H); 1.26 (s, 3H); 1.60−2.30 (m, 8H); 2.63−2.68 (m, 2H); 2.86−2.96 (m, 2H); 3.52−3.57 (m, 2H); 4.67−4.71 (m, 1H); 7.09 (d, 2H, J = 8.3 Hz); 7.24 (d, 2H, J = 9.1 Hz).

3-[4-(Trifluoromethyl)phenyl]propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (30). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.82−0.90 (m, 3H); 1.23 (s, 3H); 1.26 (s, 3H); 1.67−1.81 (m, 3H); 1.87−2.01 (m, 4H); 2.21−2.34 (m, 1H); 2.74 (t, 2H, J = 7.4 Hz); 2.91 (t, 2H, J = 6.01 Hz); 3.52−3.57 (m, 2H); 4.68−4.72 (m, 1H); 7.26 (d, 2H, J = 10.31 Hz); 7.53 (d, 2H, J = 7.96 Hz).

3-(*para*-Methoxyphenyl)propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (31). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.9 (t, 3H, J = 7.1 Hz); 1.26 (s, 3H); 1.29 (s, 3H); 1.65−2.25 (m, 8H); 2.65 (m, 2H); 2.89−2.96 (m, 2H); 3.55−3.73 (m, 2H); 3.80 (s, 3H); 4.70−4.81 (m, 1H); 6.83 (d, 2H, J = 8.0 Hz); 7.09 (d, 2H, J = 8.0 Hz).

3-(2-Naphthyl)propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidine-carbothioate (32). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.85−0.95 (t, 3H, J = 7.1 Hz); 1.21 (s, 3H); 1.24 (s, 3H); 1.70−1.85 (m, 3H); 1.91−2.23 (m, 4H); 2.23−2.40 (m, 1H); 2.80 (t, 2H, J = 7.4 Hz); 2.94 (t, 2H, J = 6.03 Hz); 3.50−3.61 (m, 2H); 4.71−4.74 (m, 1H); 7.30−7.39 (m, 1H); 7.40−7.49 (m, 2H); 7.65 (s, 1H); 7.80−7.91 (m, 3H). Mass spectrum: M^+ = 426.

4-Phenyl-1-butyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarbothioate (34). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.86 (t, 3H, J = 7.5 Hz); 1.21 (s, 3H); 1.24 (s, 3H); 1.52−1.80 (m, 6H); 1.82−2.01 (m, 2H); 2.10−2.30 (m, 2H); 2.60 (t, 2H, J = 7.2 Hz); 2.92 (m, 2H); 3.52 (t, 2H, J = 6.3 Hz); 4.67 (m, 1H); 7.13−7.28 (m, 5H).

2-Phenethyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-piperidinecarbothioate (35). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.94 (t, 3H, J = 7.5 Hz); 1.27 (s, 3H); 1.30 (s, 3H); 1.34−1.88 (m, 7H); 2.45 (m, 1H); 2.90 (t, 2H, J = 7.7 Hz); 3.26 (t, 2H, J = 7.7 Hz); 3.27 (m, 1H); 3.38 (m, 1H); 5.34 (m, 1H); 7.24−7.36 (m, 5H).

3-Phenyl-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-piperidinecarbothioate (36). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, J = 7.48 Hz); 1.24 (s, 3H); 1.28 (s, 3H); 1.43−1.92 (m, 10H); 2.69 (t, 2H, J = 7.52 Hz); 2.93 (t, 2H, J = 6.84 Hz); 3.22 (m, 1H); 3.38 (m, 1H); 5.29 (m, 1H); 7.16−7.30 (m, 5H).

3,3-Diphenyl-1-propyl (2*S*)-1-(3,3-Dimethyl-1,2-dioxopentyl)-2-piperidinecarbo-thioate (37). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, J = 7.4 Hz); 1.24 (s, 3H); 1.27 (s, 3H); 1.30−1.85 (m, 7H); 2.25−2.43 (m, 3H); 2.80−2.85 (m, 2H); 3.15−3.23 (m, 1H); 3.41 (br d, 1H, J = 14.0 Hz); 4.01 (t, 1H, J = 7.8 Hz); 5.27 (d, 1H, J = 5.2 Hz); 7.16−7.30 (m, 10H).

Biology

In Vitro Tests. Inhibition of the rotamase activity of FKBP-12 by test compounds was assayed as described by Kofron,[17] using the peptide *N*-succinyl Ala-Leu-Pro-Phe *p*-nitroanilide (Bachem) as substrate. Apparent K_i values were obtained and used as measures of relative ligand binding affinities.

Compounds were evaluated for their ability to promote neurite outgrowth in cultured sensory neurons as described previously.[10,11] Neurite outgrowth was assessed from photomicrographs of each explant culture, and all processes whose lengths exceeded the explant's diameter were counted. Dose−response curves were generated from which ED_{50} values were obtained.

Oral Dosing with Neuroimmunophilin Ligands. Drugs were solubilized in 100% ethanol at concentrations ranging from 100 to 300 mg/mL. Cremaphor EL (Sigma) was diluted in sterile saline to 10% (w/v). Just prior to dosing, each drug was diluted 20-fold with 10% Cremaphor EL and administered to mice at 10 mL/kg, to a final dose of 10 mg/kg or the appropriate doses for dose−response analysis.

MPTP Model of Parkinson's Disease in Mice. MPTP Lesions of Mice. Two different experimental paradigms were utilized as follows: (i) concurrent dosing of drugs with MPTP, to evaluate the neurotrophic effects of neuroimmunophilin ligand present during the process of nigrostriatal degeneration and (ii) delayed dosing of immunophilin ligands beginning 1 week after the onset of MPTP treatment, to evaluate potential regenerative or restorative effects of drug treatment after the peak of MPTP-induced degeneration. In both experimental paradigms, male CD1 mice (20−25 g) received five daily ip injections of MPTP (30 mg/kg) in saline vehicle. In the concurrent paradigm, daily oral and sc immunophilin ligand dosing occurred 30 min prior to each MPTP injection and on each of five subsequent days, and in the delayed post-MPTP paradigm, oral drug treatment began on the third day after cessation of MPTP treatment. Neuroimmunophilin ligands suspended in Cremaphor vehicle were given to groups of MPTP-treated mice at doses ranging from 0.4 to 100 mg/kg. All experimental mice and matching MPTP/vehicle and vehicle/vehicle control groups were sacrificed 18 days after their first MPTP injection.

TH Immunohistochemistry and Quantitative Analysis of Nigrostriatal Elements. Four animals from each group were sacrificed 18 days after their first MPTP injection for histological and immunohistochemical processing. Mice were perfused with 10% formalin, and their brains were postfixed overnight and then transferred to 30% sucrose saline. Frozen 30 μm sagittal striatal sections and 25 μm coronal nigral sections were cut on a sliding microtome, and every fourth section was reacted for free-floating immunohistochemistry using a polyclonal TH antibody (Pel Freeze, 1:2500 at 4EC for 4 nights), processed using the avidin:biotin peroxidase method (Vector Elite kit), and visualized with Diamino benzidine (DAB−HCl, Polysciences). Blinded analysis of TH fiber density in the central striatum was performed at 630× magnification. For each mouse striatum, five representative 100 μm × 100 μm fields in the central core region were photographed using a digital video camera. The percentage of sample field covered by TH positive processes and terminals was calculated using an image analysis program ("Simple," Compix Inc., Pittsburgh, PA). The mean and standard deviation of striatal innervation density were calculated for each group. The magnitude of striatal deafferentation due to the MPTP lesion was assessed by dividing the observed striatal innervation values obtained in MPTP/vehicle-treated cases by the mean striatal innervation density in vehicle/vehicle group and expressed as percent loss. The relative efficacy of neuroimmunophilin ligands at various doses in the MPTP models was compared by calculating percent recovery of striatal innervation density, determined by subtracting from the experimental observation the mean density from the analogous lesioned/untreated group and dividing this by the calculated percent loss due to neurotoxin treatment. The magnitude of neuroimmunophilin ligand-mediated increase in striatal innervation was also calculated as a function of the increased density as compared to MPTP/vehicle-treated cases (percent > sparing), obtained by dividing experimental striatal innervation density values by the mean density of striatal innervation in the MPTP group. All drug group to treatment group comparisons were evaluated using Student's t test.

In each group of experiments, compound **1** was run as a positive control compound for efficacy in the MPTP model. The *d*-isomer of compound **1** was synthesized as a control FKBP ligand, and its in vitro activity was less efficacious at FKBP 12 peptidylprolyl isomerase activity by greater than 3 orders of magnitude. This inactive stereoisomer of compound **1** was also evaluated in these MPTP paradigms at 10 mg/kg po and did not protect striatal TH innervation density.

References

(1) Galat, A. Peptidylproline cis−trans-isomerases: immunophilins. *Eur. J. Biochem.* **1993**, *216*, 689−707.
(2) Hamilton, G. S.; Steiner, J. P. Immunophilins: Beyond Immunosuppression. *J. Med. Chem.* **1998**, *41*, 5119−5143.
(3) Schreiber, S. L. Chemistry and biology of the immunophilins and their immunosuppressant ligands. *Science* **1991**, *251*, 283−287.
(4) Steiner, J. P.; Dawson, T. M.; Fotuhi, M.; Glatt, C. E.; Snowman, A. M.; Cohen, N.; Snyder, S. H. High brain densities of the immunophilin FKBP colocalized with calcineurin. *Nature* **1992**, *358*, 584−587.
(5) Lyons, W. E.; George, E. B.; Dawson, T. M.; Steiner, J. P.; Snyder, S. H. immunosuppressant FK506 promotes neurite outgrowth in cultures of PC12 cells and sensory ganglia. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3191−3195.
(6) Snyder, S. H.; Sabatini, D. M. Immunophilins in the nervous system. *Nat. Med.* **1995**, *1*, 32−37.
(7) Gold, B. G.; Storm-Dickerson, T.; Austin, D. R. The immunosuppressant FK506 increases functional recovery and nerve regeneration in rat sciatic nerve. *J. Neurosci.* **1995**, *15*, 7509−7516.
(8) Snyder, S. H.; Sabatini, D. M.; Lai, M. M.; Steiner, J. P.; Hamilton, G. S.; Suzdak, P. D. Neural actions of immunophilin ligands. *Trends Pharm. Sci.* **1998**, *19*, 21−26.
(9) Hamilton, G. S.; Steiner, J. P. Neuroimmunophilin ligands as novel therapeutics for the treatment of degenerative disorders of the nervous system. *Curr. Pharm. Des.* **1997**, *3*, 405−428.

(10) Hamilton, G. S.; Huang, W.; Connolly, M. A.; Ross, D. T.; Guo, H.; Valentine, H. L.; Suzdak, P. D.; Steiner, J. P. FKBP12-binding domain analogues of FK506 are potent, nonimmunosuppressive neurotrophic agents in vitro and promote recovery in a mouse model of Parkinson's Disease. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1785−1790.
(11) Steiner, J. P.; Hamilton, G. S.; Ross, D. T.; Valentine, H. L.; Guo, H.; Connolly, M. A.; Liang, S.; Ramsey, C.; Li, J.-H.; Huang, W.; Howorth, P.; Soni, R.; Fuller, M.; Sauer, H.; Nowotnick, A.; Suzdak, P. D. Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2019−2024.
(12) Choi, C.; Li, J.-H.; Vaal, M.; Thomas, C.; Limburg, D.; Chen, Y.; Soni, R.; Scott, C.; Ross, D. T.; Guo, H.; Howorth, P.; Valentine, H.; Liang, S.; Spicer, D.; Fuller, M.; Steiner, J.; Hamilton, G. S. Use of parallel synthesis combinatorial libraries for rapid identification of potent FKBP12 inhibitors. *Proceedings of the 216th National American Chemical Society Meeting*, 1998; Abstr. MEDI 157.
(13) Wei, L.; Wu, Y.-Q.; Wilkinson, D.; Chen, Y.; Soni, R.; Scott, C.; Ross, D. T.; Guo, H.; Howorth, P.; Valentine, H.; Liang, S.; Spicer, D.; Fuller, M.; Steiner, J.; Hamilton, G. S. Solid-phase synthesis of FKBP12 inhibitors: *N*-sulfonyl and *N*-carbamoyl prolyl and pipecolyl amides. *Proceedings of the 216th National American Chemical Society Meeting*, 1998; Abstr. MEDI 158.
(14) Hamilton, G. S. Emerging therapeutic agents for Parkinson's Disease. *Chem. Ind.* **1998**, Feb 16, 127−132.
(15) Gerlach, M.; Riederer, P.; Przuntek, H.; Youdim, M. B. MPTP mechanisms of neurotoxicity and their implications for Parkinson's Disease. *Eur. J. Pharmacol.* **1991**, *208*, 273−286.
(16) Gold, B. G. FK506 and the role of the immunopjilin FKBP-52 in nerve regeneration. *Drug Metab. Rev.* **1999**, *31*, 649−663.
(17) Kofron, J. L.; Kuzmic, P.; Kishore, V.; Colon-Bonilla, E.; Rich, D. H. Determination of kinetic constants for peptidylprolyl cis−trans isomerases by an improved spectrophotometric assay. *Biochemistry* **1991**, *30*, 6127.

JM010556C