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Synthesis, Molecular Modeling and Biological Evaluation of Aza-Proline and Aza-Pipecolic Derivatives as FKBP12 Ligands and Their In Vivo Neuroprotective Effects

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Abstract—Nonimmunosuppressant ligands, exemplified by GPI 1046 (**1**), for the peptidyl-prolyl isomerase FKBP12 have been found to unexpectedly possess powerful neuroprotective and neuroregenerative effects in vitro and in vivo. We have extensively explored the therapeutic utility of FKBP12 ligands based on analogues of proline and pipecolic acid. 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 aza-proline and aza-pipecolic acid analogues. Details of the synthetic studies, together with biological activity will be presented.

Introduction

FKBP12 is the cellular receptor for the immunosuppressant drug FK506. It belongs to a family of proteins known as the immunophilins which possess peptidyl-prolyl *cis-trans* isomerase activity (PPIase).[1–3] PPIases are also known to play critical roles in a variety of biological processes other than immunosuppression, including protein trafficking and regulation of neurite outgrowth.[4] Recently it was discovered that FKBP12 is 10–40-fold more enriched in the brain than in the immune tissues and that FK506 exhibits neurotrophic properties in vitro and in vivo.[5] FK506 possess two distinct binding domains: a FKBP12-binding domain and an effector domain, which mediates the interaction of the immunophilin–drug complex with the secondary protein target-calcineurin. Previously, we reported that small molecule ligands for FKBP12, such as **1** (GPI-1046, Fig. 1), possess neuroprotective and neuroregenerative properties in vitro and in vivo.[6,7] The neurotrophic effects of these compounds are independent of the immunosuppressive pathways because they lack the effector domain of FK506.

Azapeptides are peptides where the asymmetric C_α carbon of an amino acid is replaced by nitrogen. They are mimetics of natural peptides, however they are much more resistant to protease cleavage. There are numerous examples in the literature of azapeptides being utilized as enzyme inhibitors or receptor antagonists, such as protease papain inhibitors,[8] elastase active-site titrants,[9] and MHC II ligands.[10]

Analogues of proline and pipecolic acid have been the focus of previous work exploring the SAR of small molecules which mimic the FKBP-binding domain of FK506.[11–15] As part of our program to explore novel classes of FKBP12 ligands that may have enhanced metabolic stability and improved DMPK profile, we herein wish to report the synthesis and biological evaluation of a new class of FKBP12 ligands containing aza-proline and aza-pipecolic acid analogues (Fig. 1).

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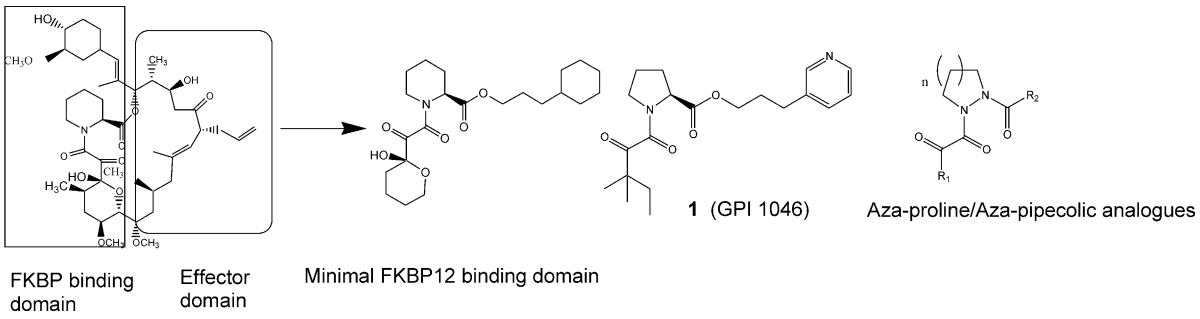
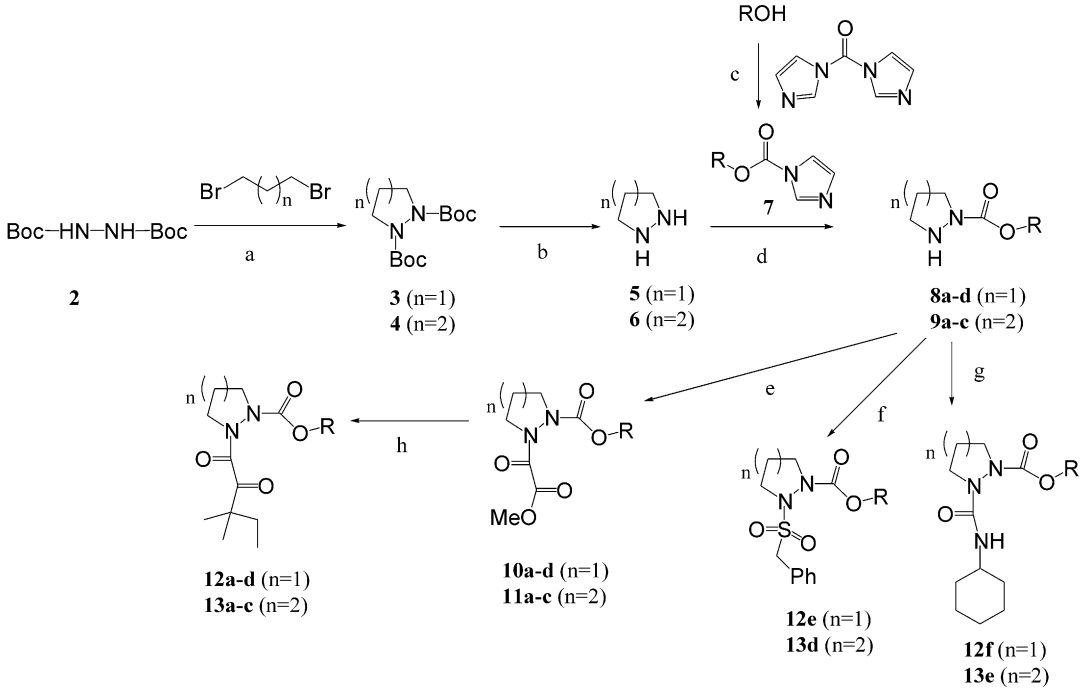


Figure 1. FK506 and small molecule mimetics of the FKBP12 binding domain.

Chemistry

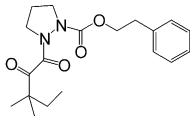
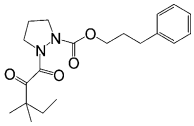
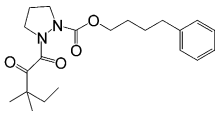
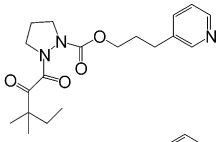
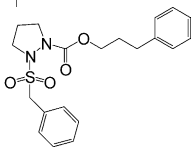
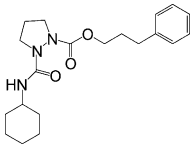
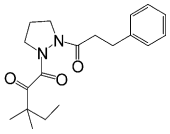
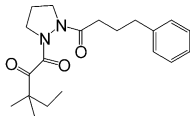
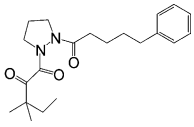
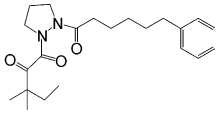
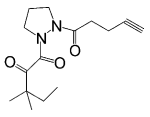
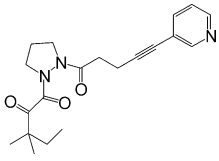
The general synthetic route to aza-prolyl and aza-pipecolyl carbamate compounds is shown in Scheme 1. Di-Boc hydrazine **2** was treated with sodium hydride, and then reacted with dibromopropane and dibromobutane to afford the five-membered ring pyzazolidine **3** and the six-membered perhydropyridazine **4**, respectively. Deprotection of the Boc group using TFA led to **5**/**6,** followed by acylation with various imidazole-1-carboxylates **7** to provide the aza-prolyl and aza-pipecolyl esters. Imidazole-1-carboxylates **7** were obtained easily by treating carbonyl diimidazole with various alcohols. Further acylation of **8**/**9** with methyl chlorooxoacetate led to the oxamate intermediates **10**/**11**. **10**/**11** were treated with Grignard reagent 1,1-dimethylpropyl magnesium bromide at −78°C yielding the desired diketoamide compounds **12a–d**/**13a–c** (see Table 1). Similarly, treatment of **8**/**9** with α-toluene sulfonyl chloride and cyclohexyl isocyanate gave the respective sulfonylamide **12e**/**13d** and urea **12f**/**13e** products. It is worth noting that compounds **8**/**9** were obtained free from bis-acylation products. Apparently, the nucleophilicity of the second nitrogen is significantly decreased after the introduction of the first carbonyl group. Consequently, the second acylation reactions require either more electrophilic reagents (sulfonyl chloride, oxylate chloride) or base promotion (KF on Celite), such as in the case of **12f**/**13e**.

The general synthetic route to the aza-prolyl and aza-pipecolyl amide compounds is shown in Scheme 2. Benzyl carbazate **14** was protected by a Boc group to yield asymmetric di-protected hydrazine **15**. Similarly, the five-membered ring pyrazolidine **16** and the six-membered perhydropyridazine **17** were obtained from di-protected hydrazine when treated with sodium hydride, followed by dibromopropane and dibromobutane in DMF. Mono deprotection of the Boc group using TFA resulted in **18**/**19**, which were then acylated by methyl chlorooxoacetate yielding oxamate intermediates **20**/**21**. The reaction of **20**/**21** with 1,1-dimethylpropylmagnesium bromide led to the formation of diketoamides **22**/**23**. The deprotection of the Cbz group under



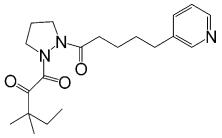
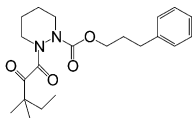
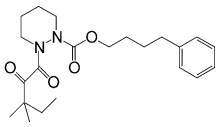
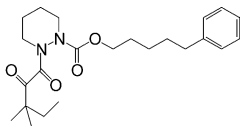
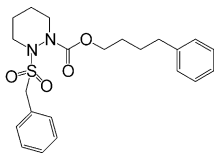
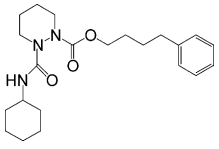
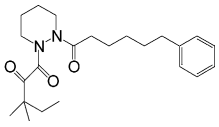
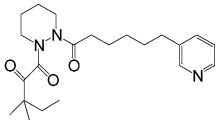
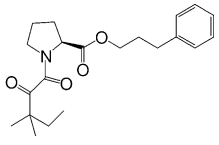
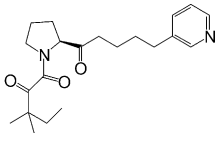
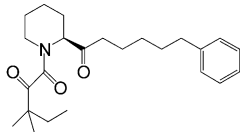
Scheme 1. Conditions: (a) NaH, DMF; (b) TFA, CH_2Cl_2; (c/d) CH_2Cl_2 rt; (e) THF, 0 °C, methyl oxalyl chloride; (f) CH_2Cl_2, α-toluene sulfonyl chloride; (g) CH_2Cl_2, cyclohexylisocyanate, KF-Celite; (h) THF, −78 °C, 1,1-dimethylpropylmagnesium bromide.

Table 1. Structures and in vitro activity for azal-prolyl and aza-pipecolyl compounds

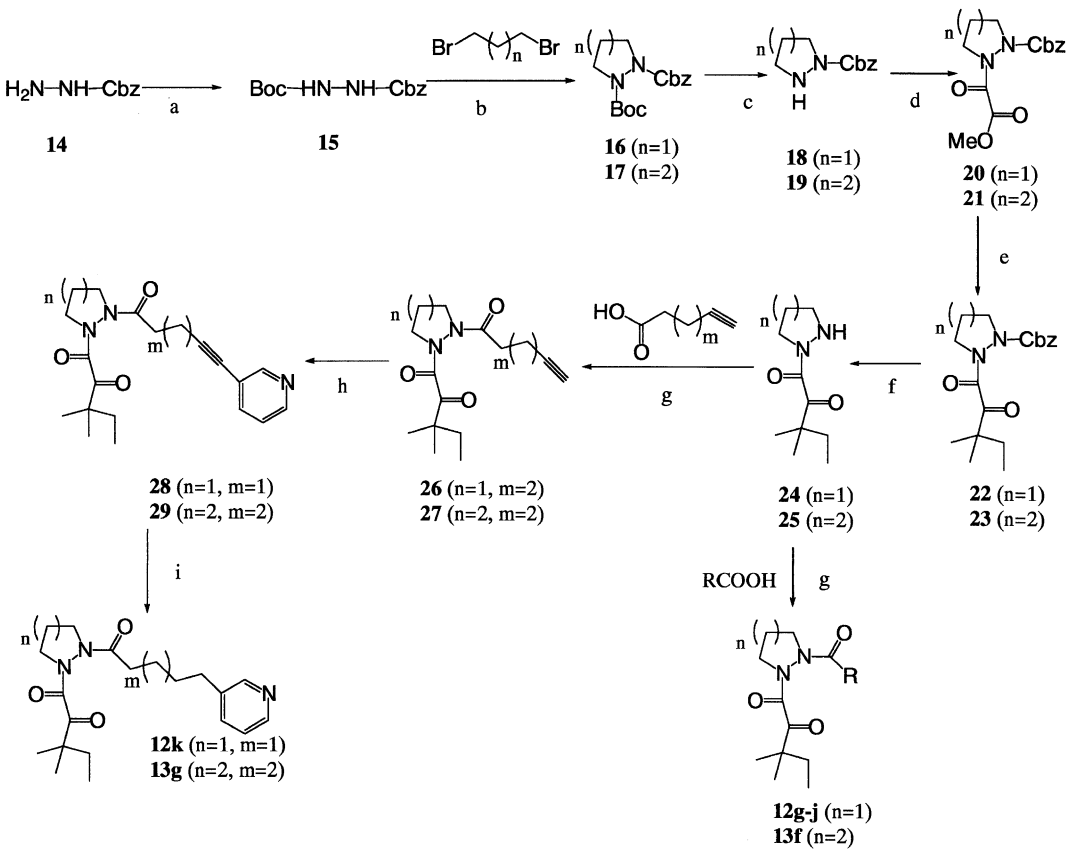
No.	Structure	Formula	Anal.	IC_{50}
12a		$C_{19}H_{26}N_2O_4$	C, H, N	15 μM
12b		$C_{20}H_{28}N_2O_4$	C, H, N	1.0 μM
12c		$C_{21}H_{30}N_2O_4$	C, H, N	3.8 μM
12d		$C_{19}H_{27}N_3O_4$	C, H, N	5.5 μM
12e		$C_{20}H_{24}N_2SO_4$	C, H, N	3.2 μM
12f		$C_{20}H_{29}N_3O_3$	C, H, N	> 25 μM
12g		$C_{19}H_{26}N_2O_3$	C, H, N	> 25 μM
12h		$C_{20}H_{28}N_2O_3$	C, H, N	9.0 μM
12i		$C_{21}H_{30}N_2O_3$	C, H, N	1.1 μM
12j		$C_{22}H_{32}N_2O_3$	C, H, N	> 25 μM
26		$C_{15}H_{22}N_2O_3$	C, H, N	> 25 μM
28		$C_{20}H_{25}N_3O_3$	C, H, N	5.5 μM

(continued)

Table 1 (*continued*)

No.	Structure	Formula	Anal.	IC_{50}
12k		$C_{20}H_{29}N_3O_3$	C, H, N	750 nM
13a		$C_{21}H_{30}N_2O_4$	C, H, N	900 nM
13b		$C_{22}H_{32}N_2O_4$	C, H, N	1.9 μM
13c		$C_{23}H_{34}N_2O_4$	C, H, N	5.0 μM
13d		$C_{22}H_{28}N_2S_1O_4$	C, H, N	2.1 μM
13e		$C_{22}H_{28}N_3O_3$	C, H, N	>25 μM
13f		$C_{23}H_{34}N_2O_3$	C, H, N	5.0 μM
13g		$C_{22}H_{33}N_3O_3$	C, H, N	3.3 μM
30				120 nM[a]
31				210 nM[a]
32				780 nM[a]

[a]Refs 14 and 15.



Scheme 2. Conditions: (a) $(Boc)_2O$, Et_3N; (b) NaH, DMF; (c) TFA, Ch_2Cl_2; (d) THF, 0 °C, methyl oxalyl chloride; (e) THF, −78 °C. 1,1-dimethylpropylmagnesium bromide; (f) Pd/C, H_2; (g) RCOOH, Et_3N, *i*-butylchloroformate; (h) 3-bromopyridine, Pd catalyst; (i) PtO_2, H_2.

hydrogenation conditions resulted in **24**/**25**, which, in turn, were coupled to various commercially available phenylalkyl acids under mixed anhydride peptide coupling conditions, affording final compounds **12g–j**/**13f** (see Table 1). For the synthesis of compounds **12k**/**13g**, no corresponding acids were commercially available. Therefore, **24**/**25** were first converted to **26**/**27** after coupling to acids containing a terminal alkyne. Then **26**/**27** were subjected to a facile palladium catalyzed coupling with 3-bromopyridine, resulting **28**/**29**. The final hydrogenation yielded the desired **12k**/**13g**. Overall, the reactions described above were smooth and of high yield, and suitable to run in parallel synthesis.

Structure–Activity Relationship (SAR)

Aza-apeptides arise when asymmetric α-carbon of an amino acid is replaced by nitrogen. It has been well documented that constrained cyclic aza-prolyl and aza-pipecolyl compounds adopt an asymmetric configuration (induced chirality) at both nitrogens, although the degree of pyramidalization at either nitrogen is not the same as a tetrahedral carbon.[16,17] It was expected that the aza nitrogen in our inhibitors would need to be pyramidalized in order to retain good affinity towards FKBP12. It has already been shown that only the L-stereoisomer of the prolyl/pipecolyl parent compounds show significant inhibitory activity while D-stereoisomers have no inhibitory activity. When our aza compounds were evaluated for in vitro inhibitory activity against the PPIase activity of recombinant purified human FKBP12, they were found to show significant activity, though generally reduced, when compared to their non-aza analogues, by at least an order of magnitude.

As the in vitro data shown in Table 1 indicate, the compounds strongly preferred a 'side arm' aromatic ring (e.g., **12b**, **12i** and **12k** vs **26**) and displayed an optimal 'side-arm' chain length of five atoms from the ring (**12i** vs **12g** and **12j**). Heterocycles such as a pyridine ring can replace the phenyl ring (**12k** and **13g** vs **12i** and **13f**). In addition, sulfonyl amides in both aza-prolyl and aza-pipecolyl series were essentially as potent as their diketoamides counterparts (**12e** and **13d** vs **12b** and **13b**). These results were consistent with previous SAR results in the regular prolyl and pipecolyl series (e.g., **30**, **31** and **32**).[14,15] One noticeable difference was urea compounds, such as **12f**, which did not show appreciable activity up to 25 μM, while urea-based prolyl ligands showed good activity[4] (e.g., prolyl analogue of **12f** having 1.8 μM activity).

Molecular Modeling

There are many different effects that play a role in the differential affinity of two ligands for the same target protein. For example, differences in the enthalpic interactions between the ligands and the target, entropy

effects and solvation effects can all play a role. The goal of the modeling experiments was to identify the most important effects in order to better understand the SAR of the aza 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.[18] SB3 was used as a template to build the aza compounds into the active site of FKBP12. Each compound was minimized in the active site with FKBP12 held rigid.[19]

All of the aza compounds fit well into the active site of FKBP12. The pharmacophore binding elements in the active site for these types of ligands can best be described as 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 aza compounds are predicted to fill both hydrophobic pockets and bind the Tyr82 OH (see Fig. 2) as well as their non-aza analogues. The interaction with the Ile56 backbone NH is similar to the non-aza analogues but requires further pyramidalization of the aza nitrogen for the interaction to remain optimal.

In the non-aza analogues that have a tetrahedral carbon in place of the aza nitrogen, the carbonyl interacts with the Ile56 NH, and is perfectly located for this interaction. In order for the aza compounds to reproduce the Ile56 NH interaction, the aza nitrogen would need to be pyramidalized $\sim 55^\circ$ from planarity. The crystal structures of the aza-tripeptides where the central amino acid is replaced by an aza-proline have been solved. The aza nitrogen is pyramidalized, on average, 40°,[16,17] which if extrapolated back to our aza compounds binding to FKBP12, this would not result in an optimal interaction between the carbonyl and the Ile56 NH. Since the forcefield used in these calculations is not explicitly parameterized for this type of molecule, it is unclear from those calculations what the energetic consequences of the required pyramidalization of the aza nitrogen are. In order to better determine the consequence of additional pyramidalization of the aza nitrogen required for optimal binding, we have quantum mechanically determined the structure of 1,2-diformylpyrazolidine at the MP2/6-31G(d)//MP2/6-31G(d) level of theory.[19] In this structure the aza nitrogen is pyramidalized 35° from planarity. In order to optimize the Ile56 NH interaction, the aza nitrogen would need to pyramidalize $\sim 20^\circ$ more. We have approximated the energetic consequences of this additional pyramidalization by freezing the appropriate torision angle while optimizing the rest of the molecule. At the MP2/6-31G(d)//MP2/6-31G(d) level of theory the additional pyramidalization of the aza nitrogen requires an additional 2.8 kcal/mol.

An additional consideration is the fact that there are two pyramidalized conformation in which each of the aza compounds exists. One conformation optimizes the binding interactions to the target (productive) via further pyramidalization; while the other one has the wrong 'stereochemistry' (non-productive) and would not result in significant binding to the target without first interconverting to the productive conformation. We believe that the energy required to further pyramidalize the nitrogen in the aza compounds to optimize the binding interactions, along with the conformational issue described above, primarily account for the order of magnitude difference in the activity between the aza and non-aza compounds.

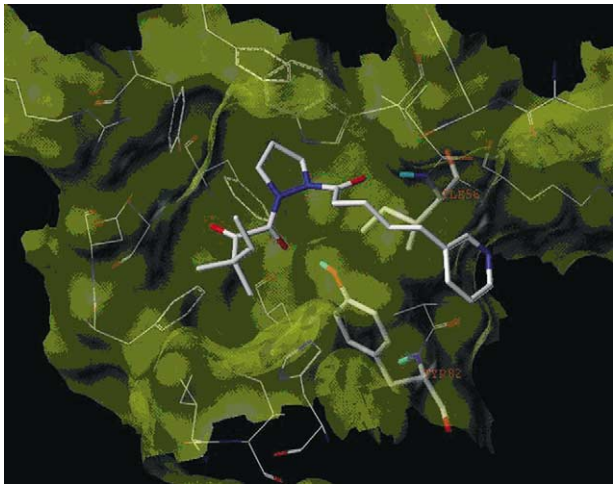


Figure 2. The predicted binding mode of **12k** complexed to FKBP12. **12k** and FKBP12 residues Ile56 and Tyr82 are shown in capped sticks. The surface of FKBP12 is shown in yellow.

In Vivo Biology

The neuroprotective properties of the aza compounds 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 the degeneration of this motor pathway and the subsequent decrease in dopaminergic transmission.[20] *N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a neurotoxin which selectively destroys dopaminergic neurons.[21] Lesioning of the nigrostriatal pathway in animals with MPTP has been utilized extensively as an animal model of Parkinson's disease. In a study utilizing a 'protective' or concurrent dosing protocol, mice were treated with MPTP and the aza compounds concurrently for 5 days.[13] The aza compounds were given for an additional 5 days. After 18

Table 2. In vivo data for representative examples

Compd	%Recovery 10 mg/kg, po
1	35.0 + 5.0
12k	56.8 + 6.2
13d	15.1 + 4.1
13f	34.9 + 5.9
28	25.6 + 3.4

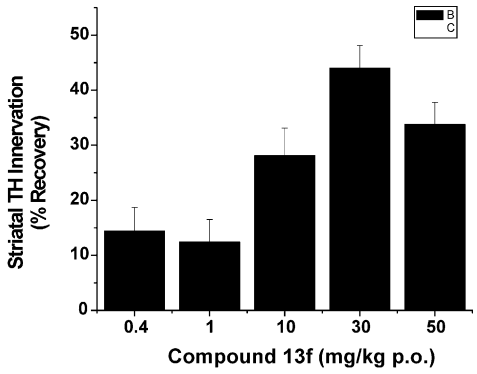


Figure 3. Dose–response curve for **13f**.

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 the survival of dopaminergic neurons.

Table 2 presents the data from representative aza compounds given to MPTP-treated animals in these experiments as the percent recovery of striatal dopaminergic innervation relative to MPTP-treated animals that received vehicle. In animals treated with MPTP and vehicle, a substantial loss of 60–70% of functional dopaminergic terminals was observed as compared to non-lesioned animals. Lesioned animals receiving test compounds (10 mg/kg po) concurrently with MPTP showed a striking preservation of TH-stained striatal dopaminergic terminals, demonstrating the ability of these compounds to block the degeneration of dopaminergic neurons produced by MPTP. Compounds such as **12k** and **13f** produced a remarkable preservation of striatal dopaminergic innervation up to 56.8 and 34.9% of control levels, respectively. The dose-response curve for **13f** following oral administration in the protective model is shown in Figure 3. It produced significant effects at 10 mg/kg dose and produced maximum preservation of about 45% at dose of 30 mg/kg.

Conclusions

We have developed an efficient synthetic approach to the synthesis of aza-prolyl and aza-pipecolyl compounds. We believe these compounds are the first reported examples of aza-proline and aza-pipecolic derivatives as FKBP12 ligands. Further more, our preliminary biological results suggest that some of these compounds are potent neuroprotective agents with potential therapeutic utility in treating degenerative disorders of the nervous system, such as Parkinson's disease.

Experimental

General methods

All commercially available starting materials and solvents were reagent grade. Anhydrous tetrahydrofuran (THF) and diethyl ether were used as obtained from Sigma-Aldrich Inc. (*S*)-Pipecolic acid was obtained as the (*R*)-tartrate salt from Oxford Asymmetry. Analytical thin-layer chromatography 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 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, USA. Elemental analyses were determined by Atlantic Microlab, Norcross, GA, USA and are within $\pm 0.4\%$ of the calculated values unless otherwise noted.

General procedure for Scheme 1

***tert*-Butyl 2-[(*tert*-butyl)oxycarbonyl]perhydropyridazinecarboxylate (4).** A solution of di-Boc hydrazine (20 g, 84.4 mmol) in 150 DMF was added dropwise to a suspended solution of 6.75 g (168.8 mmol) NaH in 75 mL DMF under nitrogen. After the mixture was stirred for 30 min at room temperature, a solution of 1,4 dibromobutane (18.2 g, 84.4 mmol) in 25 mL DMF was added dropwise. The reaction was allowed to stir overnight at room temperature. The reaction was then concentrated and partitioned between 200 mL CH_2Cl_2 and 200 mL water. The aqueous layer was extracted with an additional 200 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and filtered, and concentrated. The crude product was further purified by silica gel chromatography to yield 20.2 g (82% yield) product. The product was analyzed by GC/MS as pure compound with M^+ 286.

Perhydropyridazine (6). 2.83 mL (36.7 mmol) TFA was added dropwise to a solution of *tert*-butyl 2-[(*tert*-butyl)-oxycarbonyl]perhydropyridazinecarboxylate **4** (1.5 g, 5.2 mmol) in 7 mL CH_2Cl_2, and the mixture was stirred overnight. At this time, the reaction was completed and 5.85 mL (42 mmol) triethylamine was added to quench the reaction. The reaction was concentrated and the residue, which contained product, was used without further purification.

4-Phenylbutyl perhydropyridazinecarboxylate (9b). A solution containing 1,1′-carbonyl diimidazole (0.893 g, 5.5 mmol) in 5 mL CH_2Cl_2 was added slowly to a solution of CH_2Cl_2 containing phenylbutyl alcohol (0.89 mL, 5.77 mmol). After stirring at room temperature for 1 h, this solution was then added slowly to the solution containing perhydropyridazine **6** mentioned above. The reaction was allowed to stir overnight. The crude mixture was then concentrated and used without further purification.

Methyl 2-oxo-2-{2-[(4-phenylbutyl)oxycarbonyl]perhydropyridazinyl} acetate (11b). A solution of CH_2Cl_2 containing previous crude product of 4-phenylbutyl perhydropyridazinecarboxylate **9b** from the last step was cooled to 0 °C, and a solution of methyl oxalyl chloride (0.74 g, 5.77 mmol) in 5 mL CH_2Cl_2 was added dropwise over 0.5 h. The resulting mixture was stirred at 0°C for 4 h, and then warmed up to room temperature.

The reaction mixture was diluted with 50 mL CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was further purified by silica chromatography to yield 1.8 g (62% overall yield for three steps) product. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.39 (m, 2H); 1.69 (m, 6H); 2.62(t, 2H, $J=8$); 2.83 (m, 1H); 3.1 0(m, 1H); 3.79 (s, 3H); 4.16 (m, 3H); 4.31 (m, 1H); 7.22 (m, 5H).

4-Phenylbutyl 2-(3,3-dimethyl-2-oxopentanoyl)perhydropyridazine carboxylate (13b). A solution of methyl 2-oxo-2-{2-[(4-phenylbutyl)oxycarbonyl]perhydropyridazinyl} acetate **11b** (1.2 g, 3.45 mmol) in 15 mL dry THF was cooled to −78 °C and treated with 5.2 mL of 1.0 M solution of 1,1-dimethylpropylmagnesium chloride in THF. After stirring the resulting homogeneous mixture at −78 °C for 4 h, the mixture was poured into saturated ammonium chloride (20 mL) and extracted into ethyl acetate. The organic layer was washed with water, dried and concentrated. The crude material was purified by silica gel column, eluting with 25% ethyl acetate in hexane, to obtain 0.98 g product (73% yield). $R_f=0.73$ (2:1 hexane: EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.81 (t, 3H, $J=7.1$); 1.13 (s, 3H); 1.20 (s, 3H); 1.64 (m, 10H); 2.64 (m, 2H); 2.86 (m, 1H); 3.20 (m, 1H); 3.99 (m, 1H); 4.19 (m, 2H); 4.35 (m, 1H); 7.24 (m, 5H). Anal. calcd for $C_{22}H_{32}N_2O_4$: C, 68.01; H, 8.30; N, 7.21. Found: C, 68.10; H, 8.29; N, 7.15.

4-Phenylbutyl 2-[benzylsulfonyl]perhydropyridazinecarboxylate (13d). A solution of α-toluene sulfonyl chloride (1.12 g, 5.77 mmol) in CH_2Cl_2 was added to a CH_2Cl_2 solution containing 4-phenylbutyl perhydropyridazinecarboxylate **9b** (1.37 g, 5.2 mmol) and triethylamine (0.83 mL, 6 mmol). The reaction was stirred overnight at room temperature under nitrogen, and then diluted to 50 mL CH_2Cl_2. The organic layer was washed with water, dried, and concentrated. The crude material was purified by silica gel column to yield 1.4 g (64%) final product as clear oil. $R_f=0.60$ (2:1 hexane: EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.68 (m, 8H); 2.67 (m, 2H); 2.90 (m, 1H); 3.38 (m, 2H); 4.22 (m, 5H); 7.32 (m, 10H). Anal. calcd for $C_{22}H_{28}N_2S_1O_4$ C, 63.44; H, 6.78; N, 6.73, S, 7.70. Found: C, 63.86; H, 6.83; N, 6.41, S, 7.58.

4-Phenylbutyl 2-(*N*-cyclohexylcarbamoyl)perhydropyridazinecarboxylate (13e). Cyclohexylisocyanate (0.38 g, 3.0 mmol) was added to a CH_2Cl_2 solution containing 4-phenylbutyl perhydropyridazinecarboxylate **9b** (0.72 g, 2.75 mmol) and 50 wt.% KF-celite (348 mg, 3 mmol). The reaction was stirred overnight at room temperature under nitrogen atmosphere, and then diluted to 50 mL CH_2Cl_2. The organic layer was washed with water, dried, and concentrated. The crude material was purified by silica gel column to yield 0.95 g (89%) final product as clear oil. $R_f=0.28$ (2:1 hexane:EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.10 (m, 3H); 1.33 (m, 3H); 1.69 (m, 10H); 1.88 (m, 2H); 2.62 (m, 2H); 2.72 (m, 1H); 2.87 (m, 1H); 3.60 (m, 1H); 4.13 (m, 3H); 4.38 (m, 1H); 5.11 (d, 1H, $J=8.3$ Hz); 7.23 (m, 5H). Anal. calcd for $C_{22}H_{28}N_3O_3\cdot0.14H_2O$: C, 67.75; H, 8.60; N, 10.77. Found: C, 67.75; H, 8.45; N, 10.90.

2-Phenylethyl 2-(3,3-dimethyl-2-oxopentanoyl)pyrazolidinecarboxylate (12a). $R_f=0.5$ (25% EtOAc/hexane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.83 (t, 3H, $J=7.5$ Hz); 1.18 (s, 6H); 1.63 (m, 2H); 1.99 (m, 2H); 2.97 (t, 2H, $J=7.1$); 3.60 (br s, 4H); 4.35 (t, 2H, $J=6.6$ Hz); 7.19–7.30 (m, 5H). Anal. calcd for $C_{19}H_{26}N_2O_4$: C, 65.88; H, 7.56; N, 8.09. Found: C, 65.82; H, 7.51; N, 8.02.

3-Phenylpropyl 2-(3,3-dimethyl-2-oxopentanoyl)pyrazolidinecarboxylate (12b). $R_f=0.4$ (25% EtOAc/Hexane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, $J=7.4$ Hz); 1.22 (s, 6H); 1.66 (t, 2H, $J=7.5$ Hz); 2.00–2.12 (m, 4H); 2.72 (t, 2H, $J=7.4$ Hz); 3.60 (br s, 4H); 4.18 (t, 2H, $J=6.5$); 7.18–7.31 (m, 5H). Anal. calcd for $C_{20}H_{28}N_2O_4$: C, 66.64; H, 7.83; N, 7.77. Found: C, 66.73; H, 7.81; N, 7.72.

4-Phenylbutyl 2-(3,3-dimethyl-2-oxopentanoyl)pyrazolidinecarboxylate (12c). $R_f=0.6$ (25% EtOAc/Hexane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.83 (t, 3H, $J=7.5$ Hz); 1.19 (s, 6H); 1.67 (t, 2H, $J=7.5$ Hz); 1.60–1.69 (m, 4H); 2.07 (t, 2H, $J=7.4$ Hz); 2.62 (t, 2H, $J=6.4$); 3.60 (br s, 4H); 4.13 (t, 2H, $J=6.1$ Hz); 7.28–7.15 (m, 5H). Anal. calcd for $C_{21}H_{30}N_2O_4$: C, 67.35; H, 8.07; N, 7.48. Found: C, 67.54; H, 8.31; N, 7.40.

3-(3-Pyridyl)propyl 2-(3,3-dimethyl-2-oxopentanoyl)pyrazolidinecarboxylate (12d). $R_f=0.1$ (100% EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.85 (t, 3H, $J=7.5$ Hz); 1.21 (s, 6H); 1.67 (t, 2H, $J=7.5$ Hz); 2.00–2.13 (m, 4H); 2.72 (t, 2H, $J=7.5$ Hz); 3.62 (br, s, 4H); 4.19 (t, 2H, $J=6.4$ Hz); 7.28 (br s, 1H), 7.54 (d, 1H, $J=7.7$ Hz); 8.48 (s, 2H). Anal. calcd for $C_{19}H_{27}N_3O_4\cdot0.35H_2O$: C, 62.06; H, 7.59; N, 11.43. Found: C, 61.77; H, 7.53; N, 11.36.

3-Phenylpropyl 2-[benzylsulfonyl]pyrazolidinecarboxylate (12e). $R_f=0.5$ (40% EtOAc/hexane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.01–2.17 (m, 4H); 2.72 (t, 2H, $J=7.8$ Hz); 3.68 (br s, 4H); 4.23 (t, 2H, $J=6.6$ Hz); 4.51 (s, 2H); 7.17–7.50 (m, 10H). Anal. calcd for $C_{20}H_{24}N_2SO_4$: C, 61.83; H, 6.23; N, 7.21; S, 8.25. Found: C, 61.63; H, 6.21; N, 7.05; S, 8.07.

3-Phenylpropyl 2-(N-cyclohexylcarbamoyl)pyrazolidinecarboxylate (12f). $R_f=0.5$ (60% EtOAc/Hexane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.09–2.00 (m, 15H); 2.69 (t, 2H, $J=7.8$ Hz); 3.70 (br s, 4H); 4.18 (t, 2H, $J=6.4$ Hz); 5.46 (d, 1H, $J=8.2$ Hz); 7.16–7.30 (m, 5H). Anal. calcd for $C_{20}H_{29}N_3O_3$: C, 66.83; H, 8.13; N, 11.69. Found: C, 66.73; H, 8.28; N, 11.59

3-Phenylpropyl 2-(3,3-dimethyl-2-oxopentanoyl)perhydropyridazine carboxylate (13a). $R_f=0.73$ (2:1 hexane/EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.82 (t, 3H, $J=7.4$ Hz); 1.16 (s, 3H); 1.22 (s, 3H); 1.67 (m, 6H); 2.00 (m, 2H); 2.69 (t, 2H, $J=7.9$ Hz); 2.86 (m, 1H); 3.23 (m, 1H); 4.00 (m, 1H); 4.20 (m, 2H); 4.37 (m, 1H); 7.23 (m, 5H). Anal. calcd for $C_{21}H_{30}N_2O_4$: C, 67.35; H, 8.07; N, 7.48. Found: C, 67.51; H, 8.11; N, 7.39.

5-Phenylpentyl 2-(3,3-dimethyl-2-oxopentanoyl)perhydropyridazine-carboxylate (13c). $R_f=0.74$ (2:1 hexane/EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.82 (t, 3H,

$J = 7.4$); 1.14 (s, 3H); 1.21 (s, 3H); 1.38 (m, 2H); 1.65 (m, 10H); 2.62 (t, 2H, $J = 7.6$ Hz); 2.83 (m, 1H); 3.20 (m, 1H); 3.98 (m, 1H); 4.15 (m, 2H); 4.33 (m, 1H); 7.23 (m, 5H). Anal. calcd for $C_{23}H_{34}N_2O_4$: C, 68.63; H, 8.51; N, 6.96. Found: C, 68.70; H, 8.47; N, 7.08.

(*tert*-Butoxy)-*N*-[benzylamino]formamide (15). A solution of benzyl carbazate (25 g, 150.4 mmol), Boc anhydride (42.7 g, 195.5 mmol), triethylamine (19.8 g, 195.5 mmol), DMAP (0.9 g, 7.5 mmol) in 650 mL CH_2Cl_2 was stirred for 24 h. The mixture was concentrated and purified by silica gel column, eluting with 20% ethyl acetate in hexane, to yield 36 g (90%) product. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.40 (m, 9H); 5.25 (m, 2H); 7.36 (m, 5H).

***tert*-Butyl 2-benzylperhydropyridazinecarboxylate (17).** A solution of (*tert*-butoxy)-*N*-[benzylamino]formamide **15** (35 g, 131 mmol) in 300 mL DMF was added dropwise to a suspended solution of 6.3 g (262 mmol) NaH in 130 mL DMF under nitrogen. After the mixture was stirred for 30 min at room temperature, a solution of 1,4 dibromobutane (28.4 g, 131 mmol) in 50 mL DMF was added dropwise. The reaction was allowed to stir overnight at room temperature. The reaction was then concentrated, followed by partition between 200 mL CH_2Cl_2 and 200 mL water. The aqueous layer was extracted with additional 200 mL CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and filtered and concentrated. The crude product was further purified by silica chromatography to yield 13.5 g (32% yield) product. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.46 (m, 9H); 1.64 (m, 4H); 2.88 (m, 2H); 4.20 (m, 2H); 5.16 (m, 2H); 7.31 (m, 5H).

Methyl 2-oxo-2-[2-benzylperhydropyridazinyl]acetate (21). 20% TFA in CH_2Cl_2 was cooled to 0°C and added dropwise to a solution of tert-butyl 2-benzylperhydropyridazinecarboxylate **17** (13.34 g, 41.7 mmol) in 10 mL CH_2Cl_2. The mixture was stirred overnight. At this time, the mixture was cooled to 0 °C and 12.66 mL (125 mmol) triethylamine was added, followed by addition dropwise of methyl oxalyl chloride (5.62, 45.9 mmol) in 5 mL CH_2Cl_2. The mixture was allowed to stir 2 h at 0 °C and warm up to room temperature overnight. The reaction was diluted with addition of CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was further purified by silica chromatography to yield 9.2 g (72.4% yield) product as clear oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.72 (m, 4H); 2.85(m, 1H); 3.12(m, 1H); 3.67 (s, 3H); 4.15 (m, 1H); 4.35 (m, 1H); 5.20 (m, 2H); 7.35 (m, 5H).

3,3-Dimethyl-1-[2-benzylperhydropyridazinyl]pentane-1,2-dione (23). A solution of methyl 2-oxo-2-[2-benzylperhydropyridazinyl]acetate **21** (9.0 g, 29.4 mmol) in 30 mL dry THF was cooled to −78 °C and treated with 35 mL of 1.0 M solution of 1,1-dimethylpropylmagnesium chloride in THF. After stirring the resulting homogeneous mixture at for 5 h, the mixture was poured into saturated ammonium chloride (150 mL) and extracted into ethyl acetate. The organic layer was washed with water, dried and concentrated. The crude material was purified by silica gel column, eluting with 10% ethyl acetate in hexane, to obtain 7.0 g product (69% yield) as clear oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.76 (t, 3H, $J = 7.0$ Hz); 1.06 (s, 6H); 1.69 (m, 6H); 2.80 (m, 1H); 3.15 (m, 1H), 4.03 (m, 1H); 4.13 (m, 1H), 5.18 (m, 2H), 7.36 (m, 5H).

3,3-Dimethyl-1-perhydropyridazinylpentane-1,2-dione (25). 1 g 10% Pd/C was added to a solution of 3,3-dimethyl-1-[2-benzylperhydropyridazinyl] pentane-1,2-dione (7.0 g, 20.2 mmol) in 70 mL EtOH. The mixture was under hydrogenation at room pressure (1 atm) overnight. The product was obtained as white solid after filtering Pd catalyst and concentration (3.8 g, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, $J = 7.0$ Hz), 1.19 (s, 6H), 1.65 (m, 4H), 1.79 (m, 2H), 2.85 (m, 2H), 3.42 (m, 1H), 3.56 (m, 1H).

3,3-Dimethyl-1-[2-(6-phenylhexanoyl)perhydropyridazinyl]-pentane-1,2-dione (13f). To a solution of 5-phenylvalaric acid (0.2 g, 1.1 mmol) in 3 mL CH_2Cl_2 was added triethylamine (0.15 mL, 1.1 mmol), followed by isobutyl chloroformate (0.15 g, 1.1 mmol) at 0°C. After stirring for 5 min, a solution of 3,3-dimethyl-1-perhydropyridazinylpentane-1,2-dione **25** (0.212 g, 1 mmol) in 1 mL CH_2Cl_2 was added. The reaction was gradually warmed up to room temperature. The crude material was subject to silica gel purification to yield final product as clear oil (0.20 g, 55%). $R_f = 0.58$ (33% EtOAc/hexane). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, 3H, $J = 7.5$ Hz), 1.24 (s, 6H), 1.37 (m, 2H), 1.68 (m, 6H), 1.74 (m, 4H), 2.23 (m, 2H), 2.62 (t, 2H, $J = 7.60$ Hz), 2.80 (m, 2H), 4.53 (m, 2H), 7.21 (m, 5H). Anal. calcd for $C_{23}H_{34}N_2O_3$: C, 71.47; H, 8.87; N, 7.25. Found: C, 71.54; H, 8.80; N, 7.32.

1-(2-Hex-5-ynoylperhydropyridazinyl)-3,3-dimethylpentane-1,2-dione (27). To a solution of 5-hexynoic acid (0.467 g, 4 mmol) in 10 mL CH_2Cl_2 was added triethylamine (0.56 mL, 4 mmol), followed by isobutyl chloroformate (0.53 mL, 4 mmol) at 0 °C. After stirring for 5 min, a solution of 3,3-dimethyl-1-perhydropyridazinyl pentane-1,2-dione **25** (0.424 g, 2 mmol) in 1 mL CH_2Cl_2 was added. The reaction was gradually warmed up to room temperature. The crude material was subject to silica gel purification to yield final product as clear oil (0.385 g, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, $J = 7.0$ Hz); 1.26 (s, 6H); 1.76 (m, 8H); 2.28 (m, 2H); 2.50 (m, 2H); 2.88 (m, 2H); 3.60 (m, 1H); 4.50 (m, 2H).

3,3-Dimethyl-1-[2-(6-(3-pyridyl)hex-5-ynoyl)perhydropyridazinyl] pentane-1,2-dione (29). To a solution of 1-(2-hex-5-ynoylperhydropyridazinyl)-3,3-dimethylpentane-1,2-dione **27** (0.384 g, 1.25 mmol) in 10 mL CH_2Cl_2 under nitrogen was added 3-iodopyridine (0.283 g, 1.38 mmol), $(Ph_3P)_2PdCl_2$ (0.044 g, 0.06 mmol), CuI (0.0024 g, 0.013 mmol) and triethylamine (0.3 mL, 2 mmol). The reaction mixture was stirred 30 min at room temperature and then refluxed overnight. The mixture was concentrated and purified by silica gel column, eluting with 30% ethyl acetate in hexane, to yield product as light yellow oil (0.31 g, 65%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (t, 3H, $J = 7.4$ Hz); 1.21 (s, 6H); 1.70

(m, 6H); 1.96 (m, 2H), 2.52 (m, 3H); 2.90 (m, 2H); 3.60 (m, 1H); 4.42 (m, 2H); 7.20 (m, 1H); 7.66 (m, 1H); 8.49(m, 1H); 8.62 (m, 1H).

3,3-Dimethyl-1-[2-(6-(3-pyridyl)hexanoyl)perhydropyridazinyl] pentane-1,2-dione (13g). 0.1 g PtO_2 was added to a solution of 3,3-dimethyl-1-[2-(6-(3-pyridyl)hex-5 ynoyl)perhydropyridazinyl] pentane-1,2-dione **35** (0.3 g, 0.8 mmol) in 20 mL dry MeOH. The mixture was under hydrogenation at room pressure (1 atm) overnight. The product was obtained as clear oil after filtering the catalyst, concentrating and purifying on a silica gel (0.125 g, 41%). $R_f = 0.18$ (EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, 3H, $J = 7.4$ Hz); 1.24 (s, 6H); 1.38 (m, 2H); 1.66 (m, 10H); 2.14 (m, 2H); 2.63 (m, 2H); 2.82 (m, 2H); 4.60 (m, 2H); 7.23 (m, 4H). Anal. calcd for $C_{22}H_{33}N_3O_3$: C, 68.19; H, 8.58; N, 10.84. Found: C, 68.40; H, 8.52; N, 10.62.

3,3-Dimethyl-*N*-[2-(3-phenylpropanoyl)tetrahydro-1H-1-pyrazolyl]-1,2-pentane-dione (12g). $R_f = 0.60$ (2:1 hexane/EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.80–0.85 (t, 3H); 1.11–1.15 (m, 8H); 1.58–2.02 (m, 6H): 2.50–2.95 (m, 4H); 7.17–7.28 (m, 5H). Anal. calcd for $C_{19}H_{26}N_2O_3$: C, 69.06; H, 7.93; N, 8.48. Found: C, 68.98; H, 7.90; N, 8.41.

3,3-Dimethyl-1-[2-(4-phenylbutanoyl)pyrazolidinyl]-pentane-1,2-dione (12h). $R_f = 0.5$ (Hexane:EtOAc 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, $J = 7.5$ Hz); 1.22 (s, 3H); 1.26 (s, 3H); 1.64 (m, 2H); 1.92–2.07 (m, 5H), 2.20 (m, 1H), 2.63 (m, 2H); 3.25 (m, 2H); 3.80 (m, 2H); 7.27 (m 5H, aromatic). Anal. calcd for $C_{20}H_{28}N_2O_3$: C, 69.05; H, 8.27; N, 8.06. Found: C, 69.02; H, 8.22; N, 8.05.

3,3-Dimethyl-*N*-[2-(5-phenylpentanoyl)tetrahydro-1H-1-pyrazolyl]-1,2-pentane-dione (12i). $R_f = 0.25$ (2:1 hexane/EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.81–0.83 (m, 3H); 1.14 (s, 6H); 1.21 (m, 2H); 1.55–1.62 (m, 8H): 2.02 (m, 2H); 2.61 (m, 4H); 7.14–7.28 (m, 5H). Anal. calcd for $C_{21}H_{30}N_2O_3$: C, 70.36; H, 8.44; N, 7.81. Found: C, 70.10; H, 8.41; N, 7.77.

3, 3-Dimethyl-1-[2-(6-phenylhexanoyl)pyrazolidinyl]pentane-1,2-dione (12j). $R_f = 0.5$ (hexane/EtOAc 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, $J = 7.5$ Hz); 1.22 (s, 3H); 1.26 (s, 3H); 1.35 (m, 2H); 1.59 (m, 6H); 2.07 (m, 2H), 2.20 (m, 1H), 2.60 (m, 3H); 3.25 (m, 2H); 3.70 (m, 2H); 7.26 (m 5H, aromatic). Anal. calcd for $C_{22}H_{32}N_2O_3$: C, 70.65; H, 8.70; N, 7.36. Found: C, 70.94; H, 8.66; N, 7.52.

3,3-Dimethyl-1-(2-pent-4-ynoylpyrazolidinyl)pentane-1,2-dione (26). $R_f = 0.45$ (EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, $J = 7.5$ Hz); 0.90 (m, 2H); 1.22 (s, 3H); 1.26 (s, 3H); 1.64 (m, 2H); 2.03–2.20 (m, 3H), 2.52 (m, 2H), 2.63 (m, 1H); 3.69 (m, 3H). Anal. calcd for $C_{15}H_{22}N_2O_3$: C, 64.55; H, 7.98; N, 9.98. Found: C, 64.73; H, 7.97; N, 10.06.

3,3-Dimethyl-1-[2-(5-(3-pyridyl)pent-4-ynoyl)pyrazolidinyl]pentane-1,2-dione (28). $R_f = 0.2$ (EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, $J = 7.5$ Hz); 1.22 (s, 3H); 1.26 (s, 3H); 1.63 (m, 2H); 2.1 (m, 2H); 2.73 (m, 4H); 3.20–3.85 (m, 4H); 7.19 (m, 1H); 7.66 (m, 1H); 8.5 (m, 2H). Anal. calcd for $C_{20}H_{25}N_3O_3$: C, 67.67; H, 6.91; N, 10.63. Found: C, 64.58; H, 7.09; N, 10.82.

3,3-Dimethyl-1-[2-(5-(3-pyridyl)pentanoyl)pyrazolidinyl]-pentane-1,2-dione (12k). $R_f = 0.3$ (EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, $J = 7.5$ Hz); 1.22 (s, 3H); 1.26 (s, 3H); 1.37 (m, 2H); 1.65 (m, 6H); 2.1 (m, 2H); 2.30 (m, 1H); 2.62 (m, 3H); 3.20–3.85 (m, 4H); 7.19 (m, 1H); 7.66 (m, 1H); 8.5 (m, 2H). Anal. calcd for $C_{20}H_{29}N_3O_3$: C, 65.74; H, 8.06; N, 11.09. Found: C, 65.98; H, 8.20; N, 10.89.

Molecular Modeling

Most of the molecular modeling was performed using SYBYL modeling.[22] The crystal structure of (1*R*)-1,3-diphenyl-1-propyl (2*S*)-1-(3,3-dimethyl-1,2- dioxopentyl)-2-piperidinecarboxylate (SB3) bound to FKBP12 (PDB code: 1FKG) was used for all experiments.[18] SB3 was used as a template to build the aza compounds into the active site of FKBP12. Each compound was minimized in the active site with the structure of the enzyme held rigid. The Tripos forcefield[23] with Gasteiger–Huckel atomic charges[24] were used in all calculations. A non-bonded cutoff of 25 Å was utilized with a distance dependent dielectric. The ab initio quantum mechanical calculations were performed using the program GAMESS.[25] Molecules were optimized at the MP2 level of theory using the 6-31G(d) basis set.[26–29]

Biology

Inhibition of the rotamase activity of FKBP-12 by test compounds was assayed as described by Kofron,[12] using the peptide *N*-succinyl Ala-Leu-Pro-Phe *p*-nitroanilide (Bachem) as substrate.

MPTP lesioning of dopaminergic neurons in mice was used as an animal model of Parkinson's disease as previously described.[13] Four-week-old male CD1 white mice (20–25 g) were dosed ip with 30 mg/kg of MPTP in saline vehicle for 5 days. Test compounds suspended in Cremaphor or vehicle, were administered by daily oral dosing occurred 30 min prior to each MPTP injection and on each of 5 subsequent days. All experimental mice and matching MPTP/vehicle and vehicle/vehicle control groups were sacrificed 18 days after their first MPTP injection. 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.

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