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Bioorganic & Medicinal Chemistry Letters 16 (2006) 4385–4390
Bioorganic & Medicinal Chemistry Letters

Modeling and synthesis of non-cyclic derivatives of GPI-1046 as potential FKBP ligands with neurotrophic properties

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Received 1 March 2006; revised 16 May 2006; accepted 16 May 2006
Available online 5 June 2006

Abstract—Prompted by the therapeutic potential of the neuroimmunophilin FK506-binding protein (FKBP) ligand, GPI-1046, in the treatment of nerve injuries and neurodegenerative diseases, a novel series of non-cyclic derivatives of GPI-1046 were designed and synthesized. Computer modeling analysis revealed that these relatively linear derivatives could energy-favorably bind to FKBP12 with an analogous binding mode to GPI-1046. The neurotrophic activity of the target compounds was assessed in chick dorsal root ganglion (DRG) cultures. As a result, 6 out of 11 test compounds at either or both concentrations of 1 pM and 100 pM significantly promoted neurite outgrowth in DRGs in the presence of 0.15 ng/ml nerve growth factor (NGF). Compound **5c** at 100 pM exhibited the greatest neurotrophic effect in promoting both the number and length of neurite processes. However, in the absence of exogenously added NGF, all test compounds, including GPI-1046, failed to afford any positive effect on DRGs. This study suggests the intriguing potential of these compounds for further investigation.

With the initial reports of neuroregenerative and neuroprotective properties of FK506,[1,2] a natural macrolide immunosuppressant agent isolated from *Streptomyces tsukubaensis* and clinically used in inhibiting immune responses in organ transplantation,[3] investigations in immunophilins, in particular FK506-binding proteins (FKBPs) in the nervous system, have spurred a great advance in neuroscience and raised the possibility of applying FKBP ligands as a novel therapeutic strategy in the treatment of nerve injuries and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.[4–6] In comparison with a wide variety of peptidic neurotrophic factors (NTFs), which demonstrate intriguing therapeutic properties in various pre-clinical models of neurodegeneration, while their clinical development is hindered by their unfavorable pharmacokinetics nature,[7,8] FKBP ligands appear to hold a greater promise to develop into potential therapies in the light of their oral bioavailability and permeability across the blood–brain-barrier.

FK506 is composed of two domain structures, the 'FKBP-binding domain' and the 'effector domain' (see Fig. 1).[9] FK506-induced immunosuppressive effect has been associated with the formation of a triple complex with FKBP12 (the 12 kDa FKBP) and calcineurin via its 'FKBP-binding domain' and 'effector domain,' respectively.[10,11] However, it is suggested that the neural activity of FK506 is underlaid merely by its interaction with FKBP and independent of calcineurin inhibition.[5] Over the past decade, this profound notion has served as the basis for the rational design and development of neurotrophic/neuroprotective FKBP ligands devoid of undesirable immunosuppressive activity.

One typical example which resulted from the decade of development efforts was the early emergence of a small molecule, GPI-1046 (3-(3-pyridyl)-1-propyl (2*S*)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidine carboxylate), a simplified mimic structure of the 'FKBP-binding domain' of FK506 and lacking the 'calcineurin binding domain' present in FK506 (see Fig. 1).[12] GPI-1046 has been widely demonstrated to be highly efficacious in promoting morphologic and functional recovery in both in vitro and in vivo rodent models of peripheral nerve

Keywords: FK506-binding protein; GPI-1046; Nerve growth factor; Neurotrophic activity; Dorsal root ganglion; Computer modeling; Synthesis.
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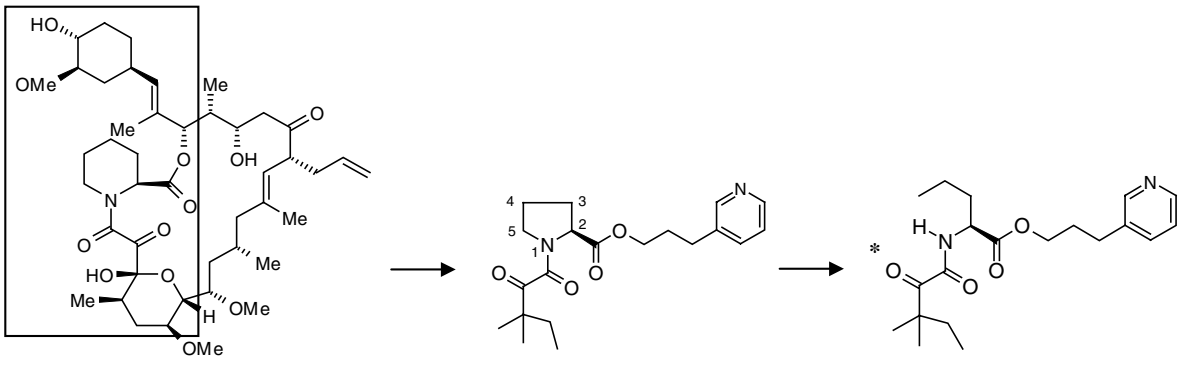
doi:10.1016/j.bmcl.2006.05.044



FK506 **GPI-1046** **GPI-1046-S**

Figure 1. (Left) Chemical structure of FK506. The circled portion represents the 'FKBP-binding domain' in FK506, which is believed to be responsible for the neural activity of FK506. (Middle) Chemical structure of GPI-1046, a mimic small molecule of the 'FKBP-binding domain' in FK506. (Right) The linear stereoisomer of GPI-1046 cleaved at the N^1–C^5 bond of the pyrrolidine ring.

injuries and neurodegenerative conditions, while having no effect in the immune system.[12–14]

In this pilot study, we sought to assess whether the non-cyclic derivatives of GPI-1046 would have the comparable therapeutic potential to GPI-1046 in promoting neurotrophism in vitro. To this end, we first conducted a computer modeling analysis to determine whether the relatively linear analogues of GPI-1046 would energy-favorably bind to FKBP. Then, based upon the promising predictive results yielded from the computer simulation, we designed and synthesized a series of target compounds that structurally resemble GPI-1046 while the pyrrolidine ring cleaved at the N^1–C^5 bond (see Fig. 1). Finally, we determined the neurotrophic activity of these relatively linear non-cyclic derivatives of GPI-1046 using a standard bioassay in chick dorsal root ganglion cultures.

FKBP exists in multiple isoforms and it is still controversial with respect to the isoform that is required for FKBP ligand-associated neural responses.[15,16] In view of the high structural homology among FKBP isoforms, we used FKBP12 as the target receptor in our computer modeling analysis. Simulation of the binding of the linear stereoisomer of GPI-1046, labeled GPI-1046-S (see Fig. 1), as a representative non-cyclic derivative of GPI-1046, with FKBP12 was composed of two consecutive steps: (1) energy minimization of GPI-1046-S; (2) docking of GPI-1046-S into the ligand binding site of FKBP12. Both simulations were performed on a SGI O2 R10000 workstation (Silicon Graphics Inc.) using the molecular modeling package InsightII 98.0 (Accelrys Inc.).

The 3D structure of GPI-1046-S was constructed on the InsightII platform, followed by a preliminary energy minimization by molecular mechanics. Since GPI-1046-S is a more flexible structure able to adopt a wide range of conformations, in the further process of energy optimization, 'template forcing' was used to force the best root mean square (RMS) match between the predicted low-energy conformation of GPI-1046-S and the experimentally resolved bound conformation of GPI-1046 on FKBP12 (PDB code: 1F40).[17] An efficient minimization strategy was designed as follows: First, 800 cycles of steepest descents minimization followed by 500 cycles of conjugate gradients minimization were performed by applying a force constant of 50 kcal mol^{-1} $Å^{-2}$. Then, three separate minimization steps of 500 cycles of conjugate gradients minimization, where the force constant was set to 25, 10, and 5 kcal mol^{-1} $Å^{-2}$, respectively, were performed. Finally, the model was relaxed by removing the constraint until the RMS derivative fell below 0.001 kcal mol^{-1} $Å^{-1}$ and a total molecular energy was measured at 152.7 kcal mol^{-1}. In comparison with the FKBP-bound conformation of GPI-1046 with a total energy of 259.1 kcal mol^{-1}, this analysis demonstrates that GPI-1046-S can adopt a close conformation to GPI-1046 with a low molecular energy, which may serve as an approximate bound conformation of GPI-1046-S on FKBP12.

The low-energy conformation of GPI-1046-S resulted from the above energy minimization was applied as the initial docking conformation. Considering the resemblance of this conformation to the bound conformation of GPI-1046 on FKBP12,[17] the two structures were first superimposed on heavy atoms in the context of FKBP12, resulting in a RMS of 2.0. Then, the structure of GPI-1046 was removed from the active site, and the resulting initial model of GPI-1046-S in complex with FKBP12 was energy optimized by 'Discover' calculation (a module integrated into InsightII), during which molecular mechanics, molecular dynamics, and then molecular mechanics were performed consecutively. As a result, the low intermolecular energy between GPI-1046-S and FKBP12 with a total of -55.5 kcal mol^{-1} (with the van der Waals and electrostatic energy at -39.0 and -16.5 kcal mol^{-1}, respectively) in the final complex model demonstrates that GPI-1046-S interacts energy-favorably with FKBP12 in a similar manner to GPI-1046 (see Fig. 2). The structural moiety in GPI-1046-S, which is in accordance with the prolyl ring in GPI-1046, is located in the same cavity in FKBP12, although a relatively greater deviation occurs with the two side moieties. Moreover, the favorable binding of

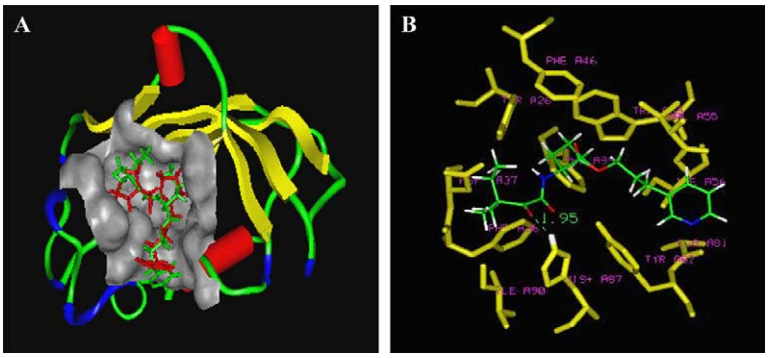
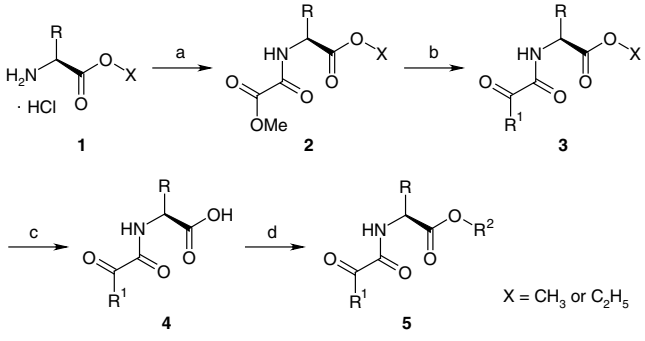


Figure 2. (A) Binding modes of GPI-1046 (red) and GPI-1046-S (green) in the active site of FKBP12. The protein is presented in secondary structure and the active site is presented by its contact surface. (B) Close-up view of GPI-1046-S in the active site of FKBP12. The protein is presented with the residues comprising the active site and depicted in yellow; the HE^2 atom in residue His^{87} is depicted in white. Ligand atoms are colored by the atom type. Intermolecular hydrogen bond is shown in green dash-line.

GPI-1046-S to FKBP12 is further enhanced by the formation of the hydrogen bond occurring between the O* atom in GPI-1046-S and the HE^2 atom in residue His^{87} in FKBP12 (refer to Figs. 1 and 2), which is postulated to be a critical factor that stabilizes the overall orientation of GPI-1046-S in the binding site of FKBP12. This analysis implicates that GPI-1046-S and related non-cyclic derivatives of GPI-1046 could be potential FKBP ligands with similar biological properties to GPI-1046.

Prompted by the favorable modeling result, we then designed a series of structural analogues to GPI-1046-S, in consideration of the structure-activity relationship revealed from our previous analyses[18] and the availability of the starting materials for preparation of the target compounds. As shown in Scheme 1, synthesis of the target compounds was started from the commercially available optically active α-alkyl amino acid ester hydrochloride **1**. The free amino group in **1** was acylated by methyl oxalyl chloride in the presence of triethylamine in methylene chloride to afford the *N*-(1,2-dioxo-2-methoxyethyl) substituted intermediate **2**. The methoxy group attached to the dicarbonyl moiety in **2** was then nucleophilically substituted by alkylating group under treatment with Grignard reagent, at the reaction temperature of −78 °C to reduce the occurrence



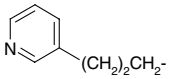
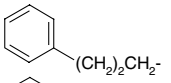
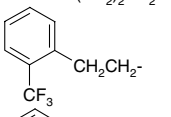
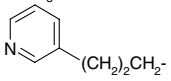
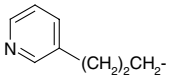
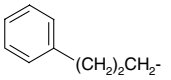
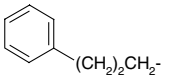
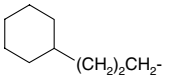
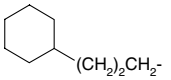
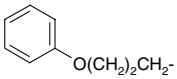
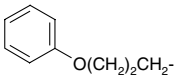
Scheme 1. Reagents and conditions: (a) $CH_3OCOCOCl$, $(C_2H_5)_3N$, CH_2Cl_2, 0 °C, 2 h; (b) R^1MgCl or R^1MgBr, THF, −78 °C, 5 h; (c) 1 N LiOH, MeOH, 0 °C, 30 min; room temperature, overnight; 1 N HCl; (d) R^2OH, DCC, DMAP, camphorsulfonic acid, CH_2Cl_2, room temperature, overnight.

of the side substitution on the monocarbonyl ester moiety. The resulting intermediate **3** underwent saponification by 1 N lithium hydroxide in methanol and then acidification by 1 N hydrochloric acid to yield the corresponding substituted amino acid **4**, which was treated with alcohols following a routine procedure to yield the target compounds **5a**–**k** (see Table 1).

The neurotrophic activity of the target compounds was assessed in vitro in chick dorsal root ganglion (DRG) cultures following a previously described procedure of Lyons et al.[1,19] Due to the poor water solubility, the compounds were first dissolved in analytically pure DMSO at 1 mM and then diluted with DMEM to the final test concentrations, in which the concentrations of DMSO were less than 10^{-7} M and had no detectable effects on DRGs. Preliminary screening of the target compounds was performed at two concentrations of 1 pM and 100 pM, which were selected upon our previous dose–response analyses.[18] The results shown in Table 1, derived from the measurement of the number of the neurite processes in DRGs, revealed that 6 out of 11 test compounds, at either or both concentrations, significantly potentiated the neurotrophic effect on neurite outgrowth induced by 0.15 ng/ml nerve growth factor (NGF), which was added to the culture to induce small neurite processes in DRGs. Compound **5c** at 100 pM exhibited the greatest effect in promoting both the number and length of neurite processes among all the treatment groups (see Fig. 3).

However, in the absence of exogenously added NGF, all test compounds, including GPI-1046, failed to afford any positive effect on neurite outgrowth in DRGs. In agreement with our previous finding,[18] together with data from other studies,[1] this observation may suggest that the FKBP ligands-inducible neurotrophic activity is dependent upon NTFs. The underlying mechanism could form a convergence with the NTF, for example, NGF-induced signal transduction pathway, leading to potentiated activity over NGF alone, and which may be mediated by the interaction between hsp90 and MAP kinase/ERK2.[16] An alternative hypothesis could result from the increased expression of NTFs induced by FKBP ligands.[20]

Table 1. Chemical structures and neurotrophic effects of compounds **5a–k** on neurite outgrowth in DRGs

Compound	R	R^1	R^2	Activity	
				1 pM	100 pM
Control	—	—		100	100
GPI-1046	—	—			120*
5a	$(CH_3)_2CH$–	$(CH_3)_2CH_3CH_2C$–		100	120*
5b	$(CH_3)_2CH$–	$(CH_3)_2CH_3CH_2C$–		39	117
5c	$(CH_3)_2CH$–	$(CH_3)_2CH_3CH_2C$–		123*	207***
5d	$(CH_3)_2CH$–	$(CH_3)_3C$–		115	136**
5e	$(CH_3)_2CHCH_2$–	$(CH_3)_3C$–		129**	65
5f	$(CH_3)_2CH$–	$(CH_3)_3C$–		65	78
5g	$(CH_3)_2CHCH_2$–	$(CH_3)_3C$–		73	92
5h	$(CH_3)_2CH$–	$(CH_3)_3C$–		136**	45
5i	$(CH_3)_2CHCH_2$–	$(CH_3)_3C$–		123*	101
5j	$(CH_3)_2CH$–	$(CH_3)_3C$–		85	93
5k	$(CH_3)_2CHCH_2$–	$(CH_3)_3C$–		108	93

DRGs were treated with compounds **5a–k** at 1 pM or 100 pM in the presence of 0.15 ng/ml NGF, and outgrowth was observed at 48 h. The control cultures were treated with the same amount of NGF. Data shown are derived from the measurement of the number of the neurite processes in the cultures and presented as the percent of the average number from NGF alone-treated control groups. A total of 10–12 ganglion cultures for each treatment group were analyzed in each experimental run.
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to NGF alone-treated control groups.

A preliminary analysis of the relationship between the chemical structures of the target compounds and their abilities to promote neurite outgrowth revealed that in the scaffold structure, R^2 appeared to have a greater impact on the neurotrophic activity of these compounds than R^1, followed by group R. Among several substituents for R^2, 2-(2-trifluoromethylphenyl)-1-ethyl seemed to be the most favorable to the activity, followed by 3-(3-pyridyl)-1-propyl. Replacement of R with 3-phenyl-1-propyl induced reduced neurotrophic activity and even an adverse effect on DRGs. Specifically, by closely comparing the structures of the most active compound, **5c**, and compound **5b**, which failed to exert a significant effect on DRGs, it can be discerned that the main structural element absent in the structure of **5b**, but present in **5c**, is the trifluoromethyl group substituted on the ortho-position of the phenyl ring. We predict that this structural element may contribute significantly to the ability of the ligand to bind to the specific FKBP isoform leading to neural responses, by eliciting an additional electrostatic interaction with the vicinal amino acid residues in the binding site of this specific FKBP isoform. Moreover, we predict that replacing the phenyl ring with the pyridyl ring could promote ligand binding to this FKBP isoform on a consistent basis, as represented by compound **5a** and GPI-1046 in comparison with **5b**, compound **5d** in comparison with

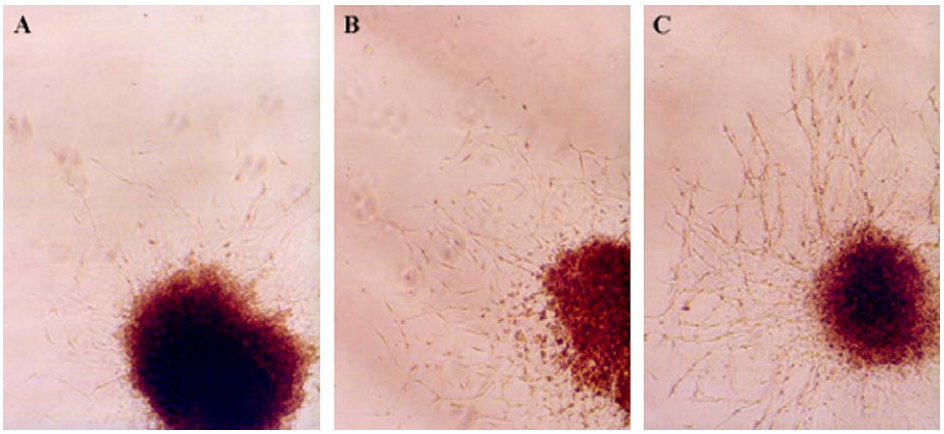


Figure 3. Representative micrographs of chick DRGs grown with NGF (0.15 ng/ml) alone or in the presence of compound **5c**. (A) NGF at 0.15 ng/ml alone only elicited small neurite processes. (B) Compound **5c** at 1 pM significantly promoted neurite processes induced by NGF. (C) Compound **5c** at 100 pM exhibited the greatest neurotrophic effect on neurite outgrowth in DRGs.

5f, and compound **5e** in comparison with **5g**. For R^1, since we have only a small dataset, it is unrealistic to have a broad discussion of its impact on the bioactivity. However, between the two substituents, the *tert*-butyl group appeared to make a slightly greater contribution to the activity than 2-methyl-2-butyl, as represented by compound **5d** in comparison with **5a**. Compared to R^1 and R^2, R substituents appeared to have less impact on the neural activity of these compounds, as demonstrated by the comparable activity of compound **5a** and GPI-1046, compounds **5d** and **5e**, compounds **5f** and **5g**, and compounds **5h** and **5i**; both compounds in each pair to be compared possess the same substituents for R^1 and R^2. Overall, these results are to some extent in accordance with those revealed from our previous analysis of a series of cyclic derivatives of GPI-1046,[18] further suggesting the analogous interaction mode of these two structurally distinct series of compounds with FKBP. An ongoing study in Dr. Li's lab is an attempt to identify this specific FKBP isoform that mediates FKBP ligand-induced neural responses by correlating the ability of these compounds to promote neurite outgrowth with their abilities to bind to different FKBP isoforms, which we anticipate will provide important insights into the nature of the FKBP isoform involved in these biological responses and the design of a specific FKBP ligand for promoting neural functions.

In summary, we described herein the computer modeling, synthesis, and biological evaluation of a novel series of non-cyclic derivatives of GPI-1046. These relatively linear compounds can energy-favorably adopt an analogous binding mode to GPI-1046 on FKBP12. Six out of 11 test compounds were effective in promoting neurite outgrowth in DRGs, suggesting the intriguing potential of these compounds as novel candidate leads for further investigation.

Acknowledgments

This work was supported by a grant from the National '973' Project Foundation of China (G1998051107) to S.L., and grants from the National Natural Science Foundation of China (30000215) and China Postdoctoral Science Foundation to L.Z.

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19. Chick dorsal root ganglion (DRG) cultures. DRGs were dissected from chick embryos of 8 d gestation and inoculated in rat tail collagen-coated culture flasks. Following a 1 h of attachment at 37 °C in a humidified 5% CO_2 atmosphere, the DRGs were treated with the test compounds plus 0.15 ng/ml of nerve growth factor (NGF) in Dulbecco's modified Eagle's medium (DMEM). The control groups received the same amount of NGF only. The DRGs were further incubated in a 37 °C, 5% CO_2 incubator for 48 h prior to evaluation. The ganglia were observed and recorded under phase contrast with an Olympus CK2 inverted microscope. A total of 10–12 ganglia for each treatment were analyzed in each experimental run. Statistically significant differences between groups were determined by a one-way analysis of variance followed by a Student-Newman–Keuls post hoc analysis.
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