FKBP Ligands as Novel Therapeutics for Neurological Disorders

C. Christner[‡*], T. Herdegen[§], and G. Fischer[†]

[‡] *Pintex Pharmaceuticals Inc., 86 Rosedale Road, Watertown, MA 02472, USA;* [§] *Institute of Pharmacology, University of Kiel, Hospitalstr. 4, 24105 Kiel, Germany;* [†] *Max-Planck Unit for Enzymology of Protein Folding, Weinbergweg 22, D-06120 Halle/Saale, Germany*

Abstract: Given their clinical importance for the treatment of acute and chronic neurodegenerative diseases in humans including nerve injuries (e.g. Alzheimer's disease, Parkinson's disease, diabetic neuropathy) a number of different approaches were pursued to obtain selectively acting FK506-binding protein (FKBP) ligands: computational methods and target-oriented screening of natural compound and synthetic product libraries. The resulting monofunctional ligands, which inhibit the peptidyl prolyl *cis*/*trans* isomerase activity of FKBPs, highlight the role of these enzymes in neuronal signaling. The exploration of the mechanisms of neuroregenerative and neuroprotective action of some of these compounds is the main focus of ongoing neuropharmaceutical research.

INTRODUCTION

Initiated by the intriguing independent reports that cyclophilins and FKBPs are abundant in the brain [1, 2], that FK506 enhances nerve regeneration in the rat sciatic nerve crush model [3] and reduces infarction following focal cerebral ischemia [4], an increasing number of research groups provided evidence for the modulation of neuronal properties by immunosuppressant drugs, previously found to interact with a particular class of folding helper enzymes. Whereas neuroregenerative capacity of cyclosporin A (CsA) is controversially discussed [5-8], the dose-dependent promotion by FK506 of neurite outgrowth with a bell-shaped dose-response curve is established in both *in vitro* and *in vivo* experiments [3, 5, 6, 9]. In addition to the effects on lesioned peripheral nerves, FK506 was shown to be beneficial in cellular and animal models for central neuronal disorders caused by dopaminergic pathway malfunction [10-12]. Neurogenic effects of FK506 have long been recognized as toxic side effects in the transplantation medicine [13].

Members of a multigene family encoding the enzyme class of peptidyl prolyl *cis/trans* isomerases (PPIases; EC 5.2.1.8) are the cytosolic receptor proteins of FK506 and cyclosporin A, natural products that reversibly inhibit subsequent to binding. However, a mechanistic picture of how these immunosuppressants mediate neurotoxic, neuroregenerative and neuroprotective effects must take into consideration the multiple biochemical functions, the high number of isoforms and the functional overlap of PPIases [14]. Thus, the application on cells or tissues of CsA and FK506 is characterized by a broad variety of biochemical manifestations visible on second to several hour time-scale. Usually, cellular events triggered by both drugs do not discriminate between affected protein functions. CsA [15], a cyclic undecapeptide, and the macrocyclic lactone FK506 [16] (Fig. **1**) are examples of drugs of microbial origin, which revolutionized the transplantation immunology due to their ability to block cellular immune response. A significant contribution to the exploration of the mechanism of immunosuppression elicited by these drugs was the identification of cyclophilin 18 (Cyp18) as receptor of CsA [17, 18] and FKBP12 as the cellular target of FK506 [19, 20]. Concommitantly, both proteins were characterized as prototypes of structurally unrelated enzyme families belonging to the class of PPIases [14, 21, 22]. However, it became evident that inhibition of the PPIase activity of the prototypic enzymes is not sufficient for immunosupressive effects of CsA and FK506. Bound to their target proteins the drugs act as a matchmaker [23] to generate a specific recognition surface for the Ca^{2+}/calmodulin-dependent protein serine/threonine phosphatase calcineurin (CaN; gain-of-function mechanism) [24].

Low molecular mass ligands of FKBPs and cyclophilins are frequently classified into two groups according to presence or absence of immunosuppressive activity in a T-cell proliferation assay. Whereas FK506 and CsA are termed immunosuppressive, many of the novel FKBP ligands with neurotrophic properties, such as GPI-1046 and V10,367, are termed non-immunosuppressive. It is generally thought that a gain-of-function mechanism acts on the biochemical level when a compound is referred to as immunosuppressive and vice versa. However, the complex nature of the drug-mediated immunosuppression, which includes the transport of the drugs to the cytosol, the drug stability, for example, does not allow for definite conclusions about the action of the low molecular mass ligands on cellular constituents. Therefore, the term bifunctional inhibitor is used in this review for a compound that expresses both, inhibtion of the PPIase activity and a gain-of-function activity. The latter may lead to inhibition of calcineurin, for example. A monofunctional PPIase inhibitor inactivates the catalysed prolyl isomerization but lacks the gain-of function activity, whereas a monofunctional calcineurin inhibitor is inert towards the PPIase activity. Furthermore, dependent on the cellular abundance of cyclophilins and FKBPs, these drugs are acting multifunctionally regarding their primary

*Address correspondence to this author at the Pintex Pharmaceuticals Inc., 86 Rosedale Road, Watertown, MA 02472, USA; Tel: +1-617-924-9200; Fax: + 1-617-924-9290; E-mail: cchristner@pintexpharm.com

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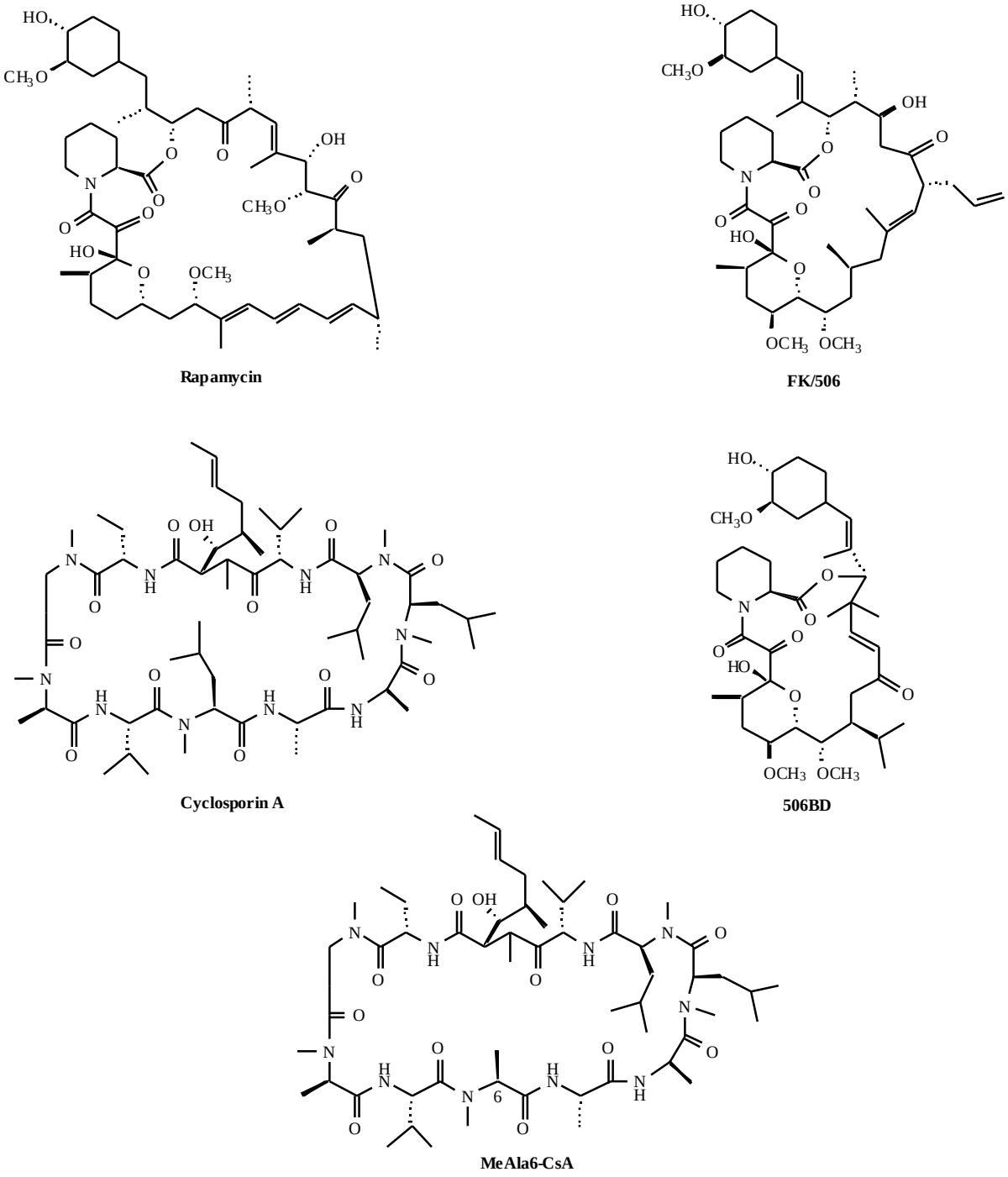


Fig (1). Immunosuppressant inhibitors of the PPIase activity of FKBP (rapamycin and FK506) and cyclophilin (CsA) and non-immunosuppressive derivatives 506BD and MeAla6-CsA.

biochemical effects, namely enzyme inhibition and facilitation of the dissociation of enzyme/substrate complexes. Based on the affinity to these immunosuppressants cyclophilins and FKBPs have been occasionally termed immunophilins or neuroimmunophilins. The latter term refers to their occurrence in neural tissues and regenerating neurons as well as to the involvement in neurological functions [1, 2]. Both immunophilin/drug complexes noncompetitively inhibit the dephosphorylation of CaN substrates such as the cytosolic subunit of NF-AT (nuclear factor of activated T cells), whose Ca^{2+}-dependent translocation to the nucleus is consequently prevented, leading to suppression of transcription of IL-2 (interleukin 2). In addition a number of mRNAs of other early T-cell-activation products are affected including IL-4, GM-CSF (granulocyte-macrophage colony-stimulating factor) and

γ−interferon [25-28]. However, the differential proteome of stimulated T cells revealed the up- and down-regulation of several hundreds of polypeptides in response to CsA [29].

Rapamycin [30] (Fig. **1**), another tight-binding, bifunctional FKBP12 inhibitor of reduced neurotoxicity, is structurally related to FK506 with regard to the composition of the FKBP12-binding segment (pyranose ring, α-ketoamide, pipecolate ester, cyclohexyleth(en)yl groups) but acts at a late, Ca^{2+}-independent stage in T-cell signaling. It is now established that the FKBP12/rapamycin complex recruits homologues of the yeast TOR (target of rapamycin) including the human RAFT/FRAP, members of the phosphatidylinositol kinase-related kinases [31-33]. As a consequence, G_1 cell cycle progression is blocked by modulation of cell cycle kinases such as cdc2 kinase, cdk2 kinase and p70 S6 kinase [34].

Beside FKBP12 and Cyp18, the human genome encodes at least 10 cyclophilins and 17 FKBPs, all of which are thought to have potential affinity to CsA and FK506, respectively [35]. With the CsA- and FK506-resistant parvulins, a new PPIase family was described, whose human member Pin1 might be involved in the development of Alzheimer's disease [36, 37]. It soon became obvious that all PPIase families are highly conserved between the organisms, are abundantly expressed in the prokaryotic and eukaryotic kingdom and have a cell-stage dependent expression pattern in virtually all mammalian tissues [35, 38].

The conformational interconversion of prolyl imide bonds, accelerated by PPIases, has been shown to represent the rate-limiting step in isomer-specific processes such as polypeptide restructuring, protein renaturation, protein phosphorylation/dephosphorylation, proteolytic degradation and peptide transporter function [37, 39-43]. In order to study the involvement of the enzyme activity of the PPIases in such biological events, a number of FK506 and CsA derivatives were developed, which contain the PPIase-binding domain but lack the structural elements required for CaN affinity, e.g. the FK506 derivative 506BD [44, 45] and the nonimmunosuppressive analog of CsA, MeAla6-CsA [46] (Fig. **1**). Among the PPIase-inhibiting drugs, FK506 has been most frequently used as an effector for studying nerve cell signals.

IMMUNOSUPPRESSIVE PPIASE LIGANDS AS NEUROGENIC AGENTS

There is accumulating evidence that low molecular mass ligands of PPIases contribute to neuroprotection and neuroregeneration through multiple (possibly overlapping) pathways. Currently, some of the above-described interactions of FK506 with FKBPs are discussed as mechanisms underlying the multiple effects of this drug on the central and peripheral nervous system [11, 12, 47, 48]. Memory formation, axonal transport, nerve growth and neurodegenerative processes were found to be affected by FKBPs and cyclophilins as could be inferred from the effects of mono- and bifunctional inhibitors as biological probes [4, 7, 8, 49].

In cell lines such as SH-SY5Y and PC12, the neurotrophic action of FK506 seems to be dependent on presence of low concentrations of nerve growth factor (NGF; 1-10 ng/mL) [6, 11, 12]. In primary cultures of chick dorsal root ganglion neurons and of hippocampal neurons FK506 increases neurite outgrowth in the absence of exogenous growth factors, although influence of endogenous growth factors cannot be excluded [6, 10].

In vivo studies using the rat sciatic nerve crush model revealed augmentation of the regrowth and functional recovery by FK506, as demonstrated by behavioral evaluation, electron microscopic and immunohistochemical analyses [6, 8, 11, 12]. Furthermore, the peptidomacrolide accelerates axonal sprouting following injuries to the dorsal columns of spinal cords in rats [50]. Most significantly, unlike growth factors, FK506 does not appear to act on healthy peripheral nerves [10].

In a well-established model for Parkinson's disease, dopaminergic neurons of nigrostriatal localization are destroyed by free-radical oxidative processes upon treatment with the neurotoxin 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). As assessed by anti-tyrosin hydroxylase (TH) Ig immunostaining, orally administered FK506 partially protected against MPTP-induced loss of striatal TH+ axonal density in mice [51]. Similarly, repetitive application (2 mg/kg/day for 8 days) reduced the death of axotomized neurons in the substancia nigra pars compacta following transection of the medial forebrain bundle [52].

Whereas neuroprotection of peripheral and central axotomized neurons by FK506 and derivatives can be considered as accepted knowledge, contradicting findings are published for neuroregeneration of central neurons. The positive effects reviewed above are challenged by negative reports on abortive sprouting following optic nerve cut [50, Herdegen *et al*., unpublished observation] and spinal cord injury [50, 53]. Thus, it remains to be elucidated whether the neuroregenerative effect of FK506 is restricted to peripheral and central extrinsic neurons whereas central intrinsic neurons are insensitive to FK506.

Besides protection and regeneration following nerve fiber injury, FK506 and derivatives can exert further beneficial effects including alteration of neurotransmitter release [54], protection against ischemic brain injury [4, 48, 55-57] and glutamate neurotoxicity *in vitro* [58], prevention of N-methyl-D-aspartate (NMDA)-receptor desensitization [59], modulation of long-term potentiation (LTP) [60-62] and blockade of long-term depression (LTD) in the rat hippocampus [63], prevention of LTP and LTD in the visual cortex [64, 65].

The fundamental incidences in various neurodegenerative processes such as neuronal injuries, amyotrophic lateral sclerosis and ischemia are rather divergent and range from enhanced glutamate release, rapid depolarization, reactive oxygen species (ROS) to cellular Ca^{2+} increase with subsequent activation of Ca^{2+}-dependent enzymes [66]. Some recent findings have uncovered several physiological pathways with frequent involvement in neurotrophic FK506/CsA effects.

CaN and ROS

A major issue of ongoing discussion is the role of CaN in neuroprotection, since CaN regulates the activity of various intracellular proteins including nitric oxide synthase (NOS), the NMDA receptor complex, the IP_3 receptor and a variety of ion channels [48]. Noticably, both, FK506 and CsA have been shown to be effective in a wide range of cerebral ischemia models, although conflicting data have also been reported.

In the initial study of Sharkey *et al*. (1994), FK506 but not CsA or rapamycin alleviated the outcome of focal cerebral ischemia, arguing against involvement of CaN. However, the effect of FK506 was blocked by pretreatment with rapamycin [4], which would support a role of CaN inhibition. Subsequent studies of Bochelen *et al*. (1999) revealed protective effects in a model of focal cerebral ischemia for both ascomycin derivative SDZ ASM 981 and CsA. Rapamycin proved to be inactive [67]. Anti-ischemic properties of the immunosuppressants CsA and FK506 were also described by Kuroda *et al*. (1999) [68]. Recently, FK506 as well as rapamycin were reported to significantly decrease the infarct volume when administered 10 min after occlusion of the left middle cerebral artery in rats, whereas the monofunctional FKBP ligand GPI-1046 was completely inactive [69]. The poor blood-brain barrier permeability of CsA requires the use of relatively high dosages for neuroprotection and may contribute for the observed ineffectiveness in some experimental models of ischemia [4, 48].

One of the most accepted mechanisms for FK506- and CsA-mediated neuroprotection is by diminution of free radical production via inhibition of neuronal NOS (nNOS) [70], a Ca^{2+}/calmodulin-dependent enzyme, which produces nitric oxide (NO) by oxidative cleavage of arginine. The complex physiological and pathophysiological actions of NO are not fully understood and are essentially determined by the NOS isoform and the amount of NO. It is well established that NO acts as a neurotransmitter within the CNS. However, NO can also combine with superoxide radicals to form highly reactive hydroxyl and peroxynitrite radicals with subsequent cellular damage by oxidation of nucleic acids, proteins and membrane lipids [71, 72]. Dephosphorylation, e.g. by CaN, is thought to cause inactivation of NOS. Several groups reported that both, FK506 and CsA inhibited NMDA-stimulated NOS activity and blocked NMDA neurotoxicity in neuronal cell cultures. Rapamycin antagonized the neuroprotective effects of FK506 [48, 58, 73], indicating a CaN-dependent mechanism. Unlike NMDA receptor antagonists (e.g. MK801) [57] and NOS inhibitors (e.g. L-N-nitroarginine) [74], however, FK506 and CsA failed to display neuroprotectivity in animal models of NMDA- or quinolinate-induced neurotoxicity [57]. Recently, Toung *et al*. did not observe inhibition of NO production at FK506 concentrations which provide robust neuroprotection against transient focal cerebral ischemia. Thus, NOS seems less likely to be involved in neuroprotective effects of FK506 *in vivo* [75].

The hydroxyl radical (OH·) is another member of the ROS family with neurodegenerative properties that is generated by H_2O_2 [76]. Enhanced apoptosis of neurons mediated by familial amyotrophic lateral sclerosis-associated mutant Cu/Zn superoxide dismutase (SOD1) can be antagonized by high cellular Cyp18 concentration whereas CsA and FK506 (1 μM) promote cell death. Obviously, the PPIase activity of Cyp18 is involved in this mechanism, because the PPIase activity-deficient Cyp18 variant R55A did not prevent neuronal cell death [77].

Mitochondrial Targeting

As summarized in a recent review [48], beside CaN-triggered immunosuppression and suppression of apoptosis, stabilization of the mitochondrial function is suggested to account for the anti-ischemic activities of FK506 and CsA. By blocking MTP (mitochondrial permeability transition pore) formation in ischemia, CsA may prevent mitochondrial depolarization, calcium accumulation, uncoupling of mitochondrial respiration and release apoptosis-related enzymes such as cytochrome C and apoptosis inducing factor (AIF) [48, 78]. Although FK506 does not target the MTP, it was reported to inhibit secondary deterioration in mitochondrial respiration as well as the run-down of ATP following reperfusion [79] and to maintain cellular Ca^{2+} homeostasis in ischemia [80].

Apoptotic Pathway

Preincubation with CsA and FK506 reduced susceptibility to apoptosis, induced by virus-mediated high-level constitutive activity of calcineurin in PC12 cells [81]. Asai *et al*. (1999) showed that CaN can facilitate both, Ca^{2+}- and non-Ca^{2+}-mediated apoptosis in PC12 cells [82]. CaN-triggered neuronal apoptosis may share common mechanisms with other apoptotic pathways, such as cytochrome C release and caspase-3 activation. Thus, CaN-induced apoptosis can be antagonized by either Bcl-2, which prevents cytochrome C release; CrmA (cytokine response modifier A), which blocks caspase cascades; or caspase-3 inhibitors.

Furthermore, apoptosis can be initiated by application of the pro-apoptotic phospholipid ceramide, which is released in the post-ischemic rat brain. Recently, FK506 was reported to diminish this release and the expression of the death inducing ligand CD95-ligand in both, human neuroblastoma cells and the rat brain following MCA occlusion [83]. Intracellular targets for ceramide are stress-activated kinases (e.g. JNK, c-Jun N-terminal kinases) with subsequent activation of the transcription factor c-Jun. The role of c-Jun in neuroregeneration and neurodegeneration is rather complex and multifunctional, but it is certainly involved in neuronal apoptosis.

The increased expression and phosphorylation of c-Jun has been noted in various models for neurodegenerative diseases [52, 83, 84]. FK506 augments the regeneration of axotomized motor and sensory neurons as indicated by its ability to enhance c-Jun-like protein immunoreactivity [85]. As opposed to its effect on peripheral neurons, within the CNS, FK506 counteracted both the expression and

phosphorylation of c-Jun *in vivo* following axotomy [52] and ischemia as well as *in situ* after serum deprivation or hydrogen peroxide-triggered cell death [86, 87]. It remains to be elucidated whether FK506 directly interferes with JNK or with its upstream-activators or antagonistic proteins. Thus, FK506 enhanced the expression of the JNK- and ERK-(extracellular response kinases) inactivating MAP (mitogen-activated protein) kinase phosphatase 1 (MKP-1) in surviving mamillary neurons following axotomy but failed to do so in degenerating nigral neurons [88]. In H_2O_2-stressed PC12 and Neuro2A cells, FK506 prevented activation of JNK [87] independent of PPIase inhibition, arguing that the protection of neural cells by FKBP-ligands is apparently independent of JNK activity.

Mechanistic Insights from Immunosuppressive PPIase Inhibitors

Considering the potential therapeutic strategy for treating neurological disorders with PPIase inhibitors, research efforts have been intensified to determine the molecular basis underlying the promising neuronal effects of FK506. The major aim depends on the dissection of effects caused by PPIase inactivation, CaN phosphatase inhibition and depletion of proline-directed protein/protein interactions.

Colocalization of neuroimmunophilins with CaN [1, 2] initially suggested that CaN mediates the neuroprotective and neuroregenerative properties of FK506. This theory was supported by the finding that expression and phosphorylation of phosphatase substrates such as GAP-43 (growth-associated protein of 43 kDa, also known as neuromodulin, P-57, B-50 and F-1) [89] and nNOS are increased upon FK506 and CsA administration to brain tissue [58, 90, 91]. The monofunctional FKBP12 ligand V-10,367 had similar effects on GAP-43 expression and functional outcome after CNS injury, indicating that CaN inhibition does not account for this effect of FK506 and CsA [92]. It is well established that GAP-43 mRNA levels are elevated during development and regeneration of the vertebrate nervous system. In addition, GAP-43 and its phosphorylated form have been linked to persistence of LTP, synaptic plasticity and neurotransmitter release [89].

However, conflicting results are published on the effects of the immunosuppressive drugs on neurite extension *in vitro.* On one hand, neurite outgrowth in cultured PC12 cells was increased by CsA, although less effectively than by FK506 [6]. In a recent study of Parker *et al*. (2000) FK506 was not effective in PC12 cells [69]; which may however be due to the application of a too low concentration of NGF (0.5 ng/mL) to allow differenciation [6, 11, 12]. In other experiments CsA failed to promote neurite or axon elongation in SH-SY5Y cells [8], cholinergic septal neurons, explanted DRGs (dorsal root ganglia) [93] and in crushed sciatic nerve [5, 8, 94]. In contradiction to FK506 and CsA, rapamycin was revealed to reproducibly stimulate neuritogenesis, even if CaN inhibition of the FKBP12/rapamycin complex is lacking [6, 11, 12, 69]. This stimulating potential of rapamycin might be explained by an induced shift from proliferative to differentiative pathways [69], since rapamycin, unlike FK506, blocks cell cycle progression resembling cell proliferation inhibitors ciclopirox and flavopiridol.

Obviously, the multifunctional reactivity of these immunosuppressive drugs prevents their use in the treatment of neurological disorders, and makes the molecular analysis of the effects difficult. Cell-penetrating, tight-binding drug derivatives with the sole function of either PPIase or CaN inhibition would provide a straightforward path to success.

MONOFUNCTIONAL FKBP AND CAN INHIBITORS

Compelling evidence ruling out a CaN-dependent mechanism in neuroregeneration and neuroprotection was obtained by means of monofunctional FKBP inhibitors such as V-10,367 [95], GPI-1046 [6, 10, 11, 12] and cycloheximide-*N*-(ethyl ethanoate) [96]. In particular, ring-substituted phenylgyoxylamides and 2-pyrrolidine carboxylates, such as V-10,367 and GPI-1046, respectively, have been extensively studied [51, 11, 12, 95, 97-100].

In SH-SY5Y and PC12 cells, V-10,367, which inhibits the PPIase-activity of FKBP12 as potently as FK506 (K_i = 0.5 nM) [95] but leaves CaN activity fully intact, increased lengths of neurite processes at concentrations in the lower nanomolar range, comparable to FK506. In addition, V-10,367 (5-400 mg/kg) was shown to speed up regeneration of crushed rat sciatic nerves as well as functional recovery when given subcutaneously or orally [98]. Cycloheximide-*N*-(ethyl ethanoate), directly administered to the site of sciatic nerve lesion, had beneficial effects at dosages of 30 mg/kg [96]. GPI-1046, which also represents a monofunctional FK506-derivative was reported to exert neurotrophic and regenerative actions on cultured neuronal cells, DRG neurons and crushed sciatic nerves [6, 10, 11, 12], although only marginally reproducible by a number of research groups [69, 101]. Additional studies suggest that GPI-1046 protects against the *p*-chloroamphetamine-induced destruction of central serotoninergic neurons, senescence-related atrophy of medial septal cholinergic neurons [100] as well as against loss of dopaminergic neuronal function provoked by MPTP [10, 11, 12, 99], whereas the compound was inactive in rescuing nigral neurons after transection of the rat medial forebrain bundle [52]. Recently, Emborg *et al*. (2001) failed to confirm the reported beneficial effect of GPI-1046 after MPTP administration in nonhuman primates [102].

Neuroprotection was exerted by V-10,367 in the MPTP model with complete restoration of the dopaminergic innervation of the striatum, while FK506 failed to do so [51]. The obvious difference between both compounds is additional CaN inhibition by FK506. Thus, the lack of neuroprotective FK506 effects may indicate a functional interplay between the FKBP- and the CaN-mediated pathways. However, mitochondrial dysfunction also involves the malregulation of the MTP pore that is subject to monofunctional PPIase inhibitor-induced pore closing that is not related to CaN.

The above-described neuroregenerative and neuroprotective properties of FK506 suggested an exciting

new therapeutic approach for the treatment of neurological disorders. Promising drug candidates are small molecular FKBP ligands with oral bioavailibility, which are devoid of immunosuppressive activity and are able to cross the blood-brain-barrier. Different strategies have been aimed at the identification and development of selective acting FKBP inhibitors such as structure-based rational design and target-orientated screening.

Design and synthesis of low-molecular mass, monfunctional FKBP inhibitors first provided compounds with structural modification of the FKBP-binding domain of FK506, which is supposed to interact with the active site of the PPIase. Minimal binding requires the central pipecolic acid ring with the α-dicarbonyl amide linkage connected with the pyranose ring [103] (Fig. **2**). In the crystal structure, both FK506 and rapamycin are complexed in a similar fashion regarding the deep binding of the pipecolinyl ring in the cavity defined by Trp-59 and the side chains of Tyr-26, Phe-46, Val-55, Ile-56 and Phe-99, whereas the α-dicarbonyl amide is hydrogen bonded to NH of Ile-56 and the Tyr-82 OH group. The pyranose ring is buried in the hydrophobic pocket formed by Phe-36, Asp-37, Tyr-82, His-87, Ile-90 and Ile-91, and the cyclohexyl ester chain is engaged in hydrophobic interactions within a shallow groove on the surface of the FKBPs [104]. By preserving the above-described structural elements of the minimal FKBP binding domain, an open chain compound (K_d = 10 nM) can be synthesized, which has approximately 10-fold weaker affinity for the enzyme as compared to FK506 and rapamycin [105, 106] (Fig. **2**). Subsequently, structure-activity relationship (SAR) studies were performed for a large number of simple, analogous molecules (Fig. **3**) in terms of their ability to inhibit FKBP12 [95, 107] and reviewed recently [11, 12]. As a result, the α-dicarbonyl amide functionality was identified as being essential for enzyme inhibition because derivatives obtained by replacement of either or both of the carbonyl groups corresponding to positions C-8 and C-9 of FK506 such as peptides ($K_i \geq 1$ μM) [108], sulfonamides ($K_i \geq 160$ nM) [103, 109] and ureas ($K_i \geq 120$ nM) [110] were substantially less potent. Similarly, only very limited structural modification of the pipecolic acid ring is allowed since inhibition constants of ring-opened derivatives were in the middle to upper micromolar range [103]. High-affinity FKBP12 ligands were especially characterized as belonging to the pipecolate and *N*-(glyoxyl) prolyl esters. [11, 12]. Among these compounds, a prerequisite for nanomolar inhibition ($K_{i,app}$ = 1 – 100 nM) were bulky hydrophobic alkyl groups such as 1,1-dimethyl-propyl and (3,4,5-trimethoxy)phenyl as substituents for the pyranose ring region of FK506, as well as simple alkyl or alkyl aryl esters, instead of the lead cyclohexylethyl moiety (e.g. the above-mentioned GPI-1046 and V10,367 (Fig. **4**) [11, 12, 95, 103, 111]). However, the binding properties for some of these α-dicarbonyl amides have been critically discussed. As confirmed by free energy perturbation techniques in Monte Carlo statistical mechanics simulations as well as a linear response method [112, 113], a favorable contribution to binding previously shown for pyridyl substituents as in the case of GPI-1046 is not supported.

Cyclical pipecolate esters (Fig. **5**), which are as potent as rapamycin and FK506 were designed by Adalsteinsson and Bruice (1999) using the crystal structures of the complexes FKBP12/rapamycin and FKBP12/rapamycin/FRAP as the basis for molecular dynamics [114, 115]. Since the gene clusters of the rapamycin producing polyketide synthetase (PKS) have been cloned, such tetrakidic and pentakidic FKBP ligands may be accessible by means of respective protein variants [115].

Although computational methods are commonly applied for lead optimization, there are still only a few examples of completely new chemical entities discovered by such approaches [116]. In a recent paper, Burkhard *et al*. (1999)

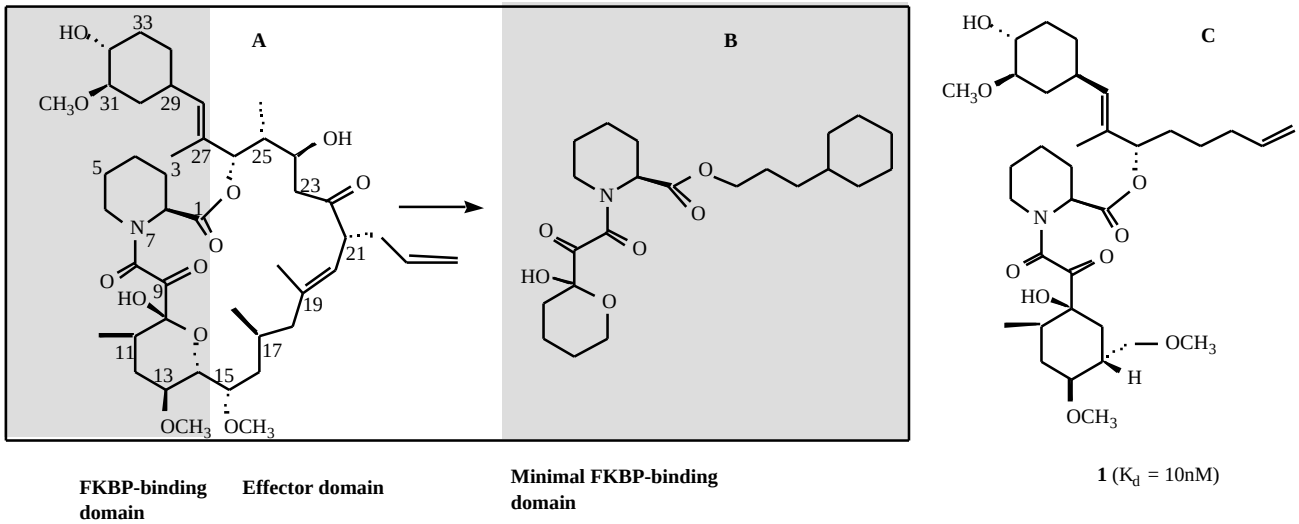


Fig. (2). Towards the minimal FKBP-binding domain. (A): Immunosuppressant FKBP ligands such as FK506 are composed of an immunophilin-binding domain and an effector domain. (B) Theoretical minimal FKBP-binding domain according to Holt *et al*. (1994). (C): Synthetic FKBP-binding domain derivative (**1**).

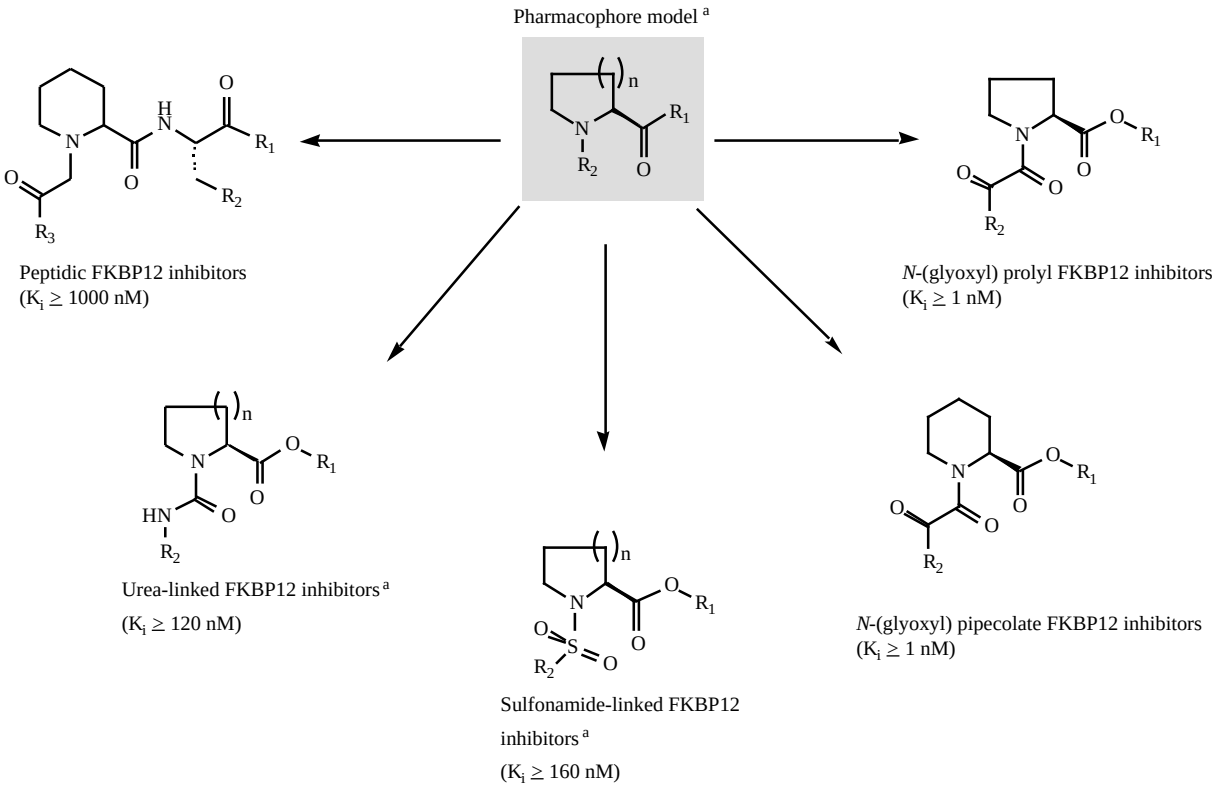


Fig. (3). SAR studies on the minimal FKBP-binding domain. Pharmacophore model for FKBP ligands and synthesized derivatives: peptidic, sulfonamide- and urea linked FKBP12 inhibitors, *N*-(gyoxyl) prolyl and *N*-(glyoxyl) pipecolyl esters [11, 12]. [a] n = 1,2

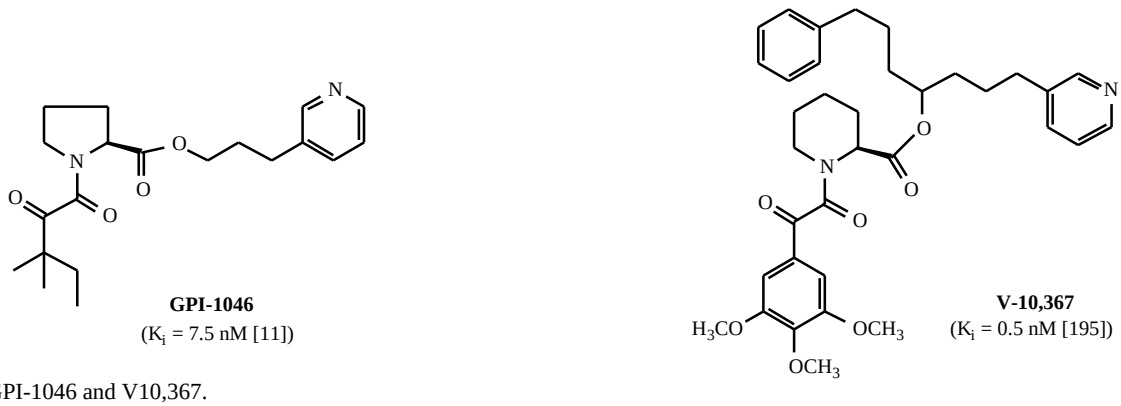
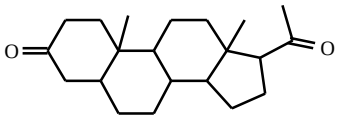


Fig. (4). GPI-1046 and V10,367.

Fig. (5). Pentakidic and tetrakidic rapamycin derivatives. The LIE free energy of binding for IV-A (7.0 kcal/mol) and V-B (7.1 kcal/mol) to FKBP12 was comparable to the value calculated for rapamycin (6.6 kcal/mol).

utilized the molecular docking computer program SANDOCK to screen small molecule three-dimensional databases for FKBP ligands. As confirmed by fluorescence quenching tests, several compounds with micromolar K_d values were identified by this method. Interestingly, several steroids were characterized to be novel leads for FKBP12 inhibitors, for example 5β Pregnan 3,20 dion (K_d = 7 μM, Fig. **6**). The biological significance of these FKBP-steroid interactions remains to be elucidated. However, so far there is no evidence that the binding of steroids to FKBPs accounts for neuronal actions of steroids.



5β Pregnan 3,20 dion
(K_d = 7 μM)

Fig. (6). 5β Pregnan 3,20 dion.

In search of novel lead structures for enzyme inhibitors, biological screening techniques like high-throughput screening of compound libraries have usually been employed. Recently, as a result of our screening for new inhibitors of hFKBP12, the glutarimide antibiotic cycloheximide (4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione) [117, 118] was identified [96]. Cycloheximide differs from known FKBP12 ligands in terms of its structural composition and its easy accessibility to chemical modification. Therefore it was further characterized by detailed binding studies as well as by SAR investigations in a series of synthesized cycloheximide derivatives in relation to cytotoxicity against eukaryotic cells (mouse L-929 fibroblasts, K-562 leukemic cells). Cycloheximide competitively inhibited activity of hFKBP12 (K_i = 3.4 μM, Fig. **7**) and exhibits an inhibitory specificity for FKBP-like PPIases that are known to bind FK506, such as *E. coli* FKBP26, rabbit FKBP52, *P. phosphoreum* FKBP22 and *L. pneumophila* FKBP25 (Mip).

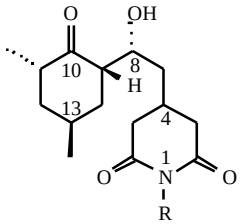
In the course of our SAR studies, several less or non-toxic cycloheximide derivatives were identified by *N*-substitution of the glutarimide moiety with IC_{50} values between 22.0 μM to 4.4 μM for inhibition of hFKBP12. In contrast, replacements of the C-10 carbonyl and the C-8 hydroxyl functionalities dramatically reduced or abolished the affinity for FKBP12 demonstrating the significance of these moieties for inhibition of hFKBP12. Among the *N*-substituted compounds, the best effect was seen with cycloheximide-*N*-(ethyl ethanoate). Cycloheximide-*N*-(ethyl ethanoate) exerted FKBP12 inhibition to an extent comparable to cycloheximide (K_i = 4.1 μM, Fig. **7**). Importantly, the derivative (IC_{50} = 115 μM) caused an approximately 1000-fold weaker inhibition of eukaryotic protein synthesis than cycloheximide (IC_{50} = 0.1 μM) as revealed by the rabbit reticulocyte type I translation assay.

For elucidation of their neurobiological effects, the majority of the above-discussed FKBP ligands were evaluated with respect to protection and regeneration of damaged neurons *in vitro* and *in vivo* [11, 12, 95, 98]. Particularly striking is that the neurotrophic potency of many of these compounds does not correlate linearly with their ability to inhibit the PPIase activity of FKBP12 *in vitro* [11, 12]. A considerable number of these FKBP ligands promote neurite outgrowth at 10- to 100-fold lower concentrations as compared to their K_i values for FKBP12 inhibition.

Limited success has been obtained in the development of specific, monofunctional CaN inhibitors. Inhibitory peptides have limited applicability due to degradation and transport inefficency [119]. Interestingly, both the multifunctional FK506 and the monofunctional FKBP inhibitor V-10,367 protect against H_2O_2-induced cell death. In contrast, the specific, monofunctional, low-molecular mass CaN inhibitor Lie120, which reversibly inhibits the CaN activity in the lower micromolar range, is not protective, suggesting a CaN-independent mechanism of protection by FKBP ligands (84, 120). Importantly, Lie120 does not interact with either FKBPs or cyclophilins, and it is this novel property, which renders Lie120 a promising tool in the analysis of the role of CaN in neurodegeneration.

FKBPS IN NEURONAL SIGNALING

Since monofunctional FKBP inhibitors can have a direct neuroregenerative potential in response to nerve injury, FKBPs are suggested to be critical in nerve defects. Initially, the concomitant up-regulation of FKBP12 in the facial nucleus following facial nerve axotomy as well as in lumbar motor neurons and DRG following sciatic nerve crush [90] strongly indicated an FKBP12-dependent mechanism. However, recent studies of Gold *et al*. (1999) cast serious



R	hFKBP12 K_i (μM)	Cytotoxicity IC_{50} (μg/mL) L-929	Cytotoxicity IC_{50} (μg/mL) K-562	*S.c.* α-factor synthesis IC_{50} (μM)
H	3.4	<0.39	<0.39	0.1
$CH_2C(O)OC_2H_5$	4.1	76.6	64.9	115.0

Fig. (7). Effect of cycloheximide and cycloheximide-*N*-(ethyl ethanoate) on the activity of hFKBP12, cytotoxicity against eukaryotic cell lines L-929 and K-562 and inhibition of the protein synthesis of the α-1 mating pheromone (α-factor) from *S. cerevisiae*.

doubt on FKBP12 being the target of neuronal effects of FKBP ligands [47, 85]. Employing primary hippocampal neuronal cultures from FKBP12 knockout mice [121], they showed that FK506 retains its neurite outgrowth-promoting potential [47, 85]. In addition, a FKBP52 antibody blocked both, the ability of FK506 and NGF to increase neurite outgrowth in SH-SY5Y cells, suggesting convergent signal transduction pathways. Furthermore, the FKBP52 antibody itself displayed neurotrophic activity. Taking into consideration the reported neurotrophic properties of steroid hormones (e.g. dexamethasone and β-estradiol) [122] and their interaction with FK506, Gold *et al*. proposed that the neural action of steroid hormones and FK506 is based on a similar mechanism involving the steroid receptor complex [85] (see discussion in the next chapter). Further evidence for this hypothesis was provided by means of geldanamycin, a benzoquinone antibiotic [123] that prevents the reassociation of the mature steroid complex and thus the nuclear translocation and activation of steroid response elements. It also destabilizes preformed Hsp90 (90-kDa heat shock protein) heterocomplexes [47]. Geldanamycin inhibited neural effects of FK506, dexamethasone and β-estradiol on SH-SY5Y cells and enhanced the action of the FKBP52 antibody. This may be explained by the different binding sites of Hsp90 for geldanamycin and FKBP52 [124]. Sodium molybdate, a transition metal oxyanion, which stabilizes the receptor complex, reduced the effectiveness of FK506, FKBP52 antibody, β-estradiol and geldanamycin to augment neural outgrowth [47].

On the basis of these results an FKBP52-related model has been hypothesized, which tries to explain some of these observations. It disregards the finding that molybdate by preserving the ATP-bound Hsp90 conformation [125] causes a modest agonistic effect on neurite outgrowth. Moreover, p23, Hsp90 and FKBP52 are suggested to be likely candidates that may mediate neural effects of FK506 [47].

p23 binds to a site different from the nucleotide binding site and stabilizes the ATP-dependent Hsp90 conformation. It was reported to be required for assembly of functional steroid aporeceptor complexes but is not essential for glucocorticoid receptor action [226]. It may play a role in estrogen receptor signal transduction [127]. However, no direct evidence for mediation of neurotrophic effects has been reported so far.

Hsp90 was recently shown to interact with several protein kinases including the Raf, MEK and Src components of the MAP kinase system [128, 129], which may account for a possible link between steroid hormone and neurotrophic factor signaling (e.g. NGF). Among the MAP kinase substrates, especially the expression and phosphorylation of c-Jun was found to correlate with nerve regeneration [130]. In rats, a single injection of FK506 increased c-Jun-like protein immunoreactivity in axotomized motor and sensory neurons [47]. According to Leppa *et al*. (1998) exposure to NGF, which causes differentiation of PC12 cells into a neuronal phenotype, results in activation of ERK-type MAP kinases and phosphorylation of c-Jun on several sites including Ser63 and Ser73 [131]. Furthermore, constitutively activated c-Jun, mimicing the MAPK-phosphorylated form of the protein, is able to induce neuronal differentiation of PC12 cells independently of upstream signals. Activation of MEKK1, which stimulates the JNK pathway is not sufficient for PC12 differentiation but co-expression of the activated protein kinase with c-Jun promotes neurite outgrowth. Thus, neural differentiation of PC12 cells may be directed by the ERK pathway. Because of the presence of an AP-1 binding site within the promoter region of the GAP-43 gene [132], elevated c-Jun expression by FK506 or steroids (e.g. dexamethasone and β-estradiol) might contribute to GAP-43 mRNA induction in regenerating neurons [47, 91, 133].

Via its interaction with dynein [134], FKBP52 may directly contribute to axonal elongation by regulating axonal transport of cytoskeletal components [129]. However, FK506 does not affect this interaction, although the PPIase domain of FKBP52 is involved in dynein coabsorption [134, 135]. The microtubular (actin) localization of cytoplasmic FKBP52 points to an involvement of FKBP52 in the dynamic process of glucocorticoid receptor movement along microtubular tracts [136]. The retrograde or anterograde direction of signaling protein movement might be determined by the interplay of FKBP52 and the protein kinase $p50^{cdc37}$ [129]. Moreover, FKBP52 was located in the nucleus with the same pattern of distribution as Hsp90 and steroid receptors [136, 137]. FKBP52 comprises a conserved sequence of eight amino acids with six negatively charged residues, which is electrostatically complementary to nuclear localization signals [138]. By means of antibodies raised against this sequence, Czar *et al*. (1995) provided evidence that FKBP52 has an NLS (nuclear localization sequence) recognition function at some stage in the glucocorticoid receptor pathway [139]. Unlike the FKBP52 antibody, FK506 is not able to dissociate the PPIase from the Hsp90 binding site but prevents the conformational change of Hsp90 and the subsequent formation of the activated adenosine diphosphate state as well as the release of p23 [140, 141].

Some observations, however, argue against the mediation of the neurotrophic action of FK506 by FKBP52. It should be noted that FKBP12 is by far more abundant in neuronal cells than FKBP52 [1,2, 38, 135]. Neuroregenerative effects of FK506 have been observed already with concentrations in the subnanomolar range [6, 11, 12, 47], whereas K_d values for FK506-binding to the active site of FKBP52 as well as K_i values for enzyme inhibition are within the 10-100 nM range [38]. In contrast, the affinity of FK506 for the active site of FKBP12 is, however, approximately two orders of magnitude higher (K_d, K_i = 0.2-1 nM) [22, 38]. Rather high concentrations of FK506 (>1 μM) are required for the potentiation of the effect of suboptimal concentrations of glucocorticoid on the expression of a glucocorticoid-inducible reporter gene in L929 cells and nuclear receptor translocation [142]. In consequence, although extracellularly applied FK506 may accumulate inside the cell, prior to any effect on FKBP52, the high-affinity FKBP12 or other present FKBP-domain proteins have to be saturated. To test the FKBP52 hypothesis of Gold *et al*., differential inhibition of FKBP12 and FKBP52 by monofunctional FKBP inhibitors can be helpful. According to recently determined inhibiton constants, rapamycin (K_i(FKBP12) = 0.6 nM); K_i(FKBP52) = 18 nM) and V10,367 (K_i(FKBP12) = 0.6

nM); K_i(FKBP52) = 755 nM) are suggested to be preferable compounds (Schubert, S.; personal communication). Interestingly, the neuroregenerative potencies of these compounds were similar to those of FK506 [6, 11, 12, 97, 98]. Most noteworthy, V13,661 (Vertex Pharmaceuticals Inc.), which is derived from the FKBP12-binding domain of FK506 but does not interact with FKBP12 can improve outcomes in animal models of peripheral neuropathies and Parkinson's disease (Cole, M.D.; XIII International Congress on Parkinsons' Disease, Vancouver, 1999; Conference "Immunophilins in the Brain", Schlangenbad, 1999). This result would exclude FKBP12 as a major target for the neural effect of FKBP12 ligands.

This was recently put into perspective by Costantini and Isacson (2000), who characterized a differential effect of FKBP12 and CaN in dopaminergic neuronal cultures from embryonic day 14 ventral mesencephalon [7]. Within this system, the bifunctional PPIase ligands FK506 and CsA clearly enhanced neurite elongation of dopaminergic neurons comparable to GDNF, whereas monofunctional FKBP inhibitors devoid of CaN inhibition, such as rapamycin and V-10,367, increased neurite branching. This led to the conclusion that elongation is dependent upon maintained phosphorylation of CaN substrates (e.g. NOS, microtubule-associated proteins, neurogranin, neuromodulin, MARCKS and NF-AT) [70], while branching is not. Interestingly, both rapamycin and V-10,367 antagonized the FK506-induced elongation and stimulated branching [7]. It should however be noted that the suggested key role of CaN in neurite elongation stands in contradiction to all previous findings. Furthermore, V-10,367 and GPI-1046 lengthened processes in other cell types (e.g. PC12 cells, SH-SY5Y cells and DRGs) and promoted regeneration without increasing the number of processes in the distal nerve following sciatic nerve crush [6, 8, 11, 12, 95, 98].

Keeping in mind the large number of FKBP encoded in the human genome, it cannot be excluded so far that neuroregenerative effects of immunophilin ligands are mediated by FKBPs other than FKBP52 and FKBP12 such as the abundant FKBP25, FKBP38 and FKBP65 [143-145]. Even if data are still lacking, the affinity for FK506 and rapamycin of these proteins should be considered. Thus, for the identification of the neurosignaling pathway targeted by the FKBP ligands, the already known biochemical levels of actions of FKBPs must be taken into consideration.

PPIASES AS SIGNAL TRANSDUCTION-TARGETED MODULATORS

Following FKBP inhibition, the neuroprotective and neuroregenerative effectiveness of monofunctional FKBP ligands may result from affecting cell functions, which are already known to be influenced by the enzyme activity of PPIases, some of which are active in nerve cells. For example, Cyp18 interacts with the thiol-specific antioxidant protein Aop1, which is thought to be part of the cellular defense against oxidative stress [146]. The binding of hCyp18 increases the enzymatic activity of Aop1 but CsA does not abolish the Cyp18 effect. Oxidative stress, relatively high Ca^{2+} and low ATP are suggested to result in the formation of the MTP from a complex of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocase and cyclophilin-D (CypD) at contact sites between the mitochondrial outer and inner membranes [147-149]. These conditions correspond to those that unfold during tissue ischaemia and reperfusion [148]. Thereby, CypD has a deleterious role in that attachment of the enzyme enhances the ability of the adenine nucleotide translocase to undergo the conformational change triggered by Ca^{2+}, which leads to the open-pore state [150]. There is increasing evidence that the VDAC-adenine nucleotide translocase-CypD complex can recruit a number of other proteins, including Bax, and that the complex is utilized in some capacity during apoptosis [148]. As shown by Halestrap *et al*. (1997) application of CsA prevents the pathogenic opening of the MTP as well as resultant cellular damage [151], and monofunctional CypD inhibitors are also effective.

The most studied member of the FKBP family, FKBP12, regulates intracellular Ca^{2+} release by interacting with multiple intracellular Ca^{2+} channels including the tetrameric skeletal muscle ryanodine receptor (RyR1) and the inositol 1, 4, 5-trisphosphate receptor (IP_3R) [152, 153]. In contrast, the cardiac ryanodine receptor, RyR2, appears to bind selectively the FKBP12 homologue, FKBP12.6 [154]. In all cases, one molecule of the PPIase is associated with each of the four receptor subunits and can be dissociated by addition of FK506, rapamycin or non-immunosuppressive analogues [38, 155]. RyR Ca^{2+} release channels, which are localized on the sarcoplasmic reticulum, are required for muscle excitation-contraction (EC) coupling [156]. This association with FKBP12 and FKBP12.6, respectively, is thought to stabilize RyR and to improve its Ca^{2+}-fluxing characteristics [157]. Phosphorylation of RyR2 by cAMP-dependent protein kinase (PKA) dissociates FKBP12.6 and regulates the channel open probability. RyR2 is PKA hyperphosphorylated in failing human hearts, leading to defective channel function due to increased sensitivity to Ca^{2+}-induced activation [158]. In FKBP12 knockout mice, the lack of FKBP12 altered the single-channel properties of skeletal RyR1 and cardiac RyR2, resulting in severe dilated cardiomyopathy [121]. The difficulty in interpreting the knockout experiments is mainly due to the lack of RyR2-associated FKBP12 (where FKP12.6 dominates as the ligand). It is underlined by the recent finding that the cell cycle of FKBP12 deficient mouse cells is arrested in the G1 phase because FFBP12 normally down-regulates TGF-ß receptor signaling [159]. IP_3R of the endoplasmic reticulum and plasma membrane regulate Ca^{2+} entry in the cell and are downstream component of hormone and neurotransmitter receptor-triggered signaling cascades with resultant IP_3 generation [160]. By anchoring CaN to the IP_3R, FKBP12 has been implicated to have a modulatory function in IP_3-mediated Ca^{2+} flux [153].

Similarly, FKBP12 was found to specifically interact with the ligand-free transforming growth factor β−type I (TGF-βI) receptor, a serine-threonine kinase, and is released upon a ligand-induced, type II receptor mediated phosphorylation of the type I receptor [161, 162]. Overexpression of FKBP12 caused inhibition of type I receptor phosphorylation by the type II receptor. At

micromolar concentrations, FK506 as well as non-immunosuppressive analogues, enhanced the functional responses elicited by TGF-β by displacing FKBP12 from the receptor, thus indicating that FKBP12 functions as an inhibitor of TGF-β-mediated signaling [162, 163]. The G89P, I90K FKBP12 protein variant, which does not inhibit CaN but retains the ability to bind FK506 [162], was incapable of blocking signaling, suggesting that FKBP12 may serve to dock CaN to the type I receptor. In contrast, recent findings of Bassing *et al*. (1998) cast doubt on a unique physiological role of FKBP12 in TGF-β receptor function [164], since the addition of excess FK506 had no effect on either TGF-β-mediated transcriptional responses or growth inhibition. In addition, dose-response curves for TGF-β-mediated signaling in primary fibroblasts and thymocytes isolated from either wild-type or FKBP12-deficient mice were identical. Obviously, the high FKBP ligand concentrations required for the impairment of receptor function raise further questions about the involvement of these receptors in the mediation of neuronal effects of these drugs. Because of the presence of high cellular concentrations of numerous FKBPs, most of which might exhibit high drug affinity, the intracellular drug concentration remains unknown, and is not identical to the administered concentration. Thus, dose-response curves are difficult to compare for different drugs.

Besides, FKBP12 was characterized as to inhibit intrinsic protein tyrosine kinase activity of the epidermal growth factor (EGF) receptor via its PPIase activity [165, 166]. This effect of FKBP12 on autophosphorylation is blocked by FK506 and rapamycin.

Three high molecular weight multidomain PPIases exist in steroid (glucocorticoid, androgen, oestrogen, progesteron) receptor/Hsp90 heterocomplexes, Cyp40 [167, 168], FKBP51 [169, 170] and FKBP52 (Hsp56, FKBP59, HBI) [135, 171-173]. Each of these PPIases contains three tetratricopeptide repeats (TPR), a conserved 34 amino acid sequence motive, which is required for binding to Hsp90 [174, 175]. FKBP52 and Cyp40 compete with each other for a common TPR acceptor site on Hsp90 and are components of independent receptor/Hsp90/FKBP52 and receptor/Hsp90/Cyp40 heterocomplexes [166, 175, 176]. Besides, other TPR domain proteins have been described to bind to the TPR site of Hsp90, e.g. the Hsp organizing protein (Hop, p60), which is considered to bring together the Hsp90 and Hsp70 components of the chaperone machinery [177-179]. The TPR acceptor site partially overlaps with a binding site for the protein kinase $p50^{cdc37}$ (vertebrate homologue of yeast cell cycle control protein Cdc37) [129]. Although the precise molecular mechanism is still an enigma, several studies provided evidence for differential modulation of steroid hormone receptor activity by FKBP52. It has been shown that in presence of steroid, the heat shock proteins and FKBP52 dissociate from the receptor [136, 180]. FKBP52 is not required for glucocorticoid receptor (GR)/Hsp90 heterocomplex assembly but is along with $p50^{cdc37}$ thought to target the retrograde or anterograde direction of signaling protein movement [129]. An additional aspect to PPIase influences on receptor signaling was recently provided by Reynolds *et al*. (1999), who showed that FKBP51 is specifically able to down-modulate the affinity of GR and PR for the steroid hormone [181]. So far, no role for PPIase activity in steroid hormone action has been established [123], and PPIase enzyme activity is not involved in interaction of FKBP52 with dynein [134]. Interferon regulatory factor-4 (IRF-4)-FKBP52 association inhibited the binding of IRF4-PU.1 to the immunoglobulin light chain enhancer E (lambda2-4) as well as IRF-4-PU.1 transactivation, effects that require functional PPIase activity [182].

CONCLUSIONS

New therapeutic approaches are required to treat neural diseases. The FKBP family of the enzyme class of the PPIases provides a novel target to screen for low-molecular weight, monofunctional FKBP inhibitors with particular clinical relevance for neurodegenerative disorders. In terms of specificity of action, bioavailability and stability, these compounds might be essentially advantageous compared to peptidic growth factors, being presently evaluated, such as NGF, BDNF, NT3 and GDNF [201-203]. Unlike the growth factors, which exert neurotrophic activities on overlapping but limited populations of CNS neurons, FKBP ligands do not cause aberrant sprouting of healthy neuronal processes *in vivo* [10]. Clinical potential of other available or presently examined neuroregenerative therapeutics, such as gangliosides [204] or the dihydropyridine Ca^{2+} antagonist nimodipine [205] is hampered by the observed adverse side effects [206, 207]. All available drugs for the treatment of frequently occuring neurodegenerative disorders such as Alzheimer's and Parkinson's diseases only alleviate symptoms and delay the neuronal atrophy by compensating or increasing impairments of the neurotransmitter metabolism [208, 209].

As an alternative approach, neural transplantation of competent neuronal cells or tissues is considered to hold promise as a future therapeutic tool to treat progressive and irreversible neural disorders [210, 211]. Whereas clear evidence is only available at present for the viability of this technique in Parkinson's disease, applications to several other diseases, including Huntington's disease, multiple sclerosis, spinal cord injury, and chronic pain are currently under investigation. To overcome associated ethical problems, cell lines and genetically engineered cells e.g. neural chimeras composed of embryonic stem (ES) cell-derived neurons and glia depict ES cells are being developed as suitable unlimited donor sources for neural repair [212]. These neural transplantation techniques, however, are still in an early developmental stage and many of the adherent problems such as viability and function of the graft remain to be solved.

In contrast, the broad spectrum of activity of FKBP ligands, their potential power and practicability is unprecedented. Although the present knowledge is still fragmentary with respect to the molecular mechanisms underlying neural actions of FKBP inhibitors, there is increasing evidence of different pathways being involved in mediation of neuroregenerative and neuroprotective properties of these compounds, which may be both additive and antagonistic. Furthermore, the interpretation of

Table 1. immunophilins: Biochemical and Functional Characterization

Cyclophilin	M_r, kDa	K_d, nM (CsA)	Localization	Ligand	Function	References
CypA	17.7	2	HIV-1 virion cytoplasma	HIV-1 p55 Gag Aop1	HIV-1 attachment, replication oxidative stress defense?	[17, 18, 183, 184] [146]
CypB	23.5	84	secretory pathway, ER	CAML	Ca^{2+} signaling	[185, 186]
CypC	22.8	4	secretory pathway, ER	77 kDa glyco-protein (CyCAP)	endotoxin and pro-inflammatory response regulation?	[187, 188]
CypD	20.0	3.6	mitochondria	MTP (ANT)	mitochondria permeability transition	[147, 148, 189]
Cyp-40	40.0	300	cytoplasm	Hsp90	steroid receptor function	[168, 169, 190]
Cyp-NK	165.7		NK cell surface		NK cell function?	[191]

			K_d, nM				
FKBP	**M_r, kDa**	**FK506**	**Rapamycin**	**Cellular localization**	**Ligand**	**Function**	**References**
FKBP12	11.8	0.4	0.2	cytoplasm SR cytoplasm ER, plasma membrane	TGF-β receptor I RyR1 EGFR IP_3R	TGF-β receptor I signaling? regulation of Ca^{2+} flux negative regulation of EGFR signaling regulation of Ca^{2+} flux	[19, 162, 164] [152] [165] [153]
FKBP12.6	11.6	0.55		SR	RyR2	regulation of Ca^{2+} flux	[154, 192]
FKBP13	13.2	38	3.6	secretory pathway, ER	4.1G	?	[193, 194]
FKBP25	25.3	160	0.9	cytoplasm, nucleus	casein kinase II, nucleolin	regulation of cell growth?	[195-197]
FKBP52	51.8	10	8	cytoplasm, nucleus	Hsp90 PAHX	steroid receptor function ?	[135, 172, 173] [198]
FKBP65	64.7 (60.5)	45 [a]		secretory pathway, ER	Tropoelastin c-Raf-1	protein folding, trafficking?	[145, 199] [200]

[a] (IC_{50})

neurobiological results is complicated by great discrepancies in the reported inhibition constants for FKBP ligands (e.g. GPI-1046) and unaccessible data of the pharmacokinetic properties. Finally, CaN plays a pivotal role in a variety of neuroprotective and anti-apoptotic pathways stimulated by FK506 and CsA in that it exerts protective and toxic effects [70]. Thus, FKBP12 and Cyp18 being target proteins of FK506 and CsA are likely to participate in the prevention of neuronal death by these compounds, especially during ischemia [48]. In contrast, present results obtained under various conditions of neuronal growth, repair but also degeneration strongly argue against contributions of a FKBP12-related pathway, including Ca^{2+} channel, TGF-β receptor and EGF receptor signaling and suggest mediation by other FKBPs such as FKBP52 [47, 85]. The multiple pathways activated by neuroimmunophilin-ligands may crosstalk via common integral components such as c-Jun [47, 52, 85-87].

As a prerequisite for the design of specific acting immunophilin-ligands for different types of neuronal disorders and nerve injuries ongoing research focuses on the identification of molecular targets for neuroimmunophilin ligands and neuroimmunophilin/ligand complexes. Regardless of the mechanisms involved, the therapeutic use of monofunctional FKBP ligands nevertheless appears to be a promising area of major clinical importance.

ABBREVIATIONS

AIF	=	Apoptosis inducing factor
ANT	=	Adenine nucleotide translocase
Aop	=	Thiol-specific antioxidant protein
CAML	=	Calcium-signal modulating cyclophilin ligand
CaN	=	Ca^{2+}/calmodulin-dependent protein serine/threonine phosphatase calcineurin
CrmA	=	Cytokine response modifier A
CsA	=	Cyclosporin A
CyCAP	=	Cyclophilin C-associated protein
Cyp18	=	Cyclophilin with a molecular mass of 18 kDa
DRG	=	Dorsal root ganglia
EGF	=	Epidermal growth factor
ERK	=	Extracellular response kinase
FK506	=	Binding protein
GAP-43	=	Growth-associated protein of 43 kDa
GM-CSF	=	Granulocyte-macrophage colony-stimulating factor
Hop	=	Hsp organizing protein
Hsp90	=	90-kDa heat shock protein
IL	=	Interleukin
IP_3	=	Inositol 1,4,5-trisphosphate
JNK	=	C-Jun N-terminal kinase
LTD	=	Long-term depression
LTP	=	Long-term potentiation
MAPK	=	Mitogen-activated protein kinase
MPTP	=	1-methy-4-phenyl-1,2,3,6, tetrahydropyridine
MTP	=	Mitochondrial permeability transition pore
NF-AT	=	Nuclear factor of activated T cells
NGF	=	Nerve growth factor
NLS	=	Nuclear localization sequence
nNOS	=	Neuronal nitric oxide synthase
NMDA	=	N-methyl-D-aspartate
PAHX	=	Phytanoyl-CoA alpha-hydroxylase
PKA	=	cAMP-dependent protein kinase
PPIase	=	Peptidyl prolyl *cis/trans* isomerase
ROS	=	Reactive oxygen species
RyR	=	Ryanodine receptor
SAR	=	Structure-activity relationship
SOD	=	Cu/Zn superoxide dismutase
TGF	=	Transforming growth factor
TH	=	Tyrosin hydroxylase
TOR	=	Target of rapamycin
TPR	=	Tetratricopeptide repeats
VDAC	=	Voltage-dependent anion channel

REFERENCES

[1] 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.

[2] Dawson, T. M.; Steiner, J. P.; Lyons, W. E.; Fotuhi, M.; Blue, M.; Snyder, S. H. The immunophilins, FK506-binding protein and cyclophilin, are discretly localized in the brain: relationship to calcineurin. *Neuroscience* **1994**, *62*, 569-580.

[3] Gold, B. G.; Storm-Dickerson, T; Austin, D. R.; Katoh, K. FK506, an immunosuppressant, increases functional recovery and axonal regeneration in the rat following axotomy of the sciatic nerve. *Soc. Neurosci. Abstr*. **1993**, *19*, 1316.

[4] Sharkey, J.; Butcher, S. P. Immunophilins mediate the neuroprotective effects of FK506 in focal ischemia. *Nature* **1994**, *371*, 336-339.

[5] Wang, M.-S.; Zeleny-Pooley, M.; Gold, B. G. Comparative dose-dependence study of FK506 and cyclosporin A on the rate of axonal regeneration in the rat sciatic nerve. *J. Pharmac. Exp. Therap*. **1997**, *282*, 1084-1093.

[6] Steiner, J. P.; Connolly, M. A.; Valentine, H. L.; Hamilton, G. S.; Dawson, T. M.; Hester, L. V.; Snyder, S. H. Neurotrophic actions of nonimmuosuppressive analogues of immunosuppressive drugs FK506, rapamycin and cyclosporin A. *Nature Med*. **1997**, *3*, 421-428.

[7] Costantini, L. C.; Isacson, O. Immunophilin ligands and GDNF enhance neurite branching or elongation from developing dopamine neurons in culture. *Exp. Neurol*. **2000**, *164*, 60-70.

[8] Gold B. G. FK506 and the role of immunophilins in nerve regeneration. *Mol. Neurobiol*. **1997**, *15*, 285-306.

[9] Lyons, W. E.; George, E. B.; Dawson, T. M.; Steiner, J. P. Immunosuppressant FK506 promotes neurites outgrowth in cultures of PC12 cells and sensory ganglia. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3191-3195.

[10] Steiner, J. P.; Hamilton, G. S.; Ross, D. T.; Valentine, H. L.; Guo, H.; Connolly, M. A.; Liang, S.; Ramsey, C.; Li, J.-H. J.; Huang, W.; Howorth, P.; Soni, R.; Fuller, M.; Sauer, H.; Nowotnik, A. C.; Suzdak, P. D. Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2019-2024.

[11] Hamilton, G. S.; Steiner, J. P. Neuroimmunophilin ligands as novel therapeutics for the treatment of degenerative disorders of the nervous system. *Curr. Pharmac. Design* **1997**, *3*, 405-428.

[12] Hamilton, G. S.; Steiner, J. P. Immunophilins: beyond immunosuppression. *J. Med. Chem.* **1998**, *41*, 5119-5143.

[13] Dumont, F. J.; Staruch, M. J.; Koprak, S. L.; Siekierka, J. J.; Lin, C. S.; Harrison, R.; Sewell, T.; Kindt, V. M.; Beattie, T. R.; Wyvratt, M. *et al*. The immunosuppressive and toxic effects of FK-506 are mechanistically related: pharmacology of a novel antagonist of FK-506 and rapamycin. *J. Exp. Med.* **1992**, *176*, 751-60.

[14] Fischer, G. Peptidyl-prolyl *cis/trans* isomerases and their effectors. *Angew. Chem., Int. Ed. Engl*. **1994**, *33*, 1415-1436.

[15] Ruegger, A.; Kuhn, M.; Lichti, H.; Loosli, H. R.; Huguenin, R.; Quiquerez, C.; von Wartburg, A. [Cyclosporin A, a peptide metabolite from *Trichoderma polysporum* (link ex pers.) *rifai*, with a remarkable immunosuppressive activity]. *Helv. Chim. Acta* **1976**, *59*, 1075-1092.

[16] Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishiyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, I. FK506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, physico-chemical and biological characteristics. *J. Antibiot.* **1987**, *40*, 1249-1255.

[17] Handschuhmacher, R. E.; Harding, M. W.; Rice, J.; Drugge, R. J.; Speicher, D. W. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* **1984**, *226*, 544-546.

[18] Fischer, G.; Wittmann-Liebold, B.; Lang, K.; Kiefhaber, T.; Schmid, F. X. Cyp18nd peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* **1989**, *337*, 476-478.

[19] Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber S. L. A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* **1989**, *341*, 758-760.

[20] Siekierka, J. J.; Hung, S. H.; Poe, M.; Lin, C. S.; Sigal, N. H. A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature 341*, 755-777.

[21] Fischer, G.; Bang, H.; Mech, C. [Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides.] [German] *Biomed. Biochem. Acta* **1984**, *43*, 1101-1111.

[22] Galat, A.; Metcalfe, S. M. Peptidylproline *cis/trans* isomerases. *Prog. Biophys. Molec. Biol*. **1995**, *63*, 67-118.

[23] Rosen, M. K.; Schreiber, S. L. Natural products as probes of cellular function: Studies of immunophilins. *Angew. Chem., Int. Ed. Engl*. **1992**, *31*, 384-400.

[24] Liu, J.; Farmer, J. D., Jr.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* **1991**, *66*, 807-815.

[25] McCaffrey, P. G.; Perrino, B. A.; Soderling, T. R.; Rao, A. NF-ATp, a T-lymphocyte DNA-binding protein that is a target for calcineurin and immunosuppressive drugs. *J. Biol. Chem*. **1993**, *268*, 3747-3752.

[26] Schreiber, S. L.; Crabtree, G. R. The mechanism of action of cyclosporin A and FK506. *Immunol. Today* **1992**, *13*, 136-142.

[27] Dumont, F. J. FK506, an immunosuppressant targeting calcineurin function. *Curr. Med. Chem.* **2000**, *7*, 731-748.

[28] Rovira, P.; Mascarell, L.; Truffa-Bachi, P. The impact of immunosuppressive drugs on the analysis of T-cell activation. *Curr. Med. Chem.* **2000**, *7*, 673-692.

[29] Truffa-Bachi, P.; Lefkovits, I.; Frey, J. R. Proteomic analysis of T cell activation in the presence of cyclosporin A: immunosuppressor and activator removal induces de novo protein synthesis. *Mol. Immunol.* **2000**, *37*, 21-8.

[30] Sehgal, S. N.; Baker, H.; Vezina C. Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *J. Antibiot*. **1975**, *28*, 727-732.

[31] Sabatini, D. M.; Erdjumentbromage, H.; Lui, M.; Tempst, P.; Snyder, S. H. RAFT: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* **1994**, *78*, 35-43.

[32] Dumont, F. J.; Su, Q. X. Mechanism of action of the immunosuppressant rapamycin. *Life Sciences* **1996**, *58*, 373-395.

[33] Zheng, X.-F.; Schreiber, S. L. Target of rapamycin proteins and their kinase activity are required for meiosis. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3070-3075.

[34] Cutler, N. S.; Heitman, J.; Cardenas, M. E. TOR kinase homologs function in a signal transduction pathway that is conserved from yeast to mammals. *Mol. Cell. Endocrinol.* **1999**, *155*, 135-142.

[35] Göthel, S. F.; Marahiel, M. A. Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. *Cell. Mol. Life Sci*. **1999**, *55*, 423-436.

[36] Lu, P. J.; Wulf, G.; Zhou, X. Z.; Davies, P.; Lu, K. P. The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* **1999**, *399*, 784-788.

[37] Zhou, X. Z.; Kops, O.; Werner, A.; Lu, P. J.; Shen, M. H.; Stoller, G.; Kullertz, G.; Stark, M.; Fischer, G.; Lu, K. P. Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. *Mol. Cell* **2000**, *6*, 873-883.

[38] Kay, J. E. Structure-function relationships in the FK506-binding protein (FKBP) family of peptidylprolyl *cis-trans* isomerases. *Biochem. J.* **1996**, *314*, 361-385.

[39] Weiwad, M.; Kullertz, G.; Schutkowski, M.; Fischer, G. Evidence that the substrate backbone conformation is critical to phosphorylation by p42 MAP kinase. *FEBS Lett.* **2000**, *478*, 39-42.

[40] Schmid, F. X.; Mayr, L. M.; Mucke, M.; Schonbrunner E. R. Prolyl isomerases: role in protein folding. *Adv. Prot. Chem*. **1993**, *44*, 25-66.

[41] Scherer, G.; Kramer, M. L.; Schutkowski, M.; Reimer, U.; Fischer, G. Barriers to rotation of secondary amide peptide bonds. *J. Am. Chem. Soc*. **1998**, *120*, 5568-5574.

[42] Schutkowski, M.; Bernhardt, A.; Zhou, X. Z.; Shen, M.; Reimer, U.; Rahfeld, J. U.; Lu, K. P.; Fischer, G. Role of phosphorylation in determining the backbone dynamics of the serine/threonine-proline motif and Pin1 substrate recognition. *Biochemistry* **1998**, *37*, 5566-5575.

[43] Brandsch, M.; Thunecke, F.; Kullertz, G.; Schutkowski, M.; Fischer, G.; Neubert, K. Evidence for the absolute conformational specificity of the intestinal H+/peptide symporter, PEPT1. *J. Biol. Chem.* **1998**, *273*, 3861-3864.

[44] Somers, P. K.; Wandless, T. J.; Schreiber, S. L. Synthesis and analysis of 506BD, a high-affinity ligand for the immunophilin FK506. *J. Am. Chem. Soc.* **1991**, *113*, 8045-8056.

[45] Bierer, B. E.; Somers, P. K.; Wandless, T. J.; Burakoff, S. J.; Schreiber, S. L. Probing immunosuppressant action with a nonnatural immunophilin ligand. *Science* **1990**, *250*, 556-559.

[46] Sigal, N. H.; Dumont, F.; Durette, P.; Siekerka, J. J.; Peterson, L.; Rich, D. H.; Dunlap, B. E.; Staruch, M. J.; Melino, M. R.; Koprak, S. L.; Williams, D.; Witzel, B.; Pisano, J. M. Is cyclophilin involved in the immunosuppressive and nephrotoxic mechanism of action of cyclosporin A? *J. Exp. Med*. **1991**, *173*, 619-628.

[47] Gold, B. G. FK506 and the role of the immunophilin FKBP-52 in nerve regeneration. *Drug Metab. Rev.* **1999**, *31*, 649-663.

[48] Sharkey, J.; Jones, P. A.; McCarter, J. F.; Kelly, J. S. Calcineurin inhibitors as neuroprotectants. *CNS Drugs* **2000**, *13*, 1-13.

[49] Bennett, P. C.; Singaretnam, L. G.; Zhao, W. Q.; Lawen A.; Ng, K. T. Peptidyl-prolyl-cis/trans-isomerase activity may be necessary for memory formation. *FEBS Lett*. **1998**, *431*, 386-390.

[50] Bavetta, S.; Hamlyn, P. J.; Burnstock, G.; Lieberman, A. R.; Anderson, P. N.; The effects of FK506 on dorsal column axons following spinal cord injury in adult rats: neuroprotection and local regeneration. *Exp. Neurol*. **1999**, *158*, 382-393.

[51] Costantini, L. C.; Chaturvedi, P.; Armistead, D. M.; McCaffrey, P.G.; Deacon, T. W.; Isacson, O. A novel immunophilin ligand: distinct branching effects on dopaminergic neurons in culture and neurotrophic actions after oral administration in an animal model of Parkinson's disease. *Neurobiol. Dis*. **1998**, *5*, 97-106.

[52] Winter, C.; Schenkel, J.; Burger, E.; Eickmeier, C.; Zimmermann, M.; Herdegen, T.; The immunophilin ligand FK506, but not GPI-1046, protects against neuronal death and inhibits c-Jun expression in the substantia nigra pars compacta following transection of the rat medial forebrain bundle. *Neuroscience* **2000**, *95*, 753-762.

[53] Wang, M. S.; Gold, B. G. FK506 increases the regeneration of spinal cord axons in a predegenerated peripheral nerve autograft. *J. Spinal Cord Med.* **1999**, *22*, 287-296.

[54] Steiner, J. P.; Dawson, T. M.; Fotuhi, M.; Snyder, S. H. Immunophilin regulation of neurotransmitter release. *Mol. Med*. **1996**, *2*, 325-333.

[55] Ide, T.; Morikawa, E.; Kirino, T. An immunosuppressant, FK506, protects hippocampal neurons from forebrain ischemia in the mongolian gerbil. *Neurosci. Lett*. **1996**, *204*, 157-160.

[56] Tokime, T.; Nozaki, K.; Kikuchi, H.; Neuroprotective effect of FK506, an immunosuppressant, on transient global ischemia in gerbil. *Neurosci. Lett*. **1996**, *206*, 81-84.

[57] Butcher, S. P.; Henshall, D. C.; Teramura, Y.; Iwasaki, K.; Sharkey, J. Neuroprotective actions of FK506 in experimental stroke: in vivo evidence against an antiexcitotoxic mechanism. *J. Neurosci*. **1997**, *17*, 6939-6946.

[58] Dawson, T. M.; Steiner, J. P.; Dawson, V. L.; Dinerman, J. L.; Uhl, G. R.; Snyder, S. H. Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9808-9812.

[59] Tong, G.; Shepherd, D.; Jahr, C. E. Synaptic desensitization of NMDA receptors by calcineurin. *Science* **1995**, *267*, 1510-1512.

[60] Ikegami, S.; Kato, A.; Kudo, Y.; Kuno, T.; Ozawa, F.; Inokuchi, K. A facilitatory effect on the induction of long-term potentiation *in vivo* by chronic administration of antisense oligodeoxynucleotides against catalytic subunits of calcineurin. *Mol. Brain Res.* **1996**, *41*, 183-191.

[61] Lu, Y. F.; Hayashi, Y.; Moriwaki, A.; Tomizawa, K.; Matsui, H. FK506, a Ca2+/calmodulin-dependent phosphatase inhibitor, inhibits the induction of long-term potentiation in the rat hippocampus. *Neurosci. Lett*. **1996**, *205*, 103-106.

[62] Onuma, H.; Lu, Y. F.; Tomizawa, K.; Moriwaki, A.; Tokuda, M.; Hatase, O.; Matsui, H. A calcineurin inhibitor, FK506, blocks voltage-gated calcium channel-dependent LTP in the hippocampus. *Neurosci. Res*. **1998**, *30*, 313-319.

[63] Hodgkiss, J. P.; Kelly, J. S.; Only 'de novo' long-term depression (LTD) in the rat hippocampus in vitro is blocked by the same low concentration of FK506 that blocks LTD in the visual cortex. *Brain Res.* **1995**, *705*, 241-246.

[64] Torii, N.; Kamishita, T.; Otsu, Y.; Tsumoto, T. An inhibitor for calcineurin, FK506, blocks induction of long-term depression in rat visual cortex. *Neurosci. Lett*. **1995**, *185*, 1-4.

[65] Funauchi, M.; Haruta, H.; Tsumoto, T. Effects of an inhibitor for calcium/calmodulin-dependent protein phosphatase, calcineurin, on induction of long-term potentiation in rat visual cortex. *Neurosci. Research*. **1994**, *19*, 269-278.

[66] Beal, M. F. Oxidative damage in neurodegenerative diseases. *Neuroscientist* **1997**, *3*, 21-27.

[67] Bochelen, D.; Rudin, M.; Sauter, A. Calcineurin inhibitors FK506 and SDZ ASM 981 alleviate the outcome of focal cerebral ischemic/reperfusion injury. *J. Pharm. Exp. Therap.* **1999**, *288*, 653-659.

[68] Kuroda, S.; Janelidze, S.; Siesjo, B. K. The immunosuppressants cyclosporin A and FK506 equally ameliorate brain damage due to 30-min middle cerebral artery occlusion in hyperglycemic rats. *Brain Research.* **1999**, *835*, 148-153.

[69] Parker, E. M.; Monopoli, A.; Ongini, E.; Lozza, G.; Babij, C. M. Rapamycin, but not FK506 and GPI-1046, increases neurite outgrowth in PC12 cells by inhibiting cell cycle progression. *Neuropharmacology* **2000**, *39*, 1913-1919.

[70] Morioka, M.; Hamada, J.; Ushio, Y.; Miyamoto, E. Potential role of calcineurin for brain ischemia and traumatic injury. *Prog. Neurobiol.* **1999**, *58*, 1-30.

[71] Dawson, D. A. Nitric oxide and focal cerebral ischemia: multiplicity of actions and diverse outcome. Cerebrovasc. *Brain Metab. Rev.* **1994**, *6*, 299-324.

[72] Lipton, S. A.; Choi, Y. B.; Pan, Z. H.; Lei, S. Z.; Chen, H. S.; Sucher, N. J.; Loscalzo, J.; Singel, D. J.; Stamler, J. S. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* **1993**, *364*, 626-632.

[73] Kikuchi, M.; Kashii, S.; Mandai, M.; Yasuyoshi, H.; Honda, Y.; Kaneda, K.; Akaike, A. Protective effects of FK506 against glutamate-induced neurotoxicity in retinal cell culture. *Invest. Ophthal. Visual Sci*. **1998**, *39*, 1227-1232.

[74] Tagami, M.; Yamagata, K.; Nara, Y.; Fujino, H.; Kubota, A.; Numano, F.; Yamori, Y. Insulin-like growth factors prevent apoptosis in cortical neurons isolated from stroke-prone spontaneously hypertensive rats. *Lab. Invest*. **1997**, *76*, 603-612.

[75] Toung, T. J.; Bhardwaj, A.; Dawson, V. L.; Dawson, T. M.; Traystman, R. J.; Hurn, P. D. Neuroprotective FK506 does not alter in vivo nitric oxide production during ischemia and early reperfusion in rats. *Stroke* **1999**, *30*, 1279-1285.

[76] Chan, P. H. Role of oxidants in ischemic brain damage. *Stroke* **1996**, *27*, 1124-1126.

[77] Lee, J. P.; Palfrey, H. C.; Bindokas, V. P.; Ghadge, G. D.; Ma, L.; Miller, R. J.; Roos, R. P. The role of immunophilins in mutant superoxide dismutase-1-linked familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3251-3256.

[78] Nakatsuka, H.; Ohta, S.; Tanaka, J.; Toku, K.; Kumon, Y.; Maeda, N.; Sakanaka, M.; Sakaki, S. Release of cytochrome c from mitochondria to cytosol in gerbil hippocampal CA1 neurons after transient forebrain ischemia. *Brain Research.* **1999**, *849(1-2)*, 216-9.

[79] Nakai, A.; Kuroda, S.; Kristian, T.; Siesjo, B. K. The immunosuppressant drug FK506 ameliorates secondary mitochondrial dysfunction following transient focal cerebral ischemia in the rat. *Neurobiol. Dis.* **1997**, *4*, 288-300.

[80] Dhar, D. K.; Takemoto, Y.; Nagasue, N.; Uchida, M.; Ono, T.; Nakamura, T. FK506 maintains cellular calcium homeostasis in ischemia-reperfusion injury of the canine liver. *J. Surg. Res.* **1996**, *60*,142-146.

[81] Shibasaki, F.; Kondo, E.; Akagi, T.; McKeon; F. Suppression of signalling through transcription factor NF-AT by interactions between calcineurin and Bcl-2. *Nature* **1997**, *386*, 728-731.

[82] Asai, A.; Qiu, Jh.; Narita, Y.; Chi, S.; Saito, N.; Shinoura, N.; Hamada, H.; Kuchino, Y.; Kirino, T. High level calcineurin activity predisposes neuronal cells to apoptosis. *J. Biol. Chem.* **1999**, *274*, 34450-34458.

[83] Herr, I.; Martin-Villalba, A.; Kurz, E.; Roncaioli, P.; Schenkel, J.; Cifone, M. G.; Debatin, K. M. FK506 prevents stroke-induced generation of ceramide and apoptosis signaling. *Brain Res.* **1999**, *826*, 210-219.

[84] Mielke, K.; Damm, A.; Fischer, G.; Baumgrass, R.; Zhang, Y.; Buerger, E.; Herdegen, T. Neuroprotection by FK506 depends on de novo gene expression, but not on inhibition of calcineurin or JNK activation. *J. Neurochem.* **2000**, submitted.

[85] Gold, B. G.; Densmore, V.; Shou, W.; Matzuk, M. M.; Gordon, H. S. Immunophilin FK506-binding protein 52 (not FK506-binding protein 12) mediates the neurotrophic action of FK506. *J. Pharm. Exp. Therap*. **1999**, *289*, 1202-1210.

[86] Yardin, C.; Terro, F.; Lesort, M.; Esclaire, F.; Hugon, J. FK506 antagonizes apoptosis and c-jun protein expression in neuronal cultures. *Neuroreport***. 1998**, *9*, 2077-2080.

[87] Damm, A.; Herdegen, T.; Fischer, G.; Burger, E.; Mielke, K. FK506 and V10,367, but not calcineurin inhibitors, protect neuronal cell lines from H_2O_2 induced cell death. *Immunophilins in the Brain*, Prous Science, in press.

[88] Winter, C.; Schenkel, J.; Zimmermann, M.; Herdegen, T. MAP kinase phosphatase 1 is expressed and enhanced by FK506 in surviving mamillary, but not degenerating nigral neurons following axotomy. *Brain Res.* **1998**, *801*, 198-205.

[89] Oehrlein, S. A.; Parker, P. J.; Herget, T. Phosphorylation of GAP-43 (growth-associated protein of 43 kDa) by conventional, novel and atypical isotypes of the protein kinase C gene family: differences between oligopeptide and polypeptide phosphorylation. *Biochem. J.* **1996**, *317*, 219-224.

[90] Lyons, W. E.; Steiner, J. P.; Snyder, S. H.; Dawson, T. M. Neuronal regeneration enhances the expression of the immunophilin FKBP-12. *J. Neurosci*. **1995**, *15*, 2985-2994.

[91] Gold, B. G.; Yew, J. Y.; Zeleny-Pooley, M. The immunosuppressant FK506 increases GAP-43 mRNA levels in axotomized sensory neurons. *Neurosci. Lett*. **1998**, *241*, 25-28.

[92] Madsen, J. R.; MacDonald, P.; Irwin, N.; Goldberg, D. E.; Yao, G. L.; Meiri, K. F.; Rimm, I. J.; Stieg, P. E.; Benowitz, L. I. Tacrolimus (FK506) increases neuronal expression of GAP-43 and improves functional recovery after spinal cord injury in rats. *Exp. Neurol.* **1998**, *154*, 673-683.

[93] Carreau, A.; Gueugnon, J.; Benavides, J.; Vige, X. Comparative effects of FK-506, rapamycin and cyclosporin A, on the in vitro differentiation of dorsal root ganglia explants and septal cholinergic neurons. *Neuropharmacology* **1997**, *36*, 1755-1762.

[94] Lee, M.; Doolabh, V. B.; Mackinnon, S. E.; Jost, S. FK506 promotes functional recovery in crushed rat sciatic nerve. *Muscle & Nerve*. **2000**, *23*, 633-640.

[95] Armistead, D. M.; Badia, M. C.; Deininger, D. D.; Duffy, J. P.; Saunders, J. O.; Tung, R. D.; Murcko, M. A.; Yamashita, M. M.; Navia, M. A. Design, synthesis and structure of non-macrocyclic inhibitors of FKBP12, the major binding protein for the immunosuppressant FK506. *Acta Crystallogr*. **1995**, *D51*, 522-528.

[96] Christner, C.; Wyrwa, R.; Marsch, S.; Kullertz, G.; Thiericke, R.; Grabley, S.; Schumann, D.; Fischer, G. Synthesis and cytotoxic evaluation of cycloheximide derivatives as potential inhibitors of FKBP12 with neuroregenerative properties. *J. Med. Chem.* **1999**, *42*, 3615-3622.

[97] Gold, B. G.; Zeleny-Pooley, M.; Wang, M.-S.; Chaturvedi, P.; Armistead, D. M. A nonimmunosuppressant FKBP-12 ligand increases nerve regeneration. *Exp. Neurol.* **1997**, *147*, 269-278.

[98] Gold, B. G.; Zeleny-Pooley, M.; Chaturvedi, P.; Wang, M. S. Oral administration of a nonimmunosuppressant FKBP-12 ligand speeds nerve regeneration. *Neuroreport* **1998**, *9*, 553-558.

[99] 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.

[100] Sauer, H.; Francis, J. M.; Jiang, H.; Hamilton, G. S.; Steiner, J. P. Systemic treatment with GPI 1046 improves spatial memory and reverses cholinergic neuron atrophy in the medial septal nucleus of aged mice. *Brain Res.* **1999**, *842*, 109-118.

[101] Harper, S.; Bilsland, J.; Young, L.; Bristow, L.; Boyce, S.; Mason, G.; Rigby, M.; Hewson, L.; Smith, D.; O'Donnell, R.; O'Connor, D.; Hill, R. G.; Evans, D.; Swain, C.; Williams, B.; Hefti, F. Analysis of the neurotrophic effects of GPI-1046 on neuron survival and regeneration in culture and in vivo. *Neuroscience* **1999**, *88*, 257-267.

[102] Emborg, M. E.; Shin, P.; Roitberg, B.; Sramek, J. G.; Chu, Y.; Stebbins, G. T.; Hamilton, J. S.; Suzdak, P. D.; Steiner, J. P.; Kordower, J. H. Systemic administration of the immunophilin ligand GPI 1046 in MPTP-treated monkeys. *Exp. Neurol.* **2001**, *168*, 171-182.

[103] Holt, D. A.; Konialian-Beck, A. L.; Oh, H.-L.; Yen, H.-K.; Rozamus, L. W.; Krog, A. J.; Erhard, K. F.; Ortiz, E.; Levy, M. A.; Brandt, M.; Bossard, M. J.; Luengo, J. I. Structure-activity studies of synthetic FKBP ligands as peptidyl-prolyl isomerase inhibitors. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 315-320.

[104] Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. Atomic structures of the human immunophilin FKBP-12 complexes with FK506 and rapamycin. *J. Molec. Biol*. **1993**, *229*, 105-124.

[105] Teague, S. J.; Stocks, M. J. The affinity of the excised binding domain of FK-506 for the immunophilin FKBP12. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 1947-1950

[106] Teague, S. J.; Cooper, M. E.; Donald, D. K.; Furber, M. Synthesis and study of a non macrocyclic FK506 derivative. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 1581-1584.

[107] Birkenshaw, T. N.; Caffrey, M. V.; Cladingboel, D. E.; Cooper, M. E.; Donald, D. K.; Furber, M.; Hardern, D. N.; Harrison, R. P.; Marriott, D. P.; Perry, M. W. D.; Stocks, M. J.; Teague, S. J.; Withnall, W. J. Synthetic FKBP12 ligands. Design and synthesis of pyranose replacements. *Bioorg. Med. Chem. Lett*. **1994**, *4*, 2501-2506.

[108] Hauske, J. R.; Dorff, P.; Julin, S.; DiBrino, J.; Spencer, R.; Williams, R. J. Design and synthesis of novel FKBP inhibitors. *J. Med. Chem*. **1992**, *35*, 4284-4296.

[109] Duffi, J. P. Novel immunosuppressive Compounds. International Patent Application WO 92/21313, **1992**.

[110] Dragovich, P. S.; Barker, J. E.; French, J.; Imbacuan, M.; Kalish, V. J.; Kissinger, C. R.; Knighton, D. R.; Lewis, C. T.; Moomaw, E. W.; Parge, H. E.; Pelletier, L. A.; Prins, T. J.; Showalter, R. E.; Tatlock, J. H.; Tucker, K. D.; Villafranca, J. E. Structure-based design of novel, urea-containing FKBP12 inhibitors. *J. Med. Chem.* **1996**, *39*, 1872-1884.

[111] Holt, D. A.; Luengo, J. I.; Yamashita, D. S.; Oh, H.-J.; Konialian, A. L.; Yen, H.-K.; Rozamus, L. W.; Brandt, M.; Bossard, M. J.; Levy, M. A.; Eggleston, D. S.; Liang, J.; Schultz, L. W.; Stout, T. J.; Clardy, J. Design, synthesis, and kinetic evaluation of high-affinity FKBP ligands and the X-ray crystal structures of their complexes with FKBP12. *J. Am. Chem. Soc*. **1993**, *115*, 9925-9938.

[112] Lamb, M. L.; Jorgensen, W. L. Investigations of neurotrophic inhibitors of FK506 binding protein via Monte Carlo simulations. *J. Med. Chem*. **1998**, *41*, 3928-3939.

[113] Lamb, M. L.; Tirado-Rives, J.; Jorgensen, W. L. Estimation of the binding affinities of FKBP12 inhibitors using a linear response method. *Bioorg. Med. Chem*. **1999**, *7*, 851-860.

[114] Adalsteinsson, H.; Bruice, T. C. Generation and evaluation of putative neuroregenerative drugs. Part 1: virtual point mutations to the polyketide rapamycin. *Bioorg. Med. Chem*. **2000**, *8*, 617-624.

[115] Adalsteinsson, H.; Bruice, T. C. Generation and evaluation of putative neuroregenerative drugs. Part 2: screening virtual libraries of novel polyketides which possess the binding domain of rapamycin. *Bioorg. Med. Chem*. **2000**, *8*, 625-635.

[116] Burkhard, P.; Hommel, U.; Sanner, M.; Walkinshaw, M. D. The discovery of steroids and other novel FKBP inhibitors using a molecular docking program. *J. Mol. Biol*. **1999**, *287*, 853-858.

[117] Lost, J. L.; Kominek, L. A.; Hyatt, G. S.; Wang, H. Y. Cycloheximide: properties, biosynthesis, and fermentation. *Drugs Pharm. Sci.* **1984**, *22*, 531-550.

[118] Johnson, F. The chemistry of glutarimide antibiotics. *Fortschr. Chem. Org. Naturst*. **1971**, *29*, 1401.

[119] Hendey, B.; Klee, C. B.; Maxfield, F. R. Inhibition of neutrophil chemokinesis on vitronectin by inhibitors of calcineurin. *Science* **1992**, *258*, 296-299.

[120] Fischer *et al*., in preparation.

[121] Shou, W.; Aghdasi, B.; Armstrong, D. L.; Guo, Q.; Bao, S.; Charng, M. J.; Mathews, L. M.; Schneider, M. D.; Hamilton, S. L.; Matzuk, M. M. Cardiac defects and altered ryanodine function in mice lacking FKBP12. *Nature* **1998**, *391*, 489-492.

[122] Jones, K. J. Gonadal steroids as promoting factors in axonal regeneration. *Brain Res. Bull*. **1993**, *30*, 491-498.

[123] Pratt, W. B.; Toft, D. O. Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocrine Rev*. **1997**, *18*, 306-360.

[124] Scheibel, T.; Weikl, T.; Buchner, J. Two chaperone sites in Hsp90 differing in substrate specificity and ATP dependence. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 1495-1499.

[125] Grenert, J. P.; Sullivan, W. P.; Fadden, P.; Haystead, T. A. J.; Clark, J.; Mimnaugh, E.; Krutzsch, H.; Ochel, H. J.; Schulte, T. W.; Sausville, E.; Neckers, L. M.; Toft, D. O. The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. *J. Biol. Chem*. **1997**, *272*, 23843-23850.

[126] Bohen, S. P. Genetic and biochemical analysis of p23 and ansamycin antibiotics in the function of Hsp90-dependent signaling proteins. *Mol. Cell. Biol*. **1998**, *18*, 3330-3339.

[127] Knoblauch, R.; Garabedian, M. J. Role for Hsp90-associated cochaperone p23 in estrogen receptor signal transduction. *Mol. Cell. Biol*. **1999**, *19*, 3748-3759.

[128] Pratt, W. B. The role of the hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. *Ann. Rev. Pharmacol. Toxicol.* **1997**, *37*, 297-326.

[129] Pratt, W. B.; Silverstein, A. M.; Galigniana, M. D. A model for the cytoplasmic trafficking of signalling proteins involving the hsp90-binding immunophilins and p50cdc37. *Cell. Signal*. **1999**, *11*, 839-851.

[130] Herdegen, T.; Skene, P.; Böhr, M. The c-Jun transcription factor-bipotential mediator of neuronal death, survival and regeneration. *Trends Neurosci*. **1997**, *20*, 227-231.

[131] Leppa, S.; Saffrich, R.; Ansorge, W.; Bohmann, D. Differential regulation of c-Jun by ERK and JNK during PC12 cell differentiation. *EMBO J*. **1998**, *17*, 4404-4413.

[132] Eggen, B. J.; Nielander, H. B.; Rensen-de Leeuw, M. G.; Schotman, P.; Gispen, W. H.; Schrama, L. H. Identification of two promoter regions in the rat B-50/GAP-43 gene. *Molec. Brain Res.* **1994**, *23*, 221-234.

[133] Yao, G. L.; Kiyama, H. Dexamethasone enhances level of GAP-43 mRNA after nerve injury and facilitates re-projection of the hypoglossal nerve. *Mol. Brain Res.* **1995**, *32*, 308-312.

[134] Silverstein, A. M.; Galigniana, M. D.; Kanelakis, K. C.; Radanyi, C.; Renoir, J. M.; Pratt, W. B. Different regions of the immunophilin FKBP52 determine its association with the glucocorticoid receptor, hsp90, and cytoplasmic dynein. *J. Biol. Chem*. **1999**, *274*, 36980-36986.

[135] Peattie, D. A.; Harding, M. W.; Fleming, M. A.; DeCenzo, M. T.; Lippke, J. A.; Livingston, D. J.; Benasutti, M. Expression and characterization of human FKBP52, an immunophilin that associates with the 90-kDa heat shock protein and is a component of steroid receptor complexes. *Proc. Natl. Acad. Sci*. *USA* **1992**, *89*, 10974-10978.

[136] Czar, M. J.; Owens-Grillo, J. K.; Dittmar, K. D.; Hutchison, K. A.; Zacharek, A. M.; Leach, K. L.; Deibel, M. R., Jr.; Pratt, W. B. Characterization of the protein-protein interactions determining the heat shock protein (hsp90.hsp70.hsp56) heterocomplex. *J. Biol. Chem*. **1994**, *269*, 11155-11161.

[137] Perrot-Applanat, M.; Cibert, C.; Geraud, G.; Renoir, J. M.; Baulieu, E. E. The 59 kDa FK506-binding protein, a 90 kDa heat shock protein binding immunophilin (FKBP59-HBI), is associated with the nucleus, the cytoskeleton and mitotic apparatus. *J. Cell Sci*. **1995**, *108*, 2037-2051.

[138] Pratt, W. B.; Czar, M. J.; Stancato, L. F.; Owens, J. K. The hsp56 immunophilin component of steroid receptor heterocomplexes: could this be the elusive nuclear localization signal-binding protein? *J. Steroid Biochem. Mol. Biol*. **1993**, *46*, 269-279.

[139] Czar, M. J.; Lyons, R. H.; Welsh, M. J.; Renoir, J. M.; Pratt, W. B. Evidence that the FK506-binding immunophilin heat shock protein 56 is required for trafficking of the glucocorticoid receptor from the cytoplasm to the nucleus. *Mol. Endocrin*. **1995**, *9*, 1549-1560.

[140] Tai, P. K.; Chang, H.; Albers, M. W.; Schreiber, S. L.; Toft, D. O.; Faber, L. E. P59 (FK506 binding protein 59) interaction with heat shock proteins is highly conserved and may involve proteins other than steroid receptors. *Biochemistry* **1993**, *32*, 8842-8847.

[141] Miyata, Y.; Chambraud, B.; Radanyi, C.; Leclerc, J.; Lebeau, M. C.; Renoir, J. M.; Shirai, R.; Catelli, M. G.; Yahara, I.; Baulieu, E. E. Phosphorylation of the immunosuppressant FK506-binding protein FKBP52 by casein kinase II: regulation of HSP90-binding activity of FKBP52. *Proc. Natl. Acad. Sci. USA*. **1997**, *94*, 14500-14505.

[142] Ning, Y. M.; Sanchez, E. R. Potentiation of glucocorticoid receptor-mediated gene expression by the immunophilin ligands FK506 and rapamycin. *J. Biol. Chem.* **1993**, *268*, 6073-6076.

[143] Galat, A. Sequence diversification of the FK506-binding proteins in several different genomes. *Europ. J. Biochem.* **2000**, *267*, 4945-4959.

[144] Pedersen, K. M.; Finsen, B.; Celis, J. E.; Jensen, N. A. muFKBP38: a novel murine immunophilin homolog differentially expressed in Schwannoma cells and central nervous system neurons in vivo. *Electrophoresis* **1999**, *20*, 249-255.

[145] Coss, M. C.; Winterstein, D.; Sowder, R. C.; Simek, S. L. Molecular cloning, DNA sequence analysis, and biochemical characterization of a novel 65-kDa FK506-binding protein (FKBP65). *J. Biol. Chem.* **1995**, *270*, 29336-29341.

[146] Jaschke, A.; Mi, H.; Tropschug, M. Human T cell cyclophilin18 binds to thiol-specific antioxidant protein Aop1 and stimulates its activity. *J. Molec. Biol*. **1998**, *277*, 763-769.

[147] Halestrap, A. P.; Woodfield, K.-Y.; Connern, S. P. Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. *J. Biol. Chem.* **1997**, *272*, 3346-3354.

[148] Crompton, M. The mitochondrial permeability transition pore and its role in cell death. *Biochem. J*. **1999**, *341*, 233-249.

[149] Andreeva, L.; Heads, R.; Green, C. J. Cyclophilins and their possible role in the stress response. *Int. J. Exp. Pathol*. **1999**, *80*, 305-315.

[150] Halestrap, A. P.; Kerr, P. M.; Javadov, S.; Woodfield, K. Y. Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart. *Biochim. Biophys*. *Acta* **1998**, *1366*,79-94.

[151] Halestrap, A. P.; Connern, C. P.; Griffith, E. J.; Kerr, P. M. Cyclosporin A binding to mitochondrial cyclophilin inhibits the permeability transition pore and protects hearts from ischemia/reperfusion injury. *Molec. Cell. Biochem*. **1997**, *174*, 167-172.

[152] Jayaraman, T.; Brilliantes, A. M.; Timerman, A. P.; Fleischer, S.; Erdjument-Bromage, H.; Tempst, P.; Marks, A. R. FK506 binding protein associated with the calcium release channel (ryanodine receptor). *J. Biol. Chem*. **1992**, *267*, 9474-9477.

[153] Cameron, A. M.; Steiner, J. P.; Sabatini, D. M.; Kaplin, A. I.; Walensky, L. D.; Snyder, S. H. Immunophilin FK506 binding protein associated with inositol 1,4,5-trisphosphate receptor modulates calcium flux. *Proc. Natl. Acad. Sci*. *USA* **1995**, *92*, 1784-1788.

[154] Timerman, A. P.; Onoue, H.; Xin, H. B.; Barg, S.; Copello, L.; Wiederrecht, G.; Fleischer, S. Selective binding of FKBP12.6 by the cardiac ryanodine receptor. *J. Biol. Chem.* **1996**, *271*, 20385-20391.

[155] Timerman, A. P.; Wiederrecht, G.; Marcy, A.; Fleischer, S. Characterization of an exchange reaction between soluble FKBP-12 and the FKBP.ryanodine receptor complex. Modulation by FKBP mutants deficient in peptidyl-prolyl isomerase activity. *J. Biol. Chem*. **1995**, *270*, 2451-2459.

[156] Wagenknecht, T.; Grassucci, R.; Frank, J.; Saito, A.; Inui, M.; Fleischer, S. Three-dimensional architecture of the calcium channel/foot structure of sarcoplasmic reticulum. *Nature* **1989**, *338*, 167-170.

[157] Brillantes, A. B.; Ondrias, K.; Scott, A.; Kobrinsky, E.; Ondriasova, E.; Moschella M. C.; Jayaraman, T.; Landers, M.; Ehrlich, B. E.; Marks, A. R. Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* **1994**, *77*, 513-523.

[158] Marx, S. O.; Reiken, S.; Hisamatsu, Y.; Jayaraman, T.; Burkhoff, D.; Rosemblit, N.; Marks, A. R. PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): Defective regulation in failing hearts, *Cell* **2000**, *101*, 365-376.

[159] Aghdasi, B.; Ye, K. Q.; Resnick, A.; Huang, A.; Ha, H. C.; Guo, X.; Dawson, T. M.; Dawson, V. L.; Snyder, S. H. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 2425-2430.

[160] Ferris, C. D.; Snyder, S. H. Inositol 1,4,5-trisphosphate-activated calcium channels. *Ann. Rev. Physiol.* **1992**, *54*, 469-488.

[161] Wang, T.; Donahoe, P. K.; Zervos, A. S.; Specific interaction of type I receptors of the TGF-beta family with the immunophilin FKBP-12. *Science* **1994**, *265*, 674-676.

[162] Wang, T.; Li, B.-Y.; Danielson, P. D.; Shah, P. C.; Rockwell, S.; Lechleider, R. J.; Martin, J.; Manganaro, T.; Donahoe, P. K. The immunophilin FKBP12 functions as a common inhibitor of the TGFβ family type I receptors. *Cell* **1996**, *86*, 435-444.

[163] Stockwell, B. R.; Schreiber, S. L. TGF-beta-signaling with small molecule FKBP12 antagonists that bind myristoylated FKBP12-TGF-beta type I receptor fusion proteins. *Chem. Biol*. **1998**, *5*, 385-395.

[164] Bassing, C. H.; Shou, W.; Muir, S.; Heitman, J.; Matzuk, M. M.; Wang, X. F. FKBP12 is not required for the modulation of transforming growth factor beta receptor I signaling activity in embryonic fibroblasts and thymocytes. *Cell Growth & Differentiation* **1998**, *9*, 223-228.

[165] Lopez-Ilasaca, M.; Schiene, C.; Küllertz, G.; Tradler, T.; Fischer, G.; Wetzker, R. Effects of FK506-binding protein 12 and FK506 on autophosphorylation of epidermal growth factor receptor. *J. Biol. Chem*. **1998**, *273*, 9430-9434.

[166] Schiene-Fischer, C.; Chao, Yu Receptor accessory folding helper enzymes: the functional role of peptidyl prolyl cis/trans isomerases. *FEBS Lett*. **2001**, *495*, 1-6.

[167] Ratajczak, T.; Carrello, A.; Mark, P. J.; Warner, B. J.; Simpson, R. J.; Moritz, R. L.; House, A. K. The cyclophilin component of the unactivated estrogen receptor contains a tetratricopeptide repeat domain and shares identity with p59 (FKBP59). *J. Biol. Chem.* **1993**, *268*, 13187-13192.

[168] Kieffer, L. J.; Seng, T. W.; Li, W.; Osterman, D. G.; Handschumacher, R. E.; Bayney, R. M. Cyclophilin-40, a protein with homology to the P59 component of the steroid receptor complex. Cloning of the cDNA and further characterization. *J. Biol. Chem*. **1993**, *268*, 12303-12310.

[169] Smith, D. F.; Baggenstoss, B. A.; Marion, T. N.; Rimerman, R. A. Two FKBP-related proteins are associated with progesterone receptor complexes. *J. Biol. Chem*. **1993**, *268*, 18365-18371.

[170] Nair, S. C.; Rimerman, R. A.; Toran, E. J.; Chen, S.; Prapapanich, V.; Butts, R. N.; Smith, D. F. Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor. *Mol. Cell. Biol*. **1997**, *17*, 594-603.

[171] Tai, P. K.; Albers, M. W.; Chang, H.; Faber, L. E.; Schreiber, S. L. Association of a 59-kilodalton immunophilin with the glucocorticoid receptor complex. *Science* **1992**, *256*, 1315-1318.

[172] Yem, A. W.; Tomasselli, A. G.; Heinrikson, R. L.; Zurcher-Neely, H.; Ruff, V. A.; Johnson, R. A.; Deibel, M. R., Jr. The Hsp56 component of steroid receptor complexes binds to immobilized FK506 and shows homology to FKBP-12 and FKBP-13. *J. Biol. Chem*. **1992**, *267*, 2868-28671.

[173] Lebeau, M. C.; Massol, N.; Herrick, J.; Faber, L. E.; Renoir, J. M.; Radanyi, C.; Baulieu, E. E. P59, an hsp 90-binding protein. Cloning and sequencing of its cDNA and preparation of a peptide-directed polyclonal antibody. *J. Biol. Chem*. **1992**, *267*, 4281-4284.

[174] Sikorski, R. S.; Boguski, M. S.; Goebl, M.; Hieter, P. A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. *Cell* **1990**, *60*, 307-317.

[175] Ratajczak, T.; Carrello, A. Cyclophilin 40 (CyP-40), mapping of its hsp90 binding domain and evidence that

FKBP52 competes with CyP-40 for hsp90 binding. *J. Biol. Chem*. **1996**, *271*, 2961-2965.

[176] Owens-Grillo, J. K.; Hoffmann, K.; Hutchison, K. A.; Yem, A. W.; Deibel, M. R., Jr.; Handschumacher, R. E.; Pratt, W. B. The cyclosporin A-binding immunophilin CyP-40 and the FK506-binding immunophilin hsp56 bind to a common site on hsp90 and exist in independent cytosolic heterocomplexes with the untransformed glucocorticoid receptor. *J. Biol. Chem*. **1995**, *270*, 20479-20484.

[177] Johnson, B. D.; Schumacher, R. J.; Ross, E. D.; Toft, D. O.; Hop modulates Hsp70/Hsp90 interactions in protein folding. *J. Biol. Chem*. **1998**, *273*, 3679-3686.

[178] Chen, S.; Smith, D. F. Hop as an adaptor in the heat shock protein 70 (Hsp70) and hsp90 chaperone machinery. *J. Biol. Chem*. **1998**, *273*, 35194-35200.

[179] Smith, D. F. Chaperones in progesterone receptor complexes. *Sem. Cell Developm. Biol*. **2000**, *11*, 45-52.

[180] Johnson, J. L.; Toft, D. O. A novel chaperone complex for steroid receptors involving heat shock proteins, immunophilins, and p23. *J. Biol. Chem*. **1994**, *269*, 24989-24993.

[181] Reynolds, P. D.; Ruan, Y.; Smith, D. F.; Scammell, J. G. Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51. *J. Clin. Endocrinol. Metab*. **1999**, *84*, 663-669.

[182] Mamane, Y.; Sharma, S.; Petropoulos, L.; Lin, R.; Hiscott, J. Posttranslational regulation of IRF-4 activity by the immunophilin FKBP52. *Immunity* **2000**, *12*, 129-140.

[183] Luban, J.; Bossolt, K. L.; Franke, E. K.; Kalpana, G. V.; Goff, S. P. Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell* **1993**, *73*, 1067-1078.

[184] Saphire, A. C.; Bobardt, M. D.; Gallay, P. A. Host cyp18 mediates HIV-1 attachment to target cells via heparans. *EMBO J*. **1999**, *18, 6771-6785.*

[185] Price, E. R.; Zydowsky, L. D.; Jin, M. J.; Baker, C. H.; McKeon, F. D.; Walsh, C. T. Human cyclophilin B: a second cyclophilin gene encodes a peptidyl-prolyl isomerase with a signal sequence. *Proc. Natl. Acad. Sci.USA* **1991**, *88, 1903-1907.*

[186] Bram, R. J.; Crabtree, G. R. Calcium signalling in T cells stimulated by a cyclophilin B-binding protein. Nature. **1994**, *371*, 355-358.

[187] Schneider, H.; Charara, N.; Schmitz, R.; Wehrli, S.; Mikol, V.; Zurini, M. G.; Quesniaux, V. F.; Movva, N. R. Human cyclophilin C: primary structure, tissue distribution, and determination of binding specificity for cyclosporins. *Biochemistry* **1995**, *33, 8218-8224.*

[188] Trahey, M.; Weissman, I. L. Cyclophilin C-associated protein: a normal secreted glycoprotein that down-modulates endotoxin and proinflammatory responses in vivo. *Proc. Natl. Acad. Sci.USA* **1999**, *96, 3006-3011.*

[189] Connern, C. P.; Halestrap, A. P. Purification and N-terminal sequencing of peptidyl-prolyl-cis-trans-isomerase from rat liver mitochondrial matrix reveals existence of a distinct mitochondrial cyclophilin. *Biochem J*. **1992**, *284*, 381-385.

[190] Kieffer, L. J.; Thalhammer, T.; Handschumacher, R. E. Isolation and characterization of a 40-kDa cyclophilin-related protein. *J. Biol. Chem.* **1992**, *267*, 5503-5507.

[191] Anderson, S. K.; Gallinger, S.; Roder, J.; Frey, J.; Young, H. A.; Ortaldo, J. R. A. A cyclophilin-related protein involved in the function of natural killer cells. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 542-546.

[192] Lam, E.; Martin, M. M.; Timerman, A. P.; Sabers, C.; Fleischer, S.; Lukas, T.; Abraham, R. T.; O'Keefe, S. J.; O'Neill, E. A.; Wiederrecht, G. J. A novel FK506 binding protein can mediate the immunosuppressive effects of FK506 and is associated with the cardiac ryanodine receptor. *J. Biol. Chem.* **1995**, *270*, 26511-26522.

[193] Jin, Y.-J.; Albers, M. W.; Lane, W. S.; Bierer, B. E.; Schreiber, S. L.; Burakoff, S. J. Molecular cloning of a membrane-associated human FK506- and rapamycin-binding protein, FKBP-13. *Proc. Natl. Acad. Sci USA* **1991**, *88*, 6677-6681.

[194] Walensky, L. D.; Gascard, P.; Fields, M. E.; Blackshaw, S.; Conboy, J. G.; Mohandas, N.; Snyder, S. H. The 13-kD FK506 binding protein, FKBP13, interacts with a novel homologue of the erythrocyte membrane cytoskeletal protein 4.1. *J. Cell Biol.* **1998**, *141*, 143-153.

[195] Jin, Y.-J.; Burakoff, S. J.; Bierer, B. E. Molecular cloning of a 25-kDa high affinity rapamycin binding protein, FKBP25. *J. Biol. Chem.* **1992**, *267*, 10942-10945.

[196] Hung, D. T.; Schreiber, S. L. cDNA cloning of a human 25 kDa FK506 and rapamycin binding protein. *Biochem. Biophy. Res. Comm.* **1992**, *184*, 733-738.

[197] Jin, Y.-J.; Burakoff, S. J. The 25-kDa FK506-binding protein is localized in the nucleus and associates with casein kinase II and nucleolin. *Proc. Natl. Acad. Sci USA* **1993**, *90*, 7769-7773.

[198] Chambraud, B.; Radanyi, C.; Camonis, J. H.; Rajkowski, K.; Schumacher, M.; Baulieu, E. E. Immunophilins, Refsum disease, and lupus nephritis: the peroxisomal enzyme phytanoyl-COA alpha-hydroxylase is a new FKBP-associated protein. Proc. Natl. Acad. Sci. USA **1999**, *96*, 2104-2109.

[199] Davis, E. C.; Broekelmann, T. J.; Ozawa, Y.; Mecham, R. P. Identification of tropoelastin as a ligand for the 65-kD FK506-binding protein, FKBP65, in the secretory pathway. *J. Cell Biol*. **1998**, *140*, 295-303.

[200] Coss, M. C.; Stephens, R. M.; Morrison, D. K.; Winterstein, D.; Smith, L. M.; Simek, S. L. The immunophilin FKBP65 forms an association with the serine/threonine kinase c-Raf-1. *Cell Growth Diff.* **1998**, *9*, 41-48.

[201] McMahon, S. B.; Priestley, J. V. Peripheral neuropathies and neurotrophic factors: animal models and clinical perspectives. *Curr. Opin. Neurobiol.* **1995**, *55*, 616-624.

[202] Apfel, S. C. Neurotrophic factors in peripheral neuropathies: therapeutic implications. *Brain Pathol.* **1999**, *9*, 393-413.

[203] Grondin, R.; Gash**,** D. M. Glial cell line-derived neurotrophic factor (GDNF): a drug candidate for the treatment of Parkinson's disease. *J. Neurol.* **1998**, *245,P35-42*..

[204] Ekstrom, P. A.; Tomlinson, D. R. Impaired nerve regeneration in streptozotocin-diabetic rats is improved by treatment with gangliosides. *Exp. Neurol.* **1990**, *109*, 200-203.

[205] Gispen, W. H. Neuronal plasticity and function. *Clin. Neuropharmacol.* **1993**, *16*, S5-11.

[206] Candelise, L.; Ciccone, A. Gangliosides for acute ischaemic stroke. *Cochrane Database of Systematic Rev.* **2000**, *2*, *CD000094*

[207] Ahmed, N.; Nasman, P.; Wahlgren, N. G. Effect of intravenous nimodipine on blood pressure and outcome after acute stroke. *Stroke* **2000**, *31*, 1250-1255.

[208] Brodaty, H. Realistic expectations for the management of Alzheimer's disease. *Europ. Neuropsychopharmacol.* **1999**, *9*, S43-52.

[209] Grunblatt, E.; Mandel, S.; Youdim, M. B. Neuroprotective strategies in Parkinson's disease using the models of 6-hydroxydopamine and MPTP. *Ann. New York Acad. Sci.* **2000**, *899*, 262-273.

[210] Hansen, J. T.; Gash, D. M. Functional aspects of mammalian neural transplantation. *Crit. Rev. Neurobiol.* **1991**, *6*, *79-98*.

[211] Dunnett, S. B. Repair of the damaged brain. *Neuropathol. Appl. Neurobiol.* **1999**, *25*, 351-362.

[212] Brustle, O. Building brains: neural chimeras in the study of nervous system development and repair. *Brain Pathol.* **1999**, *9*, 527-545.