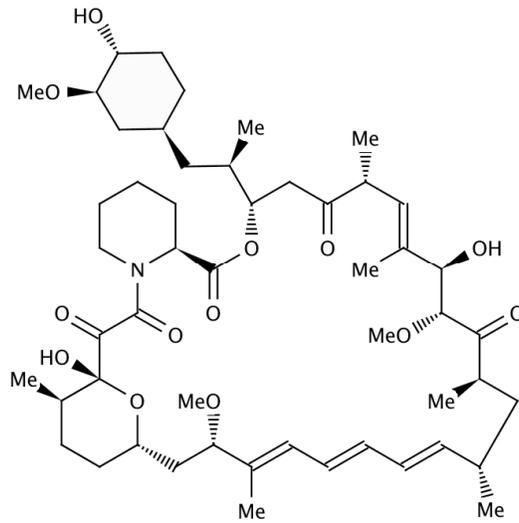


CINAPS Compound Dossier

Sirolimus



3/26/2009



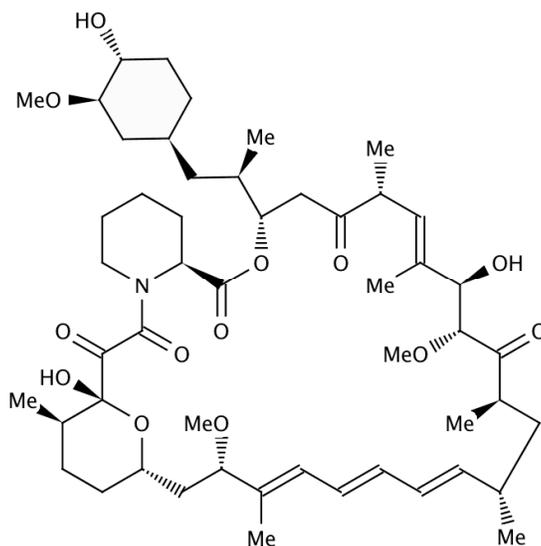
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I. Compound Information

Common name: Sirolimus

Structure:



PubChem ID: 5284616

Mol. Formula: C₅₁H₇₉NO₁₃

FW: 914.17

CASRN: 53123-88-9

Polar surface area: 195.43

logP: 7.45

IUPAC name: (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-Dihydroxy-12-[(2R)-1-[(1S,3R,4R)-4-hydroxy-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.0^{4,9}]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone

Other names: rapamycin, Rapamune®

Drug class: antibiotic, antifungal, immunosuppressant

Medicinal chemistry development potential: moderate

Notes:

II. Rationale

Ila. Scientific Rationale / Mechanism

Accumulation of unfolded or misfolded proteins leads to the production of inclusion bodies and cellular toxicity, and is associated with neuronal dysfunction and neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease. To prevent this accumulation, chaperone proteins attempt to rescue misfolded proteins by breaking up aggregates and assisting the refolding process. Proteins that cannot be rescued by refolding are tagged with ubiquitin and trafficked to the proteasome by other chaperone proteins to be degraded (**Calabrese, 2008**). In addition to these processes, autophagy provides an additional pathway for clearance of accumulating proteins. Autophagy involves the formation of double-membrane structures called autophagosomes or autophagic vacuoles, which fuse with the primary lysosomes, where their contents are degraded and then either disposed off or recycled back to the cell. These processes are highly regulated and play a significant role in cell growth, development, and homeostasis, helping to maintain a balance between the synthesis, degradation, and subsequent recycling of cellular proteins and organelles (**Ravikumar, 2002**). However, in Parkinson's disease, the capacity of these processes is exceeded and proteinaceous inclusions are formed within the central nervous system (i.e., Lewy bodies, in which the protein α -synuclein (ASN) is the major constituent), leading to neuronal dysfunction and degeneration (**Calabrese, 2008**).

Sirolimus is a macrolytic lactone produced by *Streptomyces hygroscopicus*, and has potent immunosuppressive, antimicrobial, and antitumor properties. It binds intracellularly to the immunophilin FK506 binding proteins (e.g., FKBP12 and FKBP52, a family of chaperone proteins with peptidyl prolyl-isomerase activity) and targets the protein kinase mammalian target of rapamycin (mTOR), also known as FRAP, RAFT and RAPT (**Brown, 1994**).

There are several pharmacological activities of sirolimus that hold particular relevance to neurodegenerative disorders. For example, the inhibition of phosphorylation of mTOR by sirolimus-FKBP12 activates autophagy. Studies of inclusion body formation and clearance of aggregate-prone proteins using chemical activators/inhibitors of autophagy, including sirolimus, as well as the knockdown of different autophagic genes, implicate this cellular process in the etiology of neurodegenerative diseases involving protein aggregation, including Parkinson's, Huntington's, and Alzheimer's disease (**Rubinsztein, 2006; Webb, 2003**). Sirolimus and its proautophagic analogs protect against neurodegeneration in cell, fly and mouse models of neurodegenerative proteinopathies (**Ravikumar, 2002**). Sirolimus also protects cells against pro-apoptotic insults by enhancing the clearance of mitochondria by autophagy, thereby reducing cytosolic cytochrome C release and downstream caspase activation (**Ravikumar, 2006**). Thus, sirolimus (pro-autophagic) treatment may be useful in neurodegenerative diseases where a slow but increased rate of apoptosis is evident, even if they are not associated with overt aggregate formation. It has also been reported that suppression of basal autophagy in neural cells causes neurodegenerative disease in mice (**Hara, 2006**). However, it appears unlikely that

II. Rationale (cont.)

sirolimus mediates its neuroprotective effects solely *via* autophagy, as mTOR has a significant function in several additional cellular processes. For example, it has also been shown that mTOR is involved in the control of protein synthesis, and in a cellular model of Huntington's Disease, sirolimus significantly decreased aggregation-prone polyglutamine (polyQ) proteins and expanded huntingtin inclusion bodies in both autophagy-proficient and autophagy-deficient cells (**King, 2008**). In addition to altering protein synthesis *via* mTOR, binding of sirolimus to peptidyl prolyl-isomerases inhibits their rotamase activity. Aggregation of α -synuclein is stimulated by addition of FKBP12 peptidyl prolyl isomerases, suggesting that the rotamase activity of peptidyl-prolyl isomerases accelerates the folding and subsequent aggregation of α -synuclein into inclusion bodies. It has also been shown that the peptidyl prolyl-isomerase Pin1 accumulates in Lewy bodies of Parkinson disease and facilitates formation of α -synuclein inclusions (**Ryo, 2006**). Sirolimus and other FKBP inhibitors are known to inhibit rotamase activity as well as α -synuclein aggregation, a finding further supporting their therapeutic utility in proteinopathies. Consistent data have also recently shown the important role of mTOR signaling pathway in the regulation of crucial metabolic and mitotic functions of cells, and because of these effects, it has been and continues to be evaluated in clinical trials for cancer (**Sabatini, 2006**). Sirolimus also interferes with the activation and proliferation of T-lymphocytes leading to potent immunosuppression. The early recognition of the utility of the immunosuppressive activities of sirolimus for organ transplantation led to its successful development as a valuable pharmaceutical product. Finally, sirolimus has been found to have neurotrophic effects in cultures of neurons, and nonimmunosuppressive sirolimus analogs promote neuroregeneration and protect against cellular toxicity of MPP+ and 6-OHDA in an FKBP12-independent fashion (**Guo, 2001; Hamilton, 1997**). Thus, formulations of sirolimus, when administered directly into the brain, improve both cell survival and fiber outgrowth of dopaminergic grafts (**Alemdar, 2004**).

Since drugs that bind to immunophilin-binding proteins can attenuate the detrimental effects of proteinopathies and promote nerve growth *in vitro* and *in vivo* independent of their immunosuppressive actions, compounds in this class have been considered as potential therapeutic agents for Parkinson's disease. A placebo-controlled phase II clinical trial of the immunophilin analog GPI-1485 (800 or 4,000 mg daily) in patients with mild to moderate Parkinson's disease showed this agent was well tolerated, with only a higher incidence of nausea and indigestion in those subjects taking the higher dosage. Furthermore, there was a trend toward a favorable impact on imaging measures, although there were no clear benefits on clinical measures (**Poulter, 2004**). GPI-1485 was subsequently selected for inclusion in a futility study to determine whether it is worthwhile to evaluate the possible disease-modifying effects of these compounds in future phase III trials. GPI-1485 was administered as 250 mg tablets with four tablets being taken four times daily. The study suggested that GPI-1485 should be considered in phase II clinical trials, but it was noted that the conclusion was dependent on the data used to construct the futility threshold (**NET-PD, 2007**). Thus, there are mechanistic, preclinical and clinical data that support the use of sirolimus or other immunophilin binding drugs in the treatment of Parkinson's disease and other neurological disorders.

II. Rationale (cont.)

IIb. Consistency

n/a

III. Efficacy (Animal Models of Parkinson's Disease)

IIIa. Animal Models: Rodent

Available efficacy data for immunophilin binding drugs in Parkinson's animal models are limited to studies involving analogs derived from tacrolimus (FK506) and sirolimus. This is presumably due to the desire to avoid the immunosuppressant activities of these prototypical immunophilin ligands, since both nonimmunosuppressant as well as immunosuppressive immunophilin ligands [e.g., 3-(3-pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinedinecarboxylate (GPI-1046)], have been shown to be neurotrophic in multiple neuronal systems and efficacious in promoting both morphologic and functional recovery in rodent models of peripheral nerve injury and neurodegenerative disorders. For example, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) studies in mice, concurrent or post-MPTP administration of GPI-1046 revealed enhanced striatal innervation density with significant augmentation evident at 4 mg/kg. Maximal effects were evident at 20 mg/kg with striatal innervation densities 2- to 3-fold higher than untreated MPTP/vehicle controls. In 6-OHDA-treated rats, drug treatment restored striatal dopamine to $\approx 30\%$ of control levels, and abolished the motor deficit produced in control animals (**Steiner, 1997**). Similar studies with GPI-1046 (**Zhang, 2001**) and additional immunophilin analogs have also shown similarly promising results (**Costantini, 2001**). Amide and thioester analogs of GP-1046 were as fully efficacious as previously reported immunophilin ligands in the MPTP model of Parkinson's disease, but exerted their regenerative effects at significantly lower doses (**Hamilton, 2002; Wu, 2002**). Other investigations demonstrated that FK506 and GPI-1046 treatment also significantly reduced lipid peroxidation *in vivo* and significantly increased striatal glutathione (GSH) levels by activating GSH synthesis (**Tanaka, 2002**). However, other laboratories examining FK506, sirolimus, GPI-1046 and other sirolimus analogs have failed to replicate these positive findings in MPTP and or 6-OHDA rodent studies (**Bocquet, 2001**). One possible explanation for the discrepancies in the animal studies examining utility of GPI-1046 and other immunophilins for the treatment of Parkinson's disease is the difference in the extent of the neurological lesion produced with the MPTP or 6-OHDA treatment.

IIIb. Animal Models: Non-human primates

Negative results have been reported in studies to assess whether oral administration of GPI 1046 could prevent the structural and functional consequences of MPTP administration in nonhuman primates. In one such study, 25 rhesus monkeys received daily oral administration of vehicle or one of four doses of GPI 1046. Two weeks later, all monkeys received a unilateral intracarotid injection of MPTP-HCl (3 mg). Daily drug administration continued for 6 weeks, after which time the monkeys were sacrificed. All groups of monkeys displayed similar deficits on behavioral measures and similar losses of tyrosine hydroxylase-immunoreactive nigral neurons indicating that treatment failed to have neuroprotective effects (**Emborg, 2001**). In 2002, the lack of efficacy of GPI-1046 was shown in MPTP-treated monkeys using *in vivo* brain imaging techniques,

suggesting that there may be species differences with respect to the neurotrophic effects of GPI-1046 on nigrostriatal DA neurons (**Eberling, 2002**).

IV. Efficacy (Clinical and Epidemiological Evidence)

IVa. Clinical Studies

The first clinical studies of an immunophilin-binding drug derived from tacrolimus or sirolimus in the treatment of Parkinson's disease used a Guilford pharmaceutical (GPI-1485) in low dose (800 mg daily; n=100) or high dose (4000 mg daily; n=99) treatment regimens and were completed in April 2002. The results, like those of non-human primate trials, were mixed. Using a patient population having mild to moderate symptoms (where significant substantia nigra neuronal loss has occurred), the preliminary results of the trial have demonstrated that GPI-1485 is well tolerated, but does not produce a substantial reversal of the motor symptoms of Parkinson's disease after 6 months of treatment (when the trial ended). In some patients, similar to the non-human primate trials, some improvement in motor skills was seen but overall these effects were not statistically significant (**Marshall, 2004**). This study also followed changes in striatal dopamine by evaluating dopamine transporter density in a subgroup of patients (n=105). This was measured using SPECT scanning with [¹²³I]β-CIT (DOPASCAN injection). Since dopamine transporters are located in nerve terminals, measuring transporter density measures dopaminergic innervation in the striatum. Parkinson's disease patients lose about 5%–6% of their dopamine transporters per year, thus a slowing of this loss (or reversal) would be seen a positive endpoint of this trial. However, a reduction of only 0.15% in placebo patients was found, much less than that predicted, whereas in the high dose treatment group 2.5% increase was documented. However, despite these results “looking” very promising they did not reach statistical significance (**Guilford Pharmaceuticals, 2001**). However there were enough positive aspects of this study that combined with the wealth of pre-clinical (animal data), a new clinical trial was begun by Guilford this time for 2 years on 200 patients with less severe symptoms. As well, the National Institutes of Health has now under way a study of six novel neuroprotective therapies for PD, including GPI-1485. In both trials it is hoped that the longer duration (2 years) may reveal neuroprotective effects that were statistically undetectable after 6 months in the earlier study.

In November 2003 GPI-1485 entered Phase II clinical trials for treatment of nerve damage in patients having undergone radical prostatectomy (**Guilford Pharmaceuticals, 2003**). This was based on an initial study in which Guilford conducted a series of experiments using GPI-1485 (**Guilford Pharmaceuticals, 2002**); in the same model of peripheral nerve injury (**Sezen, 2001**). At 24 h after peripheral nerve injury GPI-1485 produced a significant neuroprotective effect, preventing cavernous nerve degeneration and preserving intracavernous pressure and erectile function at greater than 90% of normal levels. Unlike the FK506 study, the Guilford study also demonstrates that when oral treatment with GPI-1485 is delayed up to 7 days after the peripheral nerve injury procedure, the treatment promotes significant restoration of function, restoring intracavernosal pressure to 90% to 100% of normal levels as measured at 1 month after injury.

IVb. Epidemiological Evidence

Acquired non-Wilsonian hepatic-cerebral degeneration may manifest as ataxia, tremor, chorea, parkinsonism, myoclonus, and dystonia. In one particular case noted in 2003, a 70-year-old man presented with a 15-month history of progressive tremor (kinetic resting). He began to drag his feet, had difficulty getting up from a chair, and required assistance in daily activities. Six months before his initial neurologic evaluation, he developed gait imbalance. Cirrhosis and liver failure were diagnosed 3 years earlier. A diagnosis of parkinsonism complicated by a prominent kinetic tremor was made. Three months after carbidopa/levodopa treatment began, the patient underwent successful hepatic transplantation. Two months later, he was taking tacrolimus, ceftazidime, ciprofloxacin, insulin, methylprednisolone, omeprazole, cisapride, levothyroxine, and carbidopa/levodopa. He reported that carbidopa/levodopa had not been effective. However, since the liver transplantation, he noticed improvement in his motor function, although tremors and gait continued to be a problem. Twenty-one months after transplantation, he reported no deterioration of his condition on a reduced carbidopa/levodopa dosage. Motor examination revealed normal tone, no resting or postural tremor, and a mild kinetic tremor of the upper extremities. His posture was erect, and gait was wide based but normal speed. One hypothesis for the reversible parkinsonian syndrome, in this patient, may be related to neuroregenerative effects of tacrolimus (**Shulman, 2003**).

V. Relevance to Other Neurodegenerative Diseases

The ability of sirolimus and other compounds to bind to the FKBP12 peptidyl-prolyl isomerase, inhibit mTOR, and promote autophagy and neuroregeneration may be useful for treatment of several other neurodegenerative diseases and age-related senility. This may be particularly true for neurodegenerative diseases that are characterized by intracellular, aggregate-prone proteins, such as expanded Huntington in Huntington's disease and mutant tau in fronto-temporal dementia. In Alzheimer's disease, activation of the mTOR pathway has been reported to increase Tau protein and contribute to disease progression. Treatment of aged mice with GPI-1046 resulted in a reversal of age-related decrease in cholinergic cell volume from 34% to 13% (**Sauer, 1999**), and it has been shown that FK506 inhibits the formation of amyloid precursor protein which is implicated in the apoptotic cell death associated with Alzheimer's (**Lee, 2000**). To what extent the inhibition of APP synthesis acts through the FKBP-12 versus some other peptidyl prolyl isomerase or other mechanism was not tested in this study. For example, in a related study FK506 and V-10,367 were shown to be neuroprotective in a model of oxidative stress, and this activity was dependent on de novo synthesis of cellular protein since actinomycin D, a drug that inhibits protein synthesis, prevented the neuroprotection (**Klettner, 2001**). Furthermore, it was shown that there was no up-regulation of the FKBP proteins 25, 38 or 52, or any genes known to be related to apoptotic cell death (caspase 3, bax, bcl-2 and bcl-xL). In later studies, it was shown that FK506 and V-10,367 rapidly induced the expression of Hsp70 and Hsp27, but not Hsp90. Moreover, their neuroprotective actions could be completely blocked by quercetin, a functional inhibitor of heat shock proteins (**Klettner, 2003**).

Tuberous sclerosis complex (TSC) represents one of the most common genetic causes of epilepsy. TSC gene inactivation leads to hyperactivation of the mammalian target of sirolimus signaling pathway, raising the possibility that mammalian target of sirolimus inhibitors might be effective in preventing or treating epilepsy in patients with TSC. Mice with conditional inactivation of the Tsc1 gene primarily in glia (Tsc1GFAPCKO mice) develop glial proliferation, progressive epilepsy, and premature death. In order to determine whether sirolimus could prevent or reverse epilepsy, as well as other cellular and molecular brain abnormalities in Tsc1GFAPCKO mice, Tsc1GFAPCKO mice and littermate control animals were treated with sirolimus or vehicle starting at postnatal day 14 (early treatment) or 6 weeks of age (late treatment), corresponding to times before and after onset of neurological abnormalities in Tsc1GFAPCKO mice. Mice were monitored for seizures by serial video-electroencephalogram and for long-term survival. Brains were examined histologically for astrogliosis and neuronal organization. Expression of phospho-S6 and other molecular markers correlating with epileptogenesis were measured by Western blotting. The results of the early treatment demonstrated that sirolimus prevented the development of epilepsy and premature death observed in vehicle-treated Tsc1GFAPCKO mice. Late treatment with sirolimus suppressed seizures and also prolonged survival in Tsc1GFAPCKO mice that had already developed epilepsy. Correspondingly, sirolimus inhibited the abnormal activation of the mammalian target of

sirolimus pathway, astrogliosis, and neuronal disorganization, and increased brain size in Tsc1GFAPCKO mice (**Zeng, 2008**). Similar results were reported in studies in mice where Tsc1 was ablated in most neurons during cortical development. Sirolimus and RAD001 [40-O-(2-hydroxyethyl)-sirolimus] were shown to improve median survival from 33 d to more than 100 d; behavior, phenotype, and weight gain were all also markedly improved. There is brain penetration of both drugs, with accumulation over time with repetitive treatment, and effective reduction of levels of phospho-S6, a downstream target of mTORC1. In addition, there is restoration of phospho-Akt and phospho-glycogen synthase kinase 3 levels in the treated mice, consistent with restoration of Akt function. Neurofilament abnormalities, myelination, and cell enlargement are all improved by the treatment. However, dysplastic neuronal features persist, and there are only modest changes in dendritic spine density and length. Strikingly, mice treated with sirolimus or RAD001 for 23 d only (postnatal days 7–30) displayed a persistent improvement in phenotype, with median survival of 78 d. In summary, sirolimus/RAD001 are highly effective therapies for this neuronal model of TSC, with benefit apparently attributable to effects on mTORC1 and Akt signaling and, consequently, cell size and myelination. Although caution is appropriate, the results suggest the possibility that sirolimus/RAD001 may have benefit in the treatment of TSC brain disease, including infantile spasms (**Meikle, 2008**).

VI. Pharmacokinetics

Via. General ADME

Sirolimus systemic bioavailability (F) is approximately 14%, and the drug shows dose proportionality with a maximal concentration (cMax) at about 1 hr. Sirolimus is widely distributed in tissues (volume of distribution = 19 L/kg) and partitions into blood cells compared with plasma, with blood/plasma ratios ranging from 36 in renal transplant recipients to 79 in healthy volunteers. The results of *in vitro* experiments using human liver microsomes suggest that cytochrome P450 3A4 is the major biotransformation pathway, generating inactive hydroxy, dihydroxy, hydroxydemethyl, didemethyl, 7-O-demethyl, and 41-O-demethyl metabolites. More than 90% of the drug/metabolites are eliminated in feces. Excretion in urine represents a minor route of elimination (2.2%). The half-life (t_{1/2}) of sirolimus is approximately 60 hr, albeit dose-independent, and shows the greatest interpatient variation, particularly among individuals with hepatic impairment (110 hr) or in the pediatric age group (as low as 10 hr). Because of this variability, therapeutic drug monitoring is recommended. High-fat meals slow the rate of but slightly increase the extent of sirolimus absorption (**Kahan, 2001**).

Vib. CNS Penetration

In a mouse pharmacokinetic study, a single dose of sirolimus given at 6 mg/kg intraperitoneally to mice aged P30–P45 led to substantial drug levels in plasma (~5000 ng/mL, liver (~5000 ng/g), and brain (~100 ng/g) at 1 h post dose administration. Brain levels remained markedly lower than systemic levels at all time points, consistent with an effect of the blood–brain barrier in reducing penetration into the CNS. Nonetheless, brain levels of each drug remained above the level required to inhibit mTORC1 (10 ng/mL when applied to cells *in vitro*) throughout the 48 h period after administration (sirolimus was at 38.6 ng/g; 48 h after drug administration). Brain levels 48 h after the last of 12 doses with sirolimus were 88.4 ng/g (**Meikle, 2008**).

Vic. Calculated log([brain]/[blood]) (Clark Model):

-1.62 (very low passive penetration of the blood-brain barrier)

VII. Safety, Tolerability, and Drug Interaction Potential

VIIa. Safety and Tolerability

Due to its mechanisms of action, there are significant implications with the use of sirolimus as a therapeutic agent, and the benefits of its use must be carefully weighed against its risks for serious adverse effects. Based upon information obtained from the use of Rapamune® (sirolimus) oral solutions and tablets, and described in the prescribing information (package insert) available from Wyeth Pharmaceuticals, sirolimus dose-related side effects include increased serum levels of cholesterol, triglycerides, and creatinine and decreased glomerular filtration rate. Hypertension, peripheral edema, rash, anemia, arthralgia (joint pain), diarrhea, constipation, headache, fever, urinary tract infection, pain, hypokalemia, leukopenia, and thrombocytopenia also may occur. Increased susceptibility to infection and the possible development of lymphoma and other malignancies may result from immunosuppression. Other adverse effects include excess mortality, graft loss, and hepatic artery thrombosis in liver transplant patients, bronchial anastomotic dehiscence in lung transplant patients, hypersensitivity reactions and exfoliative dermatitis

Sirolimus may affect response to vaccination. Therefore, during treatment with sirolimus, vaccination may be less effective. The use of live vaccines should be avoided; live vaccines may include, but are not limited to, the following: measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid.

The use of sirolimus should be avoided during pregnancy, lactation. The safety of sirolimus in children (<13) and geriatric patients (>65) has not been thoroughly evaluated.

VIIb. Drug Interaction Potential

Prominent interactions occur with other drugs that serve as substrates for CYP450 3A4 and P-glycoprotein (PGP) (**Kahan, 2001**). Sirolimus is known to be a substrate for both cytochrome P-450 3A4 (CYP3A4) and p-glycoprotein (P-gp). Inducers of CYP3A4 and P-gp may decrease sirolimus concentrations (e.g., rifampin, rifabutin, carbamazepine, phenobarbital, phenytoin, rifapentine, and St. John's Wort). Inhibitors of CYP3A4 and P-gp may increase sirolimus concentrations (e.g., ketoconazole, voriconazole, fluconazole, itraconazole, erythromycin, telithromycin, clarithromycin, bromocriptone, cimetidine, cisapride, clotrimazole, danazol, diltiazem, HIV-protease inhibitors (e.g., ritonavir, indinavir), metoclopramide, nifedipine, troleandomycin, and verapamil).

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