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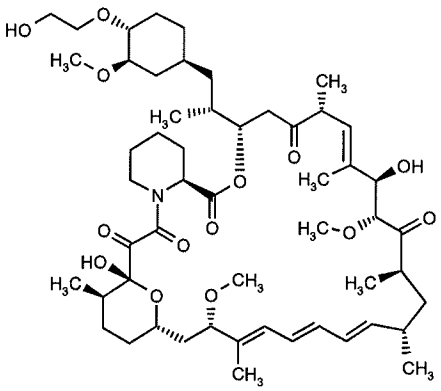
CCC: 0377-8282/99

SDZ-RAD

Immunosuppressant

[1*R*,9*S*,12*S*[1'*R*(1"*S*,3"*R*,4"*R*)],15*R*,18*R*,19*R*,21*R*,23*S*,30*S*,32*S*,35*R*]-1,18-Dihydroxy-12-[2-[4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16(*E*),24(*E*),26(*E*),28(*E*)-tetraene-2,3,10,14,20-pentaone

40-*O*-(2-Hydroxyethyl)rapamycin



$C_{53}H_{83}NO_{14}$ Mol wt: 958.2317

CAS: 159351-69-6

EN: 210424

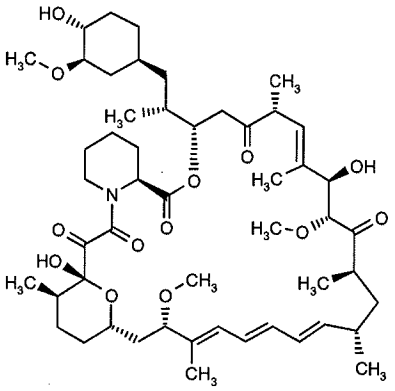
Synthesis

Alkylation of rapamycin (I) with 2-(*tert*-butyldimethylsilyloxy)ethyl triflate (II) by means of 2,6-lutidine in hot toluene gives the silylated target compound (III), which is deprotected by means of 1N HCl in methanol (1). Scheme 1.

Introduction

The macrolide rapamycin (now designated sirolimus) [I], a secondary metabolite of *Streptomyces hygroscopicus* originally described as an antifungal agent in the mid 1970s, was subsequently reported in 1989 to effectively suppress the rejection of transplanted allogenic solid organs in experimental animals (2, 3). In contrast to cyclosporine and FK-506, which act early after T cell activation by blocking transcriptional activation of early T cell-specific genes thereby inhibiting synthesis of T cell growth factors (*i.e.*, IL-2) which drive proliferation, rapamycin also suppresses proliferation at the late G_1 stage of the cell cycle. Thus, the proliferative signal provided by T cell growth factors is blocked and cells are unable to enter the S phase (4, 5). Furthermore, inhibition by rapamycin is not limited to IL-2-induced T cell proliferation since both hematopoietic and nonhematopoietic cell proliferation (*e.g.*, mast cells, fibroblasts and vascular smooth muscle cells [VSMC]) has been successfully blocked by this agent (6-9).

Rapamycin has been considered a potential candidate to prevent late graft loss resulting from graft vessel disease since the agent is capable of inhibiting proliferation of VSMC, thus avoiding intimal thickening responsible for vessel obstruction (10-12). Moreover, the nature of the differential modes of action described for cyclosporine and rapamycin has led to the discovery of synergistic interaction between the two agents, suggesting potential combination use of both for clinical transplantation (13-15). Although the efficacy of rapamycin has been



[I]

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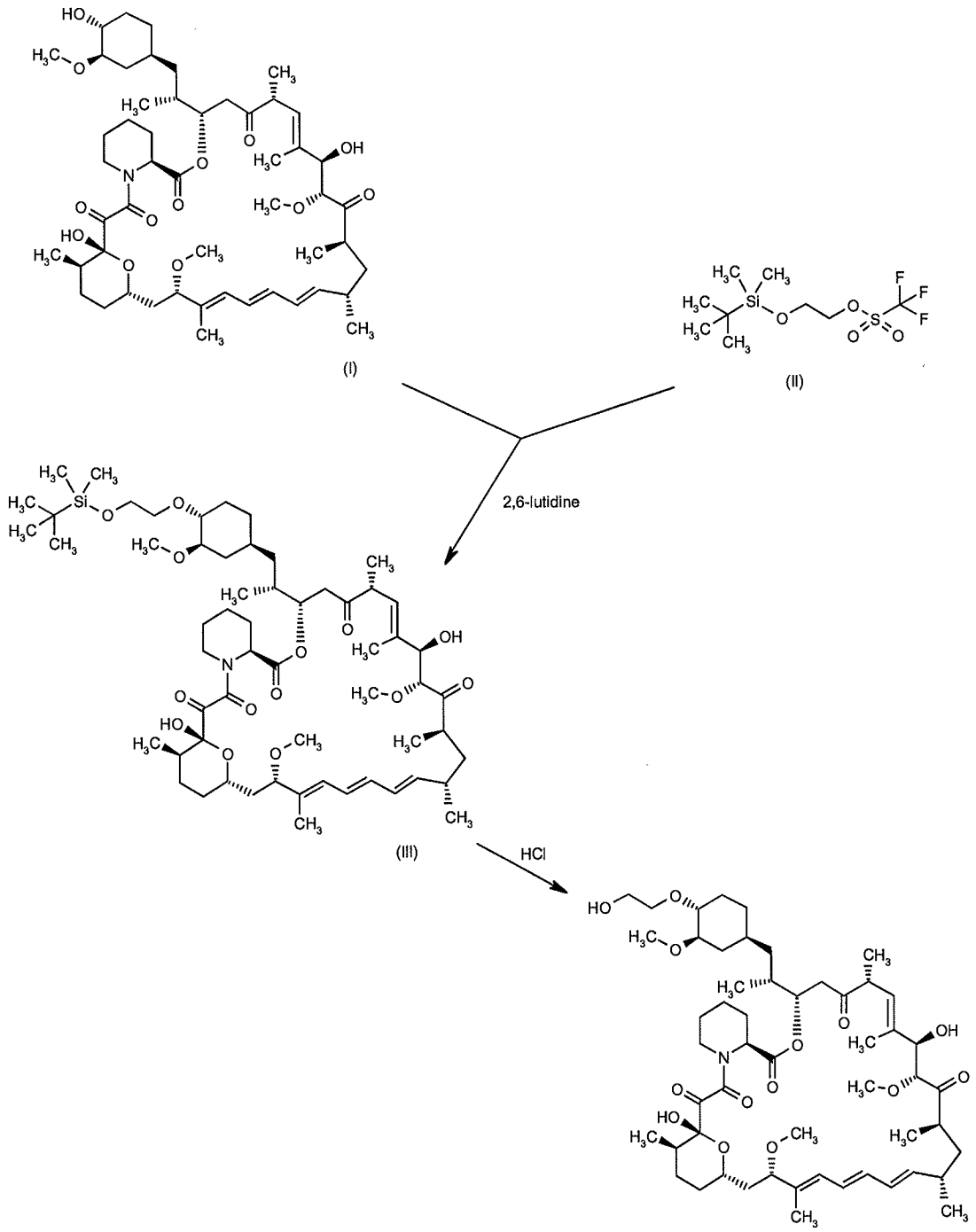
Scheme 1: Synthesis of SDZ-RAD



demonstrated after parenteral administration of the agent, difficulties have been encountered in the search for an effective oral formulation with good bioavailability and predictability (16-18). American Home Products is planning to submit an NDA for sirolimus as a treatment for immune-related anemia, using NanoCrystal technology from NanoSystems for delivery (19).

During the last years, intensive research efforts have focused on the design of new rapamycin analogs. According to the Prous Science Ensemble database, Abbott, American Home Products, Merck & Co., Novartis, Pfizer and SmithKline Beecham have been involved in the search for this type of compounds (Table I). SDZ-RAD is one such rapamycin analog that maintains the immunosuppressive activity and pharmacological properties of rapamycin. SDZ-RAD has been selected for further development for combination use with cyclosporine to prevent acute and chronic rejection following solid organ allotransplantation.

Table I: Recent patent literature on rapamycin analogs (from Prous Science Ensemble database).

Abbott	US 5373014
WO 9425022	US 5378836
WO 9514023	WO 9514696
American Home Products	WO 9514697
EP 475577	US 5385910
EP 470804	WO 9518133
US 5023264	WO 9504738
EP 467606	US 5391730
US 5100883	WO 9534565
EP 512754	US 5525610
EP 549727	WO 9616967
EP 515140	WO 9617845
EP 509795	WO 9809970
EP 516347	WO 9809972
US 5120727	**Merck & Co.**
EP 507556	US 5258389
US 5138051	US 5310903
US 5151413	**Novartis**
EP 514144	WO 9409010
WO 9305046	WO 9516691
US 5169851	WO 9641807
WO 9318043	**Pfizer**
US 5194447	WO 9221341
WO 9310122	WO 9606847
WO 9323422	**SmithKline Beecham**
US 5233036	WO 9214737
US 5260299	WO 9311130
WO 9404540	WO 9402136
WO 9411380	WO 9402137
WO 9410176	WO 9402485
EP 589703	WO 9410843
EP 593227	WO 9418206
US 5302600	WO 9418208
US 5344833	WO 9522972
WO 9528406	WO 9504060
WO 9425072	
WO 9425468	

Pharmacological Actions

SDZ-RAD was shown to have a differential mode of action as compared to cyclosporine A and FK-506 in that it inhibited growth factor-stimulated proliferation of a lymphoid cell line and VSMC. When compared to rapamycin *in vitro*, results showed that SDZ-RAD inhibition of IL-6-stimulated proliferation of a IL-6-dependent hybridoma clone (B12-29-15) was 2- to 3-fold less than that of rapamycin, with IC_{50} values of 0.2-1.4 nM and 0.07-0.5 nM, respectively. However, inhibition of proliferation of fetal calf serum (FCS)-induced proliferation of VSMC was similar for both agents (IC_{50} = 0.4-3.6 nM). Similarly, the suppressive activity of SDZ-RAD was 2- to 5-fold lower than rapamycin in the two-way mixed lymphocyte reaction using mouse spleen cells (BALB/c-CBA strain combination) (IC_{50} = 0.2-1.6 nM and 0.06-0.9 nM, respectively) and in studies using CD4-positive (helper type) human T cell clones specific for hemagglutinin peptide 307-319 derived from peripheral blood mononuclear cells (PBMC) from a healthy volunteer (IC_{50} = 0.05-0.17 nM and 0.014-0.037 nM, respectively) (20). In addition, synergistic activity was demonstrated between SDZ-RAD and cyclosporine following isobologram analysis of results from *in vitro* experiments using the same two-way mixed mouse lymphocyte reaction; results indicated an absolute index of synergy ranging between 0.3 and 0.7, while IC_{70} values of 21 and 0.3 nM were obtained for cyclosporine alone and SDZ-RAD alone, respectively (21). Synergism of SDZ-RAD (10 nM) and cyclosporine A (100 ng/ml) was also demonstrated using T cells derived from human healthy volunteers. While SDZ-RAD (0.1-100 nM) alone dose-dependently decreased anti-CD3-driven T cell proliferation, combination treatment produced an additive effect (22).

The effects of SDZ-RAD on T cell proliferation were investigated using 9 human renal allograft reciplents with stable graft function as PBMC donors. Patients receiving a combination of cyclosporine (trough levels of 100-150 ng/ml), methylprednisolone (< 12 mg/day) were administered SDZ-RAD (0.75, 2.5, 7.5 or 17.5 mg) or a placebo and blood was extracted at 0, 2, 6 and 10 h after treatment. Results showed that T cell proliferation was significantly decreased 2 and 6 h after SDZ-RAD administration with activity returning to normal after 10 h; a trend toward dose-dependent inhibition was observed although results were not statistically significant due to small sample size. No changes in T cell activity were observed in patients receiving the placebo (22).

In contrast to *in vitro* results, SDZ-RAD was found to be as effective as an immunosuppressant as rapamycin *in vivo* when administered orally in several rat allograft models including localized graft-*versus*-host reaction, autoimmune glomerulonephritis induced by mercuric chloride and orthotopic kidney or heart allotransplantation; effective doses ranged from 1-5 mg/kg/day (20). Furthermore, the synergistic action of microemulsions of SDZ-RAD and cyclosporine was demonstrated *in vivo* in rats in which orthotopic kidney or heterotopic heart allotransplantation were performed. While the minimal effective oral dose for long-term allograft survival was 5 mg/kg/day for cyclosporine and ≥ 5 mg/kg/day for SDZ-

RAD in the kidney and heart transplantations, respectively, combination therapy reduced effective doses to 1-2 mg/kg/day and 0.5-2.0 mg/kg/day for cyclosporine and SDZ-RAD, respectively (21).

SDZ-RAD (2.5 mg/kg/day by gavage) was also shown to be effective in the rat model of transplant arteriosclerosis in which rats were orthotopically transplanted with abdominal aortas exposed to 1, 4, 16 or 24 h of cold ischemia (4 °C); aortas were retrieved and analyzed after 2 months. The development of chronic rejection as indicated by intimal thickening, which increased with increased ischemic exposure, was significantly reduced in SDZ-RAD-treated animals (23, 24). Cyclosporine (12 mg/kg/day by gavage) administered alone was ineffective in reducing development of chronic rejection due to ischemic damage in similar experiments (25).

SDZ-RAD (2.5 mg/kg/day) and cyclosporine (5 mg/kg/day) administered independently for 60 days were both shown to significantly decrease the severity and incidence of transplant coronary artery disease in the genetically obese Zucker rat heart transplant model as compared to control rats. Results suggested that combination therapy may result in complete inhibition of transplant coronary artery disease in this model (26).

Studies have also demonstrated that combination therapy with cyclosporine (1.5 mg/kg/day s.c.) and SDZ-RAD (0.5 mg/kg/day p.o.) was effective in reducing chronic kidney allograft rejection in rats 16 weeks after orthotopical transplantation (27). Moreover, rat lung allograft rejection indicated by opacification was further reduced significantly with combination therapy of cyclosporine (2.5 or 7.5 mg/kg by gavage) and SDZ-RAD (2.5 mg/kg by gavage) as compared to transplanted animals receiving monotherapy with either of the agents (28).

SDZ-RAD (1.5 mg/kg p.o.) in combination with methylprednisolone (20 g p.o.) and cyclosporine (10 mg/kg p.o.) administered for 3 months was also shown to be effective in the porcine heterotopic bronchial allograft model. Luminal obstruction and complete epithelial recovery was observed in treated pigs transplanted subcutaneously with segments of terminal bronchi (29).

The efficacy of SDZ-RAD was also demonstrated in cynomolgus monkeys transplanted with lung or orthotopic kidney. In a dose-finding study in which contralaterally nephrectomized monkeys received orthotopic kidney allotransplants, SDZ-RAD (0.75, 1.5, 10 or 2.5 mg/kg/day p.o.) alone was found to be well tolerated and significantly prolonged life; longer survival was observed when SDZ-RAD was administered together with cyclosporine (10 mg/kg p.o.) (30). Moreover, rejection was completely prevented by combination administration of SDZ-RAD (0.3 mg/kg) and cyclosporine (150 mg/kg/days 1-7, 100 mg/kg/days 8-28) in animals with lung transplants; rejection continued in animals receiving monotherapy with either agent (31).

Pharmacokinetics and Metabolism

Pharmacokinetic studies using the rat model have reported similar AUC values after oral administration of SDZ-RAD (435, 1468, 6076 ng/h/ml) and rapamycin (228, 1104, 4071 ng/h/ml) with doses of 1.5, 5 and 15 mg/kg/day, respectively, for 28 days. The higher values obtained with SDZ-RAD were suggested to be due to increased bioavailability of the agent. In addition, there was no evidence of hydroxyethyl side chain cleavage of SDZ-RAD that would result in conversion to rapamycin (20).

Studies in which absorption was examined using human intestinal cell line (Caco-2) monolayers and an in situ single-pass rat perfusion model have demonstrated a 20-fold greater basolateral to apical transport of low μM concentrations of SDZ-RAD. Passive permeability for SDZ-RAD was found to be half that of rapamycin in both models (200 *vs.* 100 nm/sec in monolayers and 248 *vs.* 126 nm/sec in the rat model) (32).

Absorption and bioavailability of SDZ-RAD was also demonstrated in an intestinal first-pass metabolism study using rat jejunum. Following administration of 150 μg and 1.5 mg SDZ-RAD to rats, 50 and 30% of the parent compound, respectively, was concluded to be metabolized in the intestinal mucosa. Similar results were obtained with 150 μg rapamycin, although the higher dose of 1.5 mg resulted in only 1-14% of rapamycin metabolized by the intestine. In addition, systemic clearances of 6.2 and 3.0 ml/min were observed after intravenous administration of 1 mg/kg SDZ-RAD and rapamycin, respectively. AUC values for oral absorption of 1.5 mg/kg SDZ-RAD and rapamycin were 458 and 320 ng/ml/h, respectively, and oral absorption was determined to be 40% for SDZ-RAD as compared to 14% for rapamycin. Absolute bioavailability was calculated to be 11 and 6%, respectively (33).

Biotransformation studies using liquid chromatography coupled with mass-spectroscopic analysis of buffer samples from human liver microsomes incubated with [^{3}H]-SDZ-RAD (1, 10 and 20 μM) for 30 min revealed that the major metabolites of SDZ-RAD result from single hydroxylation and demethylation pathways. No conversion of SDZ-RAD to rapamycin was detected and 39-*O*-demethyl-RAD was identified as a metabolite (34). Other studies have identified 34-hydroxy-RAD, 34-hydroxy-RAD-dehydrate and 16-*O*-demethyl-RAD as metabolites of SDZ-RAD (35).

The pharmacokinetics of SDZ-RAD were examined in a randomized, double-blind, crossover study involving patients with and without cystic fibrosis with stable lung transplants. Patients received a single oral dose of 0.035 or 0.1 mg/kg SDZ-RAD followed by a 15 day washout period and a subsequent dose on day 16; patients were also receiving cyclosporine twice daily for a total daily dose of 225-800 mg and prednisone (up to 20 mg/day). There was a 3-fold difference in C_{max} and AUC values between high and low doses in patients without cystic fibrosis as compared to a 2-fold difference observed in patients with the disease. Cystic fibrosis patients also

Box 1: Safety and tolerability of single-dose SDZ-RAD in lung transplant recipients (39) [from Prous Science CSLine database].

Study Design	Single-center, randomized, double-blind clinical trial
Study Population	Lung transplant recipients with (n = 2) and without (n = 10) cystic fibrosis (CF)
Intervention Groups	SDZ-RAD (R) 0.035 mg/kg p.o. days 1 and 16 (2.5 mg total dose) + cyclosporine b.i.d. R 0.1 mg/kg p.o. days 1 and 16 (7.5 mg total dose) + cyclosporine b.i.d.
Adverse Events	[42% overall]; headache (17%), anemia (8%), granulocytopenia (8%), pneumonia (8%)
Results	Mean creatinine, cholesterol and platelet counts did not change appreciably from baseline Triglycerides (mg/dl) (% change): R7.5, –24.1 (CF), 18.1 (non-CF); R2.5, –4.0 (CF), 17.0 (non-CF) Leukocytes (10^9/l) (%change): R7.5, –12.5 (CF), –26.3 (non-CF); R2.5, 2.0 (CF), –9.3 (non-CF)
Conclusions	SDZ-RAD was well tolerated in stable lung transplant recipients with and without cystic fibrosis, with mild to moderate side effects

Box 2: Safety and tolerability of multiple-dose SDZ-RAD in stable renal transplant patients (40) [from Prous Science CSLine database].

Study Design	Single-center, randomized, double-blind, placebo-controlled clinical trial
Study Population	Three sequential groups of 8 patients with stable renal transplant receiving immunosuppression with prednisone and twice-daily cyclosporine
Intervention Groups	SDZ-RAD (R) 0.75 mg 1x/d x 4 weeks R2.5 mg 1x/d x 4 weeks R7.5 mg 1x/d x 4 weeks Placebo (P) x 4 weeks
Adverse Events	R0.75, back pain, seizures, renal carcinoma (n = 1) R7.5 pneumonia, multiple oral herpetic lesions, left leg pain (n = 3) At least 1 mild or moderate event: R0.75, 4/6; R2.5, 6/6; R7.5, 4/4; P, 6/6
Withdrawals [causes]	R7.5 [pneumonia], n = 1
Results	Mean creatinine, cholesterol and platelet counts did not change appreciably from baseline Triglycerides (mg/dl) (% change): R7.5, –24.1 (CF), 18.1 (non-CF); R2.5, –4.0 (CF), 17.0 (non-CF) Leukocytes (10^9/l) (%change): R7.5, –12.5 (CF), –26.3 (non-CF); R2.5, 2.0 (CF), –9.3 (non-CF)
Conclusions	SDZ-RAD administered for 4 weeks demonstrated good tolerability and dose-concentration linearity in stable renal transplant recipients

exhibited a delay in SDZ-RAD absorption and a reduction in systemic exposure. The pharmacokinetics of cyclosporine in patients without cystic fibrosis were unaffected by the doses of SDZ-RAD used and SDZ-RAD was concluded to be well tolerated (36).

Similar results were obtained in a double-blind study in which patients with stable renal transplants received ascending once-daily dosing of SDZ-RAD (0.75, 2.5 and 7.5 mg p.o.) for 4 weeks; patients were also receiving cyclosporine twice daily (trough levels of 150-300 ng/ml). The pharmacokinetics of SDZ-RAD were dose proportional with a slight potential for accumulation and steady state was achieved after 6-8 days. These results are in agreement with the 25 h reported half-life for SDZ-RAD (*vs.* 60 h for rapamycin). Slight reductions in cyclosporine C_{max} and AUC values were noted in patients administered 2.5 mg of SDZ-RAD (37).

A method to simultaneously quantify plasma SDZ-RAD and cyclosporine concentrations has been described which involves a combination of a solid-phase extraction step with an HPLC system coupled to an electrospray mass spectrometer. The sensitivity of detection of each agent was 0.05 µg/l with a range of recovery of 84.3-102.3% obtained for SDZ-RAD and 81.7-92.2% for cyclosporine. A rate of analysis of 4 samples/min was maintained for more than 500 samples (38).

Clinical Studies

SDZ-RAD treatment was determined to be well tolerated in a randomized, double-blind trial in which 12 lung transplant recipients with and without cystic fibrosis

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