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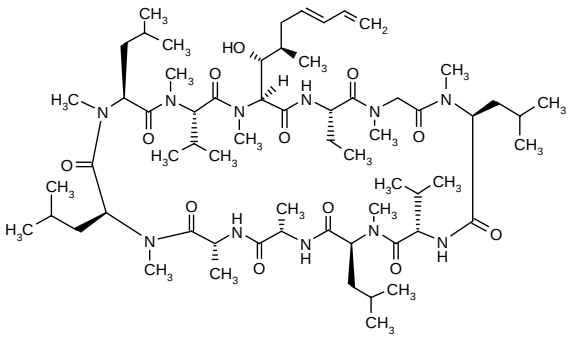
ISA-247

Calcineurin Inhibitor
Treatment of Renal Transplant Rejection
Treatment of Psoriasis

ISAtx-247
R-1524

Cyclo[[3(*R*)-hydroxy-4(*R*)-methyl-2(*S*)-(methylamino)-6(*E*),8-nonadienoyl]-L-(2-aminobutyryl)-*N*-methylglycyl-*N*-methyl-L-leucyl-L-valyl-*N*-methyl-L-leucyl-L-alanyl-D-alanyl-*N*-methyl-L-leucyl-*N*-methyl-L-leucyl-*N*-methyl-L-valyl]

6-[3(*R*)-Hydroxy-4(*R*)-methyl-2(*S*)-(methylamino)-6(*E*),8-nonadienoic acid]ciclosporin A



$C_{63}H_{111}N_{11}O_{12}$
Mol wt: 1214.635
CAS: 515814-01-4
EN: 281188

Abstract

Calcineurin inhibitors are widely used as immunosuppressants after organ transplantation, but currently available compounds are associated with toxic effects which limit their therapeutic potential. A novel calcineurin inhibitor, ISA-247 (ISAtx-247), is a more potent inhibitor than ciclosporin. *In vitro* and animal studies have demonstrated a 2-6-fold increase in potency for ISA-247 compared with ciclosporin for the inhibition of lymphocyte proliferation, cytokine production and the expression of T-cell surface antigens. Allograft survival following heart transplant in rats, renal transplant in monkeys and pancreatic islet transplant in mice was also significantly prolonged in recipients treated with ISA-247 compared with ciclosporin. Phase II studies in renal transplant patients have shown similar percent calcineurin inhibition at drug exposure levels approximately 3 times lower for ISA-247 than for ciclosporin. Post-transplant patients switched from ciclosporin to ISA-247 could be safely and effectively managed with this treatment, with no change in kidney function. A phase II study in psoriasis patients has also demonstrated the efficacy of ISA-247 in this indication, and phase III studies are now planned.

Synthesis

ISA-247 can be prepared by several related ways:

1) Acylation of ciclosporin (I) with Ac_2O and DMAP gives the acetoxy ciclosporin derivative (II), which is oxidized with O_3, $KMnO_4$, OsO_4 or $NaIO_4$ in acetonitrile to yield the carbaldehyde (III). The Wittig condensation of aldehyde (III) with either allyltriphenylphosphonium bromide (IV) by means of *t*-BuOK in THF or NaOH in toluene (1-3) or allyldiphenylphosphine oxide (V) by means of butyl lithium in THF (1, 3) provides the (*E*)-diene derivative (VI), which is finally deacylated by means of K_2CO_3 in methanol (1-3). Scheme 1.

2) Bromination of the acetoxy ciclosporin (II) with NBS and AIBN in refluxing CCl_4 yields the allyl bromide (VII), which is condensed with triphenylphosphine in toluene at 100 °C to afford the triphenylphosphonium bromide (VIII). Finally, bromide (VIII) is condensed with formaldehyde by means of NaOH followed by deacetylation with K_2CO_3 in MeOH (1-3). Scheme 2.

3) Condensation of aldehyde (III) with the silylated allylboronate (IX) in THF gives the silylated alcohol (X), which is submitted to a stereospecific elimination by means of concentrated H_2SO_4, and finally deacylated by means of K_2CO_3 in methanol (1, 3). Scheme 3.

Introduction

The enzyme calcineurin is involved in the activation of T-cells, and inhibitors of calcineurin such as ciclosporin and tacrolimus are widely used as immunosuppressants to prevent rejection after organ transplantation. However, these drugs are associated with significant side effects, particularly nephrotoxicity with ciclosporin, which may limit their therapeutic use (4-7). A novel calcineurin inhibitor, ISA-247 (ISAtx-247), has been developed which is a more potent inhibitor than ciclosporin. The compound

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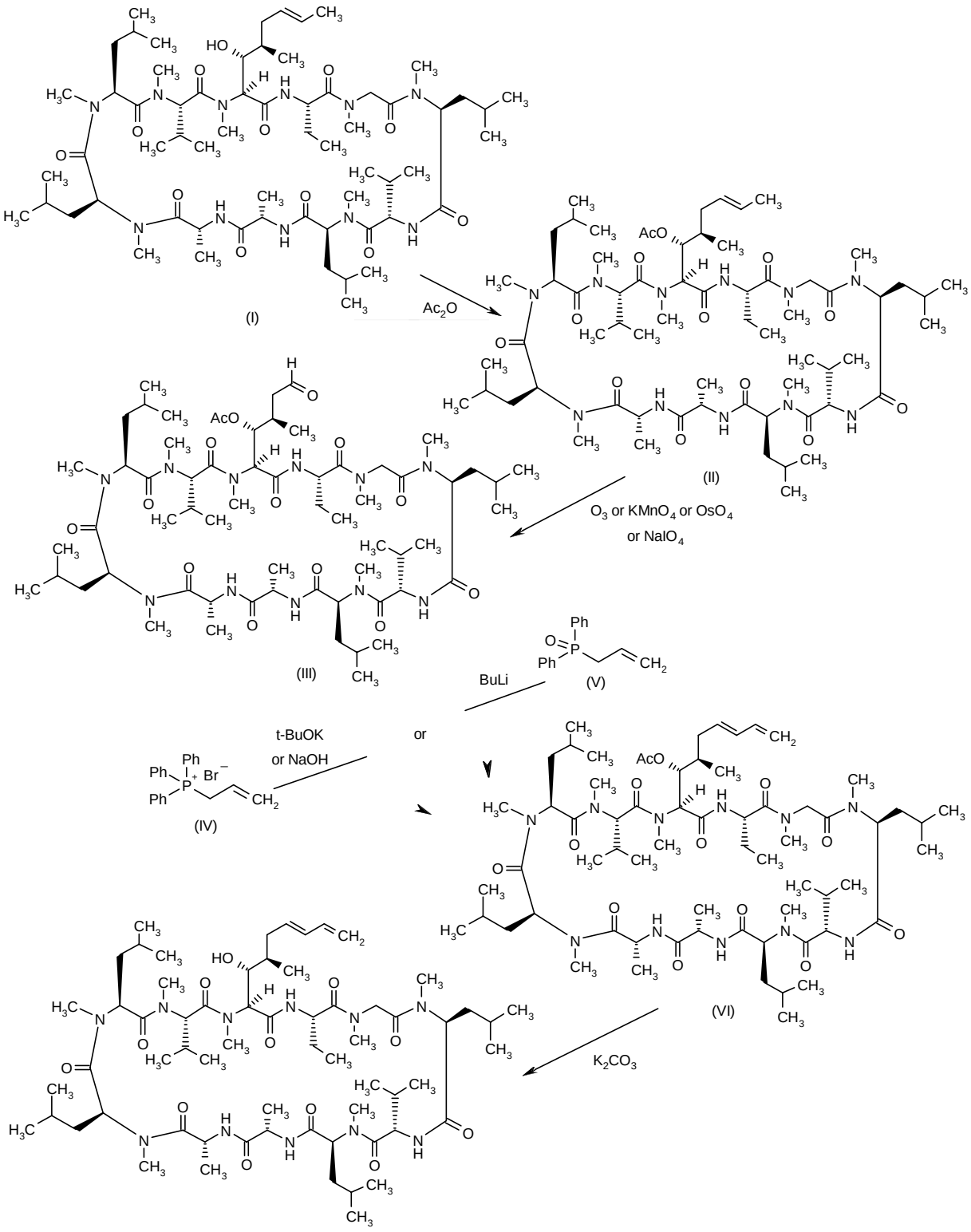
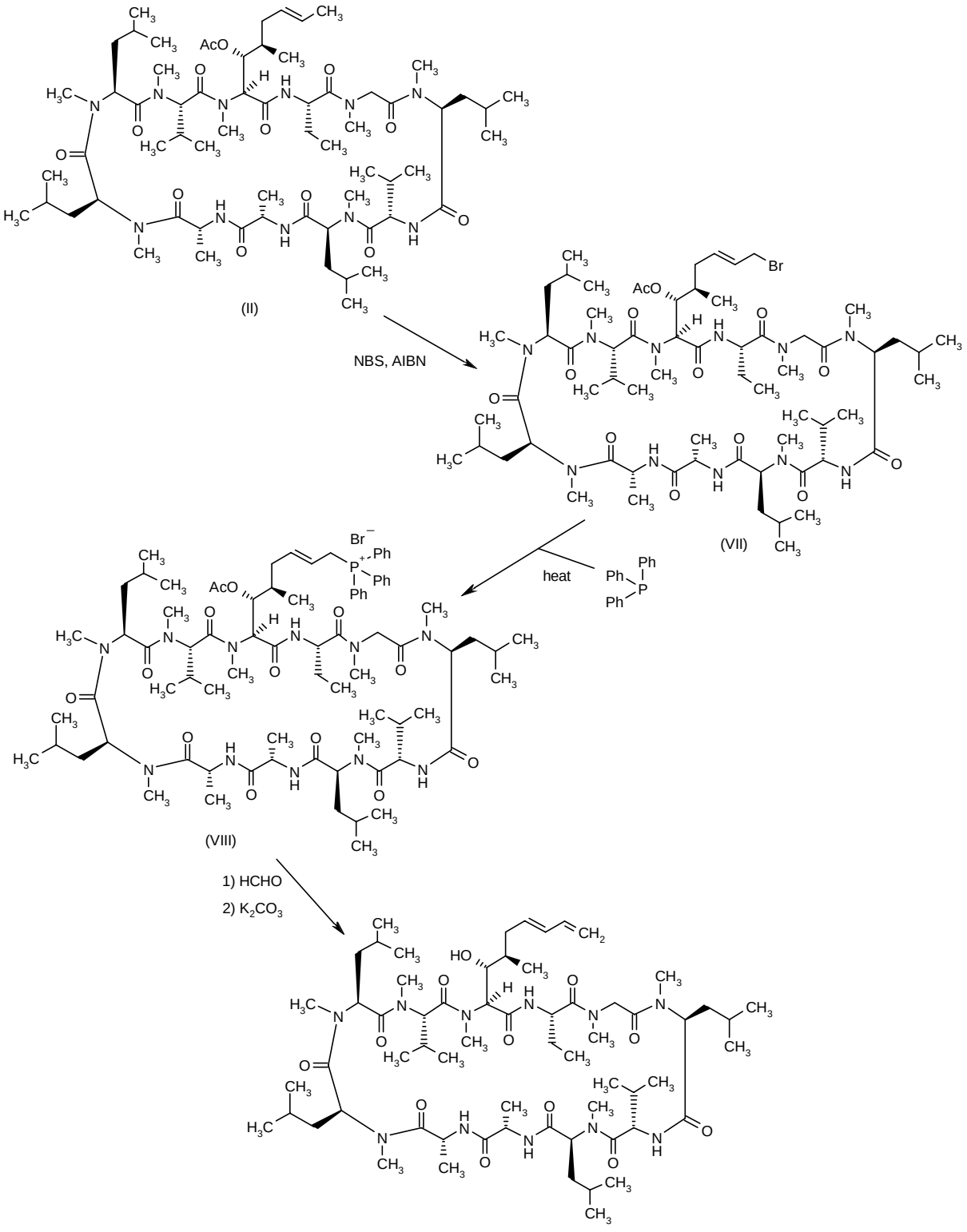
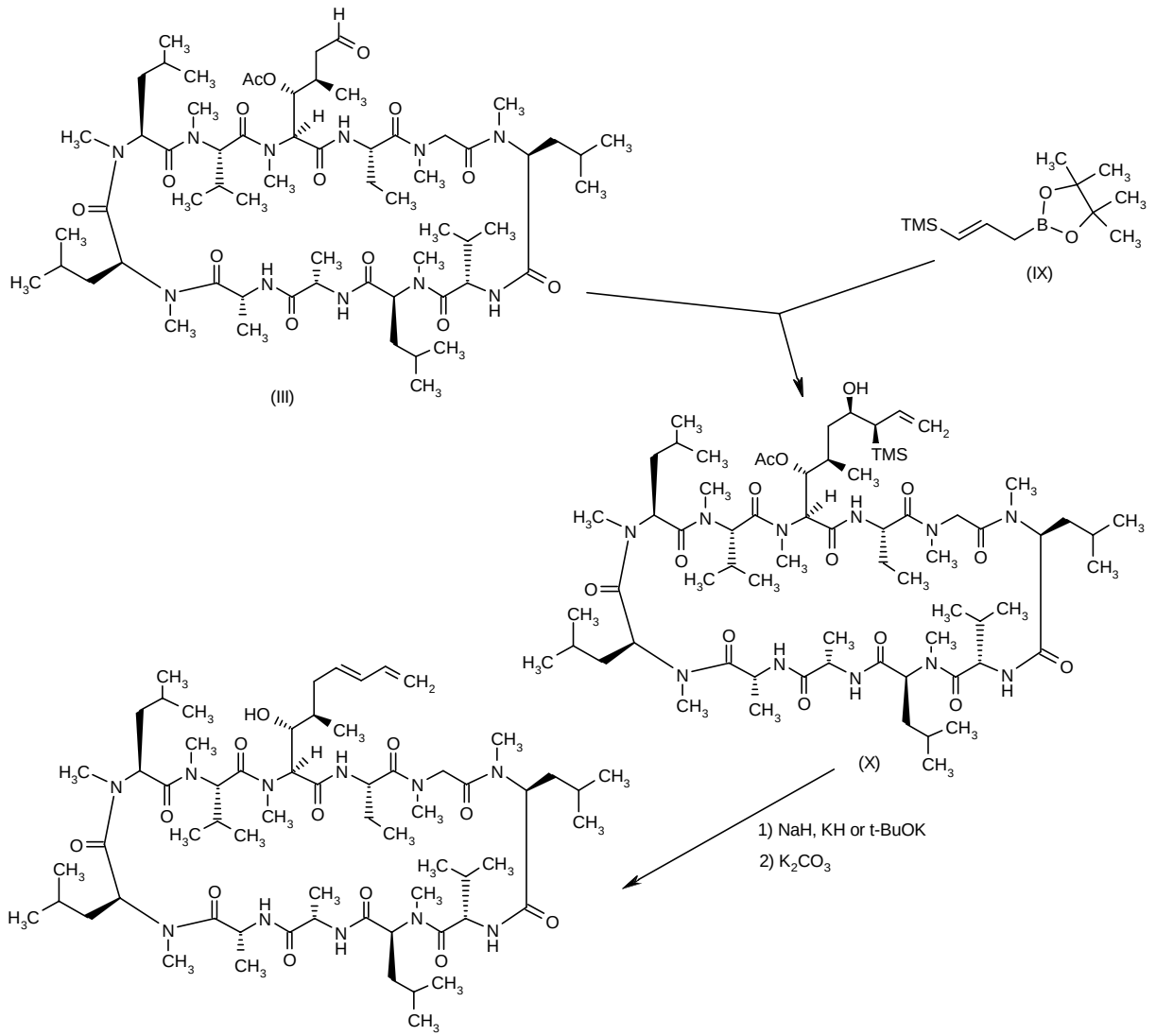
Scheme 1: Synthesis of ISA-247



Scheme 2: Synthesis of ISA-247



Scheme 3: Synthesis of ISA-247



has therapeutic potential in the management of autoimmune diseases and organ transplantation (8-12).

Pharmacological Actions

In a whole-blood calcineurin assay, the *in vitro* activity of ISA-247 (IC_{50} = 120 ng/ml) was > 2.5-fold more potent than that of ciclosporin (IC_{50} = 300 ng/ml). In a mixed-lymphocyte reaction (MLR), ISA-247 also proved to be twice as potent as ciclosporin in inhibiting proliferation (IC_{50} = 20 and 40 ng/ml, respectively), which was confirmed in a PHA-stimulated human lymphocyte proliferation assay. The *in vivo* efficacy of ISA-247 was evaluated in a heterotopic rat heart transplant model. There was a 3-fold prolongation in graft survival in rats administered ISA-247 compared with those administered ciclosporin at the equivalent dose of 1.75 mg/kg/day (56.5 days *vs.* 18.2 days). More than 80% of animals which received ciclosporin rejected their grafts during the 30-day period of dosing subsequent to transplantation, whereas only 20% of animals treated with ISA-247 rejected their grafts during the same period (8-12).

The efficacy of ISA-247 was evaluated in a model of established collagen-induced arthritis. Mice with established arthritis following type II collagen immunization were randomized to treatment with ISA-247 (125, 250 or 500 μg), ciclosporin (250 or 500 μg) or vehicle by i.p. injection for 10 days from the onset of clinical arthritis. There was a statistically significant, dose-dependent

reduction in the clinical severity of the disease by day 10 with all doses of ISA-247 compared with the control group. There were also significant improvements in paw swelling, synovial histology and articular cartilage damage scores in animals treated with ISA-247. Ciclosporin had no significant effect on either synovial inflammation or articular cartilage damage. ISA-247 was also the only treatment to significantly decrease the development of proximal interphalangeal joint erosions. ISA-247 was well tolerated in these studies, and no significant toxicity was observed (13).

The effects of ISA-247 have been studied *ex vivo* using lymphocytes from whole blood of cynomolgus monkeys. Whole blood was incubated with different concentrations of ISA-247 or ciclosporin, and flow cytometric analysis was used to assess immune function. Lymphocyte proliferation, cytokine production and the expression of T-cell surface antigens were suppressed 2.3-6 times more potently by ISA-247 than by ciclosporin (14).

The effects of ISA-247 on lymphocyte functions were examined *in vivo* in nonhuman primates. Groups of cynomolgus monkeys were treated twice daily for 7 days with ISA-247 25 or 50 mg/kg, or ciclosporin 25 mg/kg. Trough and peak drug levels were significantly higher for ciclosporin than for ISA-247, although lymphocyte proliferation was inhibited significantly more by ISA-247 at peak drug levels 3 h after dosing (80% compared with 65% for ciclosporin). There was also a similar or greater inhibition of the expression of T-cell activation surface antigens and cytokine production by ISA-247 compared with ciclosporin, despite lower blood levels. The expression of CD25 was most affected by ISA-247, with a maximal inhibition of 90%. There was a significant difference at 3 h between ISA-247 50 mg/kg and ciclosporin for CD25, CD71 and CD11a. These results indicated that ISA-247 was a more potent immunosuppressant *in vivo* overall than ciclosporin (15, 16).

The survival times of renal allografts in nonhuman primates treated with ciclosporin or ISA-247 have also been compared. Following heterotopic renal transplantation, cynomolgus monkeys were dosed twice daily with either ciclosporin or ISA-247, to maintain a 12-h trough concentration of approximately 150 ng/ml. Allografts in the monkeys treated with ISA-247 survived significantly longer than those in animals treated with ciclosporin; 50% of monkeys in the ISA-247 group survived at least 59 days post-transplant, whereas no monkeys in the ciclosporin group survived beyond 21 days. The average calcineurin inhibition at trough blood levels was 80% for ISA-247 compared with 48% for ciclosporin. This study also confirmed the increased potency of ISA-247 compared with ciclosporin at similar total drug exposure levels (17).

Pancreatic islets were transplanted beneath the renal capsule of diabetic mice and recipients were treated with ISA-247, ciclosporin or placebo i.p. for 30 days. Islet allograft survival was significantly prolonged in groups administered both 20 and 25 mg/kg/day ISA-247 compared with ciclosporin (20 mg/kg/day) or placebo (median survival of 27 and 50 days, respectively, compared with 20 days for ciclosporin). Normoglycemia was achieved in the ISA-247 groups without evidence of diabetogenicity. There was no evidence of nephrotoxicity (18).

Toxicity

Acute and chronic oral toxicity studies were performed in rats, rabbits and dogs. In all models, ISA-247 was associated with fewer side effects, particularly on the kidney, compared to ciclosporin. No renal toxicity (increase in serum creatinine, interstitial fibrosis) was seen in rabbits treated with ISA-247 at up to 15 mg/kg/day for 30 days, nor in rats treated with doses up to 80 mg/kg/day for 28 days. No morbidity or mortality was observed in rats or dogs treated with the drug for up to 13 weeks. Results suggested that ISA-247 is significantly less toxic than ciclosporin even at doses well in excess (up to 100-fold) of that required for immunosuppression (8-10, 19).

Pharmacokinetics and Metabolism

Pharmacokinetic data from rabbits administered the same (10 mg/kg/day p.o.) doses of ciclosporin and ISA-247 demonstrated similar exposure to both compounds. Data from rats and dogs indicated good oral absorption with a half-life of 6-8.8 h for all species, supporting twice-daily dosing. Absorption appeared to increase with chronic dosing. Rats, but not rabbits and dogs, showed higher drug exposure in males than females (8).

Phase I clinical trials in healthy volunteers assessed single and multiple doses of up to 6 mg/kg/day for 7 days. Pharmacokinetic data showed a significant correlation between trough levels (C_0) and area under the plasma concentration-time curve (AUC). Pharmacodynamic monitoring using a calcineurin inhibition assay demonstrated a mean inhibition of 36% at C_{max} in the 2 mg/kg/day group, which increased to 88% in the 4 mg/kg/day group. ISA-247 showed a minimum 2-3-fold potency increase for inhibition of calcineurin activity compared with ciclosporin. No significant adverse effects were noted at any dose level and no significant changes in creatinine clearance were observed (20).

Clinical Studies

A multicenter, randomized, open-label phase II study was conducted in renal transplant patients with stable renal function on ciclosporin (Neoral®). A total of 132 patients were randomized to either continue their ciclosporin treatment or switch to ISA-247 for 12 weeks. Doses were titrated using trough drug concentrations, with final mean doses being 0.65 mg/kg for ISA-247 and 1.1 mg/kg for ciclosporin. Values for C_{max} and AUC for ISA-247 were 171 ng/ml and 703 ng·h/ml, respectively, and corresponding values for ciclosporin were 650 ng/ml

and 2330 ng.h/ml. The reduced ISA-247 exposure was associated with comparable calcineurin inhibition (43% and 49% for ISA-247 and ciclosporin, respectively), demonstrating a 3-fold greater potency for ISA-247. ISA-247 trough levels directly correlated with calcineurin inhibition and AUC. Renal function as measured by calculated creatinine clearance remained stable in both groups, and the incidence of adverse events was similar, with gastrointestinal and nervous system disorders being the most frequently reported. The study demonstrated that kidney transplant patients were able to safely and effectively switch to ISA-247, with comparable immunosuppression achieved on ISA-247 at approximately one-third the drug exposure required for ciclosporin (21-24).

Another phase II study was performed in 201 patients with moderate to severe psoriasis and demonstrated that ISA-247 was well tolerated and effective in this indication. In this randomized, double-blind study, patients received ISA-247 at doses of 0.75 mg/kg (high dose) or 0.25 mg/kg (low dose), or placebo twice daily. In the high-dose group, 54% of patients achieved the primary endpoint of a 2-point reduction in patient Static Global Assessment scores, compared with 17% in the low-dose group and none in the placebo group. In the high-dose group, 74% of patients achieved a 75% reduction in the Psoriasis Area and Severity Index (PASI) scores, and 88% achieved a 50% reduction in PASI scores. The corresponding percentages for the low-dose group were 18% and 40%. There were no clinically significant changes in blood pressure, lipid levels or mean serum creatinine levels (25).

Single- and multiple-dose studies are also planned for the single-isomer compound, *trans*-ISA-247, a more bioavailable formulation than the *cis/trans* formulation currently in clinical development. Phase III trials in psoriasis and phase IIb trials in renal transplantation are anticipated to commence before the end of the year (26, 27).

Sources

Isotechnika, Inc. (CA); licensed to F. Hoffman-La Roche AG (CH).

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