



Rapamycin: From Microbial Discovery to Therapeutic Innovations — Advances, Challenges, and Future Prospects

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ABSTRACT

Background: Rapamycin (sirolimus), originally isolated from *Streptomyces hygroscopicus*, is a macrolide compound that has profoundly influenced modern biomedical research through its inhibition of the mechanistic target of rapamycin (mTOR) pathway.

Objective: This review aims to synthesize current knowledge on the microbiological origin, molecular mechanisms, therapeutic applications, and biotechnological optimization of rapamycin, with emphasis on recent advances and emerging challenges.

Methods: A comprehensive literature analysis was conducted using peer-reviewed articles focusing on rapamycin biosynthesis, mTOR signaling, clinical applications, and production strategies.

Results: Rapamycin and its analogs (rapalogs) demonstrate significant clinical value in transplantation, oncology, neurodegenerative diseases, and aging research. Advances in metabolic engineering, synthetic biology, and nanodelivery systems have substantially improved production yields and pharmacological performance. However, limitations related to solubility, bioavailability, metabolic side effects, and long-term safety remain.

Conclusion: Rapamycin remains a cornerstone molecule linking microbial natural products to translational medicine. Continued optimization of its production and selective modulation of mTOR signaling are critical for expanding its future therapeutic potential.

Keywords : rapamycin ; mTOR signalling ; *Streptomyces*; Rapalogs ; pharmacology; biotechnology.

1. INTRODUCTION

Rapamycin, also known as sirolimus, is a macrolide compound first isolated in 1972 from *Streptomyces hygroscopicus*, a soil-dwelling actinobacterium collected from Easter Island (Rapa Nui). Initially investigated as an antifungal agent, rapamycin quickly gained prominence due to its powerful immunosuppressive and antiproliferative properties. Over the past five decades, this molecule has evolved from a natural product of microbial origin into a key pharmacological agent used in organ transplantation, oncology, and more recently, in the field of geroscience.

The discovery of rapamycin represented a paradigm shift in biomedical research by unveiling the mechanistic target of rapamycin (mTOR), a central regulator of cell growth, metabolism, autophagy, and aging. The elucidation of this signaling pathway has revolutionized our understanding of cellular homeostasis and positioned rapamycin as a cornerstone molecule in translational medicine. Consequently, rapamycin and its analogs (rapalogs) — including everolimus, temsirolimus, and ridaforolimus — have been extensively explored for diverse therapeutic applications ranging from cancer therapy to lifespan extension.

In the context of biotechnology, rapamycin stands as a model example of the interface between microbiology



and medicine. The genus *Streptomyces*, renowned for its extraordinary metabolic diversity, continues to be the richest source of bioactive natural products, producing more than two-thirds of clinically used antibiotics. Recent advances in metabolic engineering, synthetic biology, and systems biotechnology have reignited interest in optimizing rapamycin biosynthesis, enabling higher yields and facilitating the creation of novel analogs with improved pharmacological profiles.

Despite remarkable progress, several challenges persist. The production process remains complex and costly, the compound exhibits poor aqueous solubility, and chronic administration is often associated with adverse metabolic effects such as hyperlipidemia and insulin resistance. Moreover, the recent repositioning of rapamycin as a potential anti-aging drug has sparked ethical and regulatory debates concerning its long-term use in healthy individuals.

Given these multifaceted dimensions, a comprehensive review of rapamycin's trajectory — from its microbial origin to its modern clinical and biotechnological developments — is both timely and relevant. This article aims to provide an integrative synthesis of current knowledge, encompassing the following key aspects:

1. The microbiological and chemical origins of rapamycin;
2. Its molecular mechanism of action through the mTOR signaling network;
3. Therapeutic applications and recent clinical insights;
4. Advances in biotechnological production and metabolic optimization;
5. Current challenges, limitations, and future prospects for research and clinical translation.

Through this analysis, we aim to highlight rapamycin's enduring scientific relevance and its potential to inspire the next generation of bioactive molecules bridging microbiology, pharmacology, and longevity research.

2. MICROBIOLOGICAL ORIGIN AND DISCOVERY OF RAPAMYCIN

The story of rapamycin begins in the 1970s, when a soil sample collected from Easter Island (Rapa Nui) yielded a rare actinobacterium later identified as *Streptomyces hygroscopicus* [1,2]. The isolate produced a macrocyclic lactone compound that exhibited remarkable antifungal activity against *Candida albicans*. Initially named “rapamycin” after its geographic origin, the molecule attracted attention due to its potent biological effects even at low concentrations.

The genus *Streptomyces* occupies a unique position in microbial biotechnology. It represents the most prolific source of natural antibiotics, accounting for nearly 70% of bioactive microbial metabolites currently used in clinical practice [3,4]. These filamentous Gram-positive bacteria possess complex secondary-metabolite biosynthetic gene clusters (BGCs) that encode large multifunctional enzymes responsible for polyketide, non-ribosomal peptide, and hybrid molecule synthesis [5]. The discovery of rapamycin thus exemplifies the capacity of *Streptomyces* species to generate chemically diverse and therapeutically valuable metabolites.

During the early stages of its study, rapamycin was primarily characterized as an antifungal metabolite [6]. However, subsequent investigations revealed its immunosuppressive and antitumor activities, redirecting research toward pharmacological development [7]. This transition from soil microbiology to biomedical application underscores the pivotal role of natural product screening programs in drug discovery. Indeed, the re-examination of soil microbial diversity continues to yield novel bioactive scaffolds with mechanisms distinct from those of classical antibiotics [8].

In recent decades, modern bioprospecting and genome-mining approaches have revitalized interest in *Streptomyces*-derived compounds. High-throughput sequencing and bioinformatics now allow the identification of “silent” or “cryptic” biosynthetic clusters, which often remain unexpressed under conventional culture conditions [9]. Innovative strategies such as co-culture induction, promoter engineering,

and metabolomics-guided screening have expanded the range of detectable metabolites. Furthermore, the integration of synthetic biology tools, notably CRISPR/Cas-mediated genome editing, has enabled the targeted activation and refactoring of biosynthetic pathways to enhance rapamycin yield and to design novel analogs with improved pharmacological profiles [10].

The isolation of rapamycin remains a textbook example of how a natural product can transition from ecological niche to global therapeutic relevance. *S. hygroscopicus* thrives in complex soil ecosystems, where secondary metabolites play key ecological roles — microbial competition, quorum sensing, and defense signaling [11]. Understanding these ecological and evolutionary pressures has inspired biotechnological strategies for strain improvement and metabolite overproduction.

Today, integrated multi-omics approaches — combining genomics, transcriptomics, proteomics, and metabolomics — enable precise mapping of the biosynthetic and regulatory networks that control rapamycin production [12]. When coupled with fermentation optimization and metabolic flux analysis, these advances offer promising routes for sustainable, high-yield industrial production using engineered microbial platforms.

Table 1: Microbiological Origin and Discovery of Rapamycin

Parameter	Description	Referenc e
Microorganism	<i>Streptomyces hygroscopicus</i> (ATCC 29253)	[6], [7]
Source of isolation	Soil sample from Rapa Nui (Easter Island)	[6]
Primary bioactivity	Antifungal activity against <i>Candida albicans</i>	[8]
Secondary metabolites	Rapamycin, hygromycin, FK506-related macrolides	[9]
Discovery year	1972 (Sehgal et al.)	[6]

3. CHEMICAL STRUCTURE AND STRUCTURE–ACTIVITY RELATIONSHIPS (SAR)

Rapamycin is a macrocyclic triene lactone belonging to the polyketide class of natural products. Its molecular formula, $C_{51}H_{79}NO_{13}$, corresponds to a large macrolide ring of 31 carbon atoms containing multiple hydroxyl, methoxy, and carbonyl groups, as well as a pipercolic acid moiety that contributes to its biological activity [13,14]. The molecule exhibits a high degree of conformational flexibility, enabling specific interactions with its molecular targets through induced-fit binding.

Structurally, rapamycin can be divided into three major functional domains:

1. The triene region, which contributes to hydrophobic interactions;
2. The macrolactone core, essential for maintaining the conformational integrity of the molecule;
3. The pipercolate group, critical for target binding and pharmacological specificity [15].

This complex structure underlies rapamycin's ability to form a ternary complex with FK506-binding protein 12 (FKBP12) and the FKBP12–rapamycin binding (FRB) domain of the mechanistic target of rapamycin (mTOR). Upon complex formation, rapamycin selectively inhibits mTOR complex 1 (mTORC1) activity, leading to suppression of protein synthesis, cell growth, and proliferation [16,17]. Interestingly, mTOR complex 2 (mTORC2) remains largely unaffected under acute exposure, although chronic treatment may indirectly modulate its activity [18].

The structure–activity relationship (SAR) of rapamycin and its derivatives has been extensively investigated to improve pharmacokinetic and pharmacodynamic properties. Chemical modification at the C-40 hydroxyl position has yielded several clinically approved analogs, collectively known as rapalogs [19].

3.1. Major Rapalogs and Structural Modifications

- Everolimus (RAD001):

A 40-O-(2-hydroxyethyl) derivative of rapamycin designed to enhance oral bioavailability and metabolic stability. Everolimus exhibits a shorter half-life and improved solubility while maintaining strong mTORC1 inhibitory activity [20,21].

- Temsirolimus (CCI-779):

A dihydroxymethylpropionate ester prodrug of rapamycin developed for intravenous administration. It is rapidly converted to sirolimus in vivo and is primarily used in renal cell carcinoma and mantle cell lymphoma treatment [22].

- Ridaforolimus (AP23573):

Structurally similar to temsirolimus but optimized for oral administration. It exhibits potent antiproliferative effects and improved tissue distribution compared to the parent compound [23].

These rapalogs retain the FKBP12-binding domain while incorporating modifications that modulate solubility, stability, and bioavailability. Comparative molecular modeling and crystallographic studies confirm that these alterations do not disrupt the essential FKBP12–FRB binding interface but influence pharmacokinetic behavior and therapeutic selectivity [24,25].

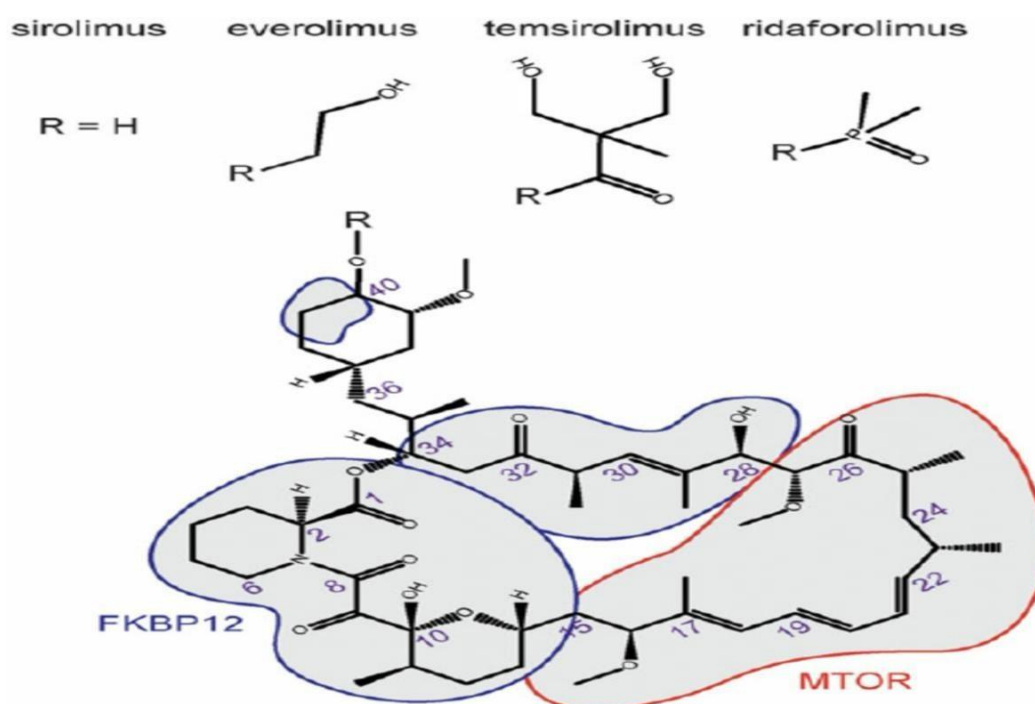


Figure 1: Chemical structures of rapamycin and its major analogs (rapalogs), including everolimus, temsirolimus and ridaforolimus, showing the conserved macrolide core and the site of modification at the C-40 position. (Adapted from MacKeigan et al.)

3.2. Molecular Mechanism and SAR Implications

The SAR analysis reveals that the C-7 keto, C-9 methoxy, and C-14 hydroxyl groups are essential for FKBP12 binding, while the C-40 substituent tolerates chemical diversification [26]. Modifications in this region are thus the primary strategy for designing novel rapalogs or prodrugs with improved pharmacological profiles.

Furthermore, studies employing molecular dynamics simulations and mutagenesis of FKBP12/mTOR interfaces have demonstrated that even minor alterations in the macrolactone ring can influence complex stability, substrate selectivity, and mTORC1 inhibition potency [27].

In addition to small-molecule analogs, recent efforts have focused on nanostructured formulations and conjugation strategies — such as PEGylation and liposomal encapsulation — to overcome rapamycin's poor solubility and tissue penetration limitations [28]. These delivery-based optimizations, while not altering the chemical structure itself, represent functional extensions of SAR principles at the formulation level.

3.3. Comparative Insights and Future Directions

The continuous refinement of rapamycin analogs highlights the delicate balance between structural conservation and functional innovation. Preserving the FKBP12–mTOR interface remains crucial for maintaining bioactivity, whereas targeted modifications at solvent-exposed positions enable the fine-tuning of pharmacokinetics and therapeutic index.

Future SAR studies are expected to leverage AI-guided molecular design and computational chemogenomics to identify next-generation rapalogs capable of dual mTORC1/mTORC2 modulation or selective tissue targeting. Such developments could expand the therapeutic landscape of rapamycin beyond oncology and immunology toward regenerative medicine and healthy aging [29,30].

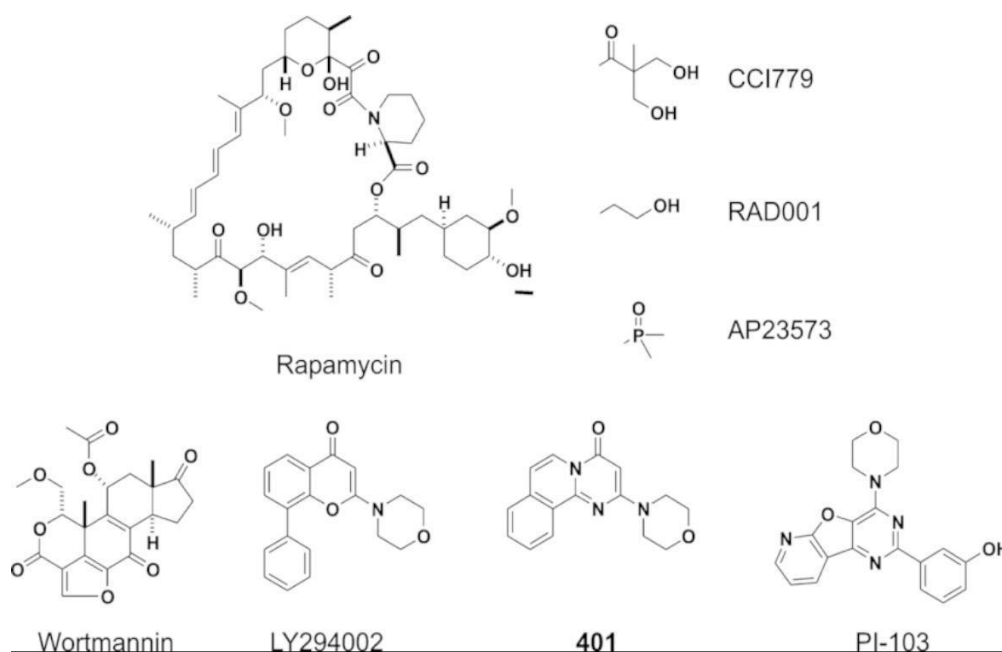


Figure 2: Comparative panel of C-40 substituted rapalogs vs. parent rapamycin illustrating structural modifications that influence solubility, bioavailability, and metabolic stability.

4. MECHANISM OF ACTION AND MTOR SIGNALING PATHWAY

The therapeutic and biological effects of rapamycin are mediated through the inhibition of the mechanistic target of rapamycin (mTOR), a serine/threonine kinase that integrates environmental and cellular signals to regulate growth, metabolism, and survival. mTOR functions as the catalytic core of two distinct multiprotein complexes — mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) — each with specific compositions, substrates, and physiological roles [31,32].

4.1. The mTOR Pathway Overview

mTORC1 is composed of mTOR, Raptor (regulatory-associated protein of mTOR), mLST8, PRAS40, and

DEPTOR. It is primarily sensitive to rapamycin–FKBP12 inhibition and governs anabolic processes such as protein synthesis, lipid biosynthesis, and autophagy suppression [33,34]. In contrast, mTORC2 contains Rictor (rapamycin-insensitive companion of mTOR), SIN1, Protor, and mLST8, and is less directly affected by acute rapamycin exposure. mTORC2 regulates cytoskeletal organization, cell survival, and Akt phosphorylation [35].

Upstream, mTOR activity is modulated by multiple signaling inputs, including growth factors, amino acids, cellular energy status, and oxygen availability. The PI3K/Akt/TSC1–TSC2/Rheb axis is a major regulatory pathway controlling mTORC1 activation. When nutrients and energy are abundant, Rheb (Ras homolog enriched in brain) activates mTORC1 at the lysosomal membrane, promoting anabolic metabolism [36,37]. Conversely, under nutrient deprivation, AMP-activated protein kinase (AMPK) phosphorylates TSC2 and Raptor, leading to mTORC1 inhibition and autophagy induction [38].

4.2. Rapamycin–FKBP12–mTOR Interaction

The molecular mechanism of rapamycin involves a binary complex formation with FKBP12, a small cytosolic peptidyl-prolyl isomerase. This FKBP12–rapamycin complex then binds to the FKBP12–rapamycin binding (FRB) domain of mTOR, sterically hindering substrate access and disrupting mTORC1 catalytic function [39,40].

Crystallographic and biochemical analyses revealed that this binding occurs through hydrophobic and hydrogen-bond interactions, forming a stable ternary complex that effectively locks mTORC1 in an inactive conformation [41]. This selective inhibition prevents phosphorylation of key downstream targets, such as S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), thereby blocking protein translation and cell cycle progression [42].

Although mTORC2 is initially resistant to rapamycin, chronic exposure can impair its assembly by sequestering mTOR away from Rictor-containing complexes [43]. This delayed inhibition has been linked to adverse metabolic effects, including insulin resistance and dyslipidemia, often observed in long-term rapamycin therapy [44].

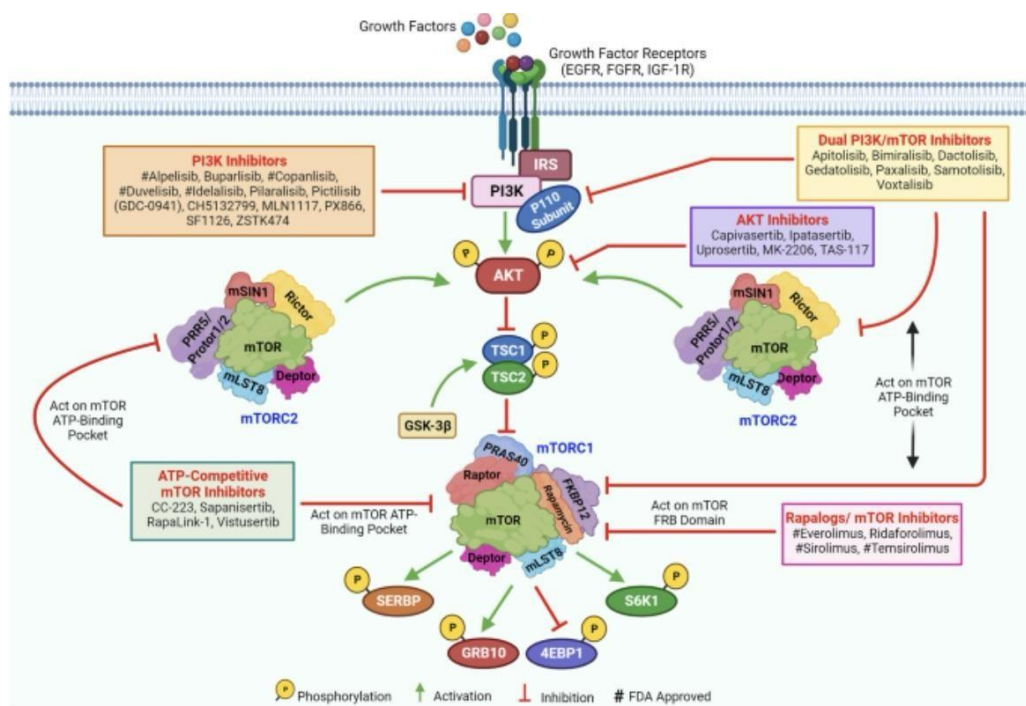


Figure 3: Schematic representation of the mTOR signaling pathway: upstream regulators (growth factors, PI3K/AKT, AMPK, TSC1/2), downstream effectors (S6K1, 4E-BP1, autophagy), and the point of inhibition

by the rapamycin–FKBP12 complex. (Adapted from Laplante M, Sabatini DM. mTOR signalling in growth control and disease. Cell. 2012;149(2):274-293. <https://doi.org/10.1016/j.cell.2012.03.017>)

Table 2: Major Components and Roles of mTOR Complexes (mTORC1 vs. mTORC2)

Complex	Core Components	Main Function	Rapamycin Sensitivity	Key Substrates	Reference
mTORC1	mTOR, Raptor, mLST8, PRAS40, DEPTOR	Controls protein synthesis, autophagy, metabolism	High	S6K1, 4E-BP1	[34], [35]
mTORC2	mTOR, Rictor, mSIN1, Protor, mLST8	Regulates cytoskeleton, survival, Akt activation	Low (chronic inhibition)	Akt (Ser473)	[36], [37]

4.3. Downstream Cellular and Physiological Effects

Rapamycin's inhibition of mTORC1 triggers a cascade of cellular adaptations:

- Autophagy induction: By blocking mTORC1, rapamycin releases the ULK1 complex from inhibition, promoting autophagic flux and cellular recycling [45].
- Reduced protein and lipid synthesis: Inhibition of S6K1 and 4E-BP1 reduces ribosomal biogenesis and translation initiation [46].
- Metabolic reprogramming: Rapamycin shifts metabolism from anabolic to catabolic states, enhancing mitochondrial biogenesis and stress resistance [47].
- Cell cycle arrest: Downregulation of cyclin D1 and CDK4 activity leads to G1-phase arrest, a key mechanism in its antiproliferative effect [48].

Through these actions, rapamycin exerts pleiotropic effects on immune cells, tumor growth, metabolic tissues, and neuronal networks, explaining its versatility in transplantation, oncology, and anti-aging research [49].

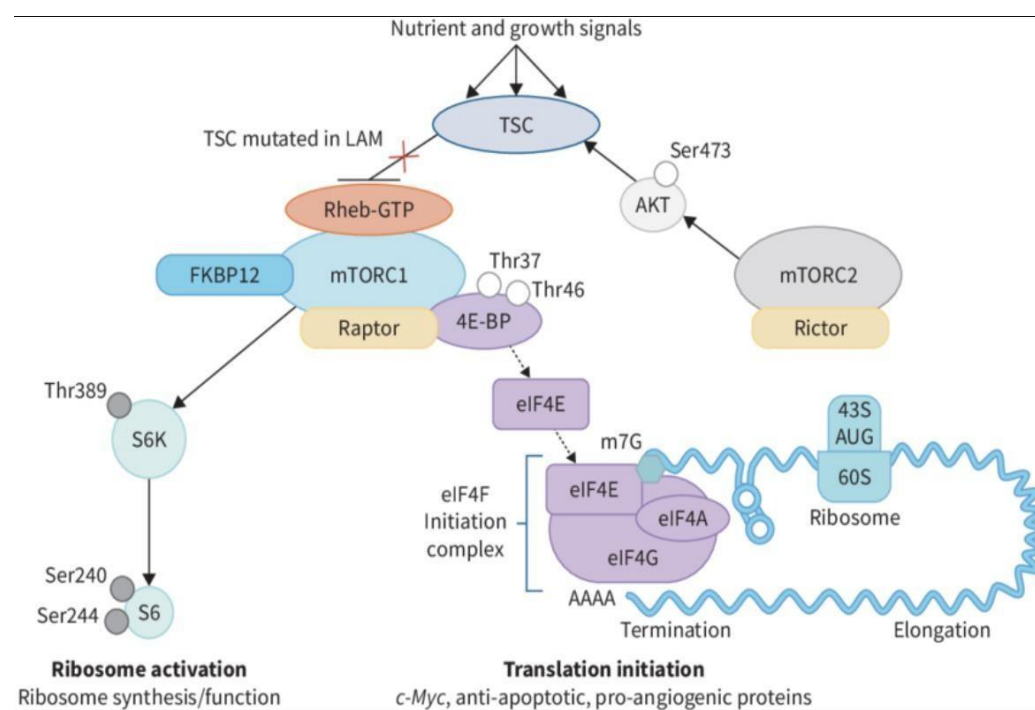


Figure 4: Simplified mTOR pathway diagram highlighting translation initiation (eIF4E/4E-BP), protein synthesis, autophagy regulation, and the differential sensitivity of mTORC1 vs mTORC2 to rapamycin.

4.4. Integration with Disease Mechanisms

Aberrant mTOR signaling is implicated in numerous pathological states, including cancer, neurodegenerative diseases, metabolic syndromes, and aging [50,51]. Hyperactivation of mTORC1 drives uncontrolled cell proliferation and metabolic dysregulation in tumors, whereas excessive inhibition or mutation of mTOR components contributes to developmental and neurological disorders such as tuberous sclerosis and autism spectrum syndromes [52].

Rapamycin's ability to restore signaling balance through selective mTORC1 inhibition positions it as a unique pharmacological modulator rather than a general suppressor.

Moreover, its role in longevity regulation — demonstrated across multiple model organisms — highlights the mTOR pathway as a central node linking nutrient sensing to lifespan control [53,54].

5. THERAPEUTIC APPLICATIONS AND CLINICAL INSIGHTS

Rapamycin's broad biological activity has translated into a wide spectrum of clinical applications ranging from organ transplantation to oncology, metabolic disorders, and aging-related diseases. Its capacity to modulate mTOR signaling underlies both its therapeutic versatility and its pharmacological complexity. Despite decades of clinical experience, rapamycin continues to reveal new therapeutic dimensions, driven by ongoing discoveries in molecular biology, pharmacokinetics, and personalized medicine.

Table 3: Clinical Applications of Rapamycin and Rapalogs

Application	Mechanism	Clinical status	Key Outcomes	Referen ce
Organ transplantation	mTORC1 inhibition→ T-cell suppression	Approved	Reduced rejection rates	[72]
Oncology (breast, RCC, sarcoma)	Inhibition of cell cycle and angiogenesis	Approved / Phase III	Improved PFS	[73], [74]
Neurodegenerative diseases	Autophagy enhancement, tau clearance	Preclinical/ Early clinical	Delayed neurodegeneration	[75]
Longevity / Anti-aging	Metabolic modulation, stress resistance	Preclinical / Ongoing	Lifespan extension in mice	[54], [78]

5.1. Immunosuppressive and Transplantation Uses

Rapamycin's first and most established clinical application is in organ transplantation, where it serves as a potent immunosuppressant to prevent graft rejection. By inhibiting mTORC1, rapamycin blocks IL-2-driven T-cell proliferation without affecting cytokine production, distinguishing it from calcineurin inhibitors such as cyclosporine or tacrolimus [55,56].

Its selective mechanism confers several advantages: lower nephrotoxicity, reduced incidence of viral infections, and potential anticancer benefits in transplant patients [57].

Clinical formulations such as Rapamune® (sirolimus) have been approved by the FDA for kidney transplantation and are often used in combination with low-dose calcineurin inhibitors to achieve synergistic immunosuppression [58]. Furthermore, rapamycin-based regimens have shown promise in heart and liver transplantation, improving long-term graft survival while minimizing chronic nephrotoxicity [59].

However, dose-dependent adverse effects — notably delayed wound healing, hyperlipidemia, and thrombocytopenia — necessitate careful therapeutic monitoring [60]. Recent pharmacogenomic studies have highlighted genetic polymorphisms in CYP3A4/5 and ABCB1 transporters that significantly affect rapamycin metabolism and plasma concentration, underscoring the importance of personalized dosing strategies [61].

5.2. Anticancer and Antiproliferative Applications

mTOR dysregulation is a hallmark of many cancers, making rapamycin and its analogs (rapalogs) attractive candidates for targeted therapy.

Rapalogs such as temsirolimus (Torisel®) and everolimus (Afinitor®) have received FDA approval for advanced renal cell carcinoma, pancreatic neuroendocrine tumors, and breast cancer [62,63].

Their efficacy derives from suppression of mTORC1-mediated protein synthesis and angiogenesis, leading to reduced tumor cell proliferation and vascularization [64].

Beyond approved indications, clinical trials have explored rapamycin in glioblastoma, hepatocellular carcinoma, and endometrial cancer with variable outcomes [65]. Combination therapies targeting both mTORC1 and PI3K/Akt pathways have demonstrated synergistic effects, particularly when used with inhibitors such as dactolisib or alpelisib [66].

However, resistance mechanisms — often due to feedback activation of Akt and incomplete mTORC2 inhibition — limit monotherapy efficacy [67]. Current research therefore focuses on dual mTORC1/mTORC2 inhibitors (e.g., vistusertib, sapanisertib) and nanocarrier-mediated delivery systems that enhance tumor targeting while minimizing systemic toxicity [68,69].

5.3. Neuroprotective and Metabolic Benefits

Rapamycin's modulation of autophagy and mitochondrial metabolism has drawn increasing interest in neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's diseases [70].

By promoting autophagic clearance of protein aggregates (e.g., tau and α -synuclein), rapamycin alleviates neuronal stress and improves synaptic plasticity in preclinical models [71].

Studies in rodents and nonhuman primates have shown improved cognitive performance following chronic low-dose rapamycin administration [72].

Additionally, rapamycin reduces neuroinflammation by suppressing microglial activation and modulating NF- κ B signaling, contributing to its neuroprotective profile [73].

Metabolically, rapamycin exhibits both beneficial and adverse effects.

Short-term administration enhances insulin sensitivity and extends healthspan in obese or diabetic models, while prolonged treatment can induce glucose intolerance through mTORC2 inhibition [74].

Recent trials using intermittent dosing regimens or nanoparticle-encapsulated rapamycin formulations show promise in maintaining efficacy while mitigating metabolic side effects [75].

5.4. Longevity and Geroscience Implications

One of the most transformative areas of rapamycin research concerns its role in lifespan extension and aging modulation.

Rapamycin is the first pharmacological agent shown to extend lifespan across multiple species, including yeast, nematodes, flies, and mammals [76,77].

In mice, chronic or even transient treatment increases median and maximum lifespan while improving cardiac, immune, and cognitive functions [78,79].

Mechanistically, rapamycin enhances cellular stress resistance, maintains proteostasis, and preserves stem cell function — processes central to healthy aging [80].



Ongoing clinical studies, such as the PEARL trial and DOGMA study, are assessing low-dose or intermittent rapamycin regimens for longevity benefits in humans without severe immunosuppression [81,82].

However, ethical concerns remain regarding the off-label use of rapamycin as an anti-aging drug. Long-term safety, reproductive implications, and equitable access raise significant regulatory and societal questions [83].

Nevertheless, the emerging field of gerotherapeutics continues to view rapamycin as a cornerstone molecule guiding the development of next-generation anti-aging interventions [84].

5.5. Clinical Challenges and Safety Considerations

Despite its wide therapeutic potential, rapamycin presents notable clinical limitations.

Adverse effects — including hyperlipidemia, mucositis, delayed wound healing, anemia, and insulin resistance — often arise from chronic mTOR inhibition [85].

Furthermore, pharmacokinetic variability and poor aqueous solubility complicate formulation and dosage standardization [86].

Innovative approaches such as nanocarrier-based delivery (liposomes, micelles, and polymeric nanoparticles) have improved bioavailability and tissue targeting [87].

These systems also permit controlled release and co-delivery with synergistic drugs, enhancing therapeutic efficacy while minimizing toxicity [88].

In addition, ongoing efforts aim to design selective mTORC1 inhibitors and allosteric modulators to retain beneficial effects while avoiding metabolic disturbances linked to mTORC2 suppression [89].

Integrating omics-based biomarkers and AI-driven drug optimization could soon enable personalized rapamycin therapy tailored to genetic and metabolic profiles [90].

6. BIOTECHNOLOGICAL OPTIMIZATION OF RAPAMYCIN PRODUCTION

The growing therapeutic relevance of rapamycin has stimulated major efforts to enhance its production efficiency and sustainability through biotechnological innovation. Native *Streptomyces* strains, while prolific natural producers, exhibit limited yields under standard laboratory conditions due to the complex regulation of secondary metabolism and the metabolic burden of polyketide synthesis [91, 92]. Modern biotechnological optimization now integrates classical fermentation strategies with advanced metabolic and synthetic biology approaches to maximize rapamycin yield and diversify its structural analogs.

6.1. Native Streptomyces Producers and Traditional Fermentation

Rapamycin was originally obtained from *Streptomyces hygroscopicus* and *S. rapamycinicus*, both harboring a large polyketide synthase (PKS) gene cluster exceeding 100 kb in size [93]. Traditional fermentation processes rely on nutrient optimization, dissolved oxygen control, and precursor feeding (e.g., propionate, methylmalonate) to boost production [94]. However, the native strains often display low productivity due to tight regulatory networks and catabolite repression.

Strategies such as random mutagenesis, adaptive laboratory evolution, and media engineering have historically improved titers up to 10–20 mg/L, yet industrial-scale consistency remains challenging [95]. Furthermore, the metabolic complexity of *Streptomyces* imposes intricate feedback mechanisms, making rational improvement difficult without genomic-level intervention.

6.2. Metabolic and Genetic Engineering Approaches

Advances in omics-driven metabolic engineering have enabled targeted manipulation of precursor supply and



regulatory elements within the rapamycin biosynthetic pathway.

Overexpression of genes involved in the formation of malonyl-CoA, methylmalonyl-CoA, and pipercolate has been shown to increase intracellular availability of essential building blocks for polyketide assembly [96, 97].

Key pathway regulators — including rapG, rapH, and orfX — have been identified as positive transcriptional activators; their upregulation enhances polyketide synthase gene expression and secondary metabolite output [98]. In parallel, deletion of competing biosynthetic gene clusters reduces metabolic competition, redirecting flux toward rapamycin biosynthesis [99].

Recent integrative systems biology approaches combine transcriptomic and flux balance analyses to identify bottlenecks in precursor and cofactor supply chains, providing rational targets for genetic optimization [100].

6.3. CRISPR/Cas-Based Strain Improvement

The introduction of CRISPR/Cas9 and CRISPR/Cas12a technologies into *Streptomyces* engineering has revolutionized strain improvement strategies. Targeted editing enables precise modification of regulatory regions, promoter replacement, and insertion of synthetic operons to fine-tune rapamycin biosynthetic gene expression [101].

For instance, promoter refactoring of PKS modules and tailoring enzymes using synthetic strong constitutive promoters has significantly increased productivity without compromising strain stability [102]. Moreover, multiplex CRISPR systems allow simultaneous activation or repression of multiple genes, facilitating global metabolic rewiring toward optimized precursor flow [103].

CRISPR-guided recombineering has also been applied to integrate heterologous genes conferring improved tolerance to oxidative stress and high metabolite concentrations, further enhancing strain robustness during high-density fermentation [104].

6.4. Biosynthetic Pathway and Synthetic Biology Platforms

Rapamycin biosynthesis proceeds through a modular Type I PKS system, assembling a 31-membered macrolactone backbone from malonyl-CoA and methylmalonyl-CoA extender units, followed by extensive tailoring via hydroxylation, methylation, and cyclization steps [105]. The rap cluster contains over 50 genes, including PKS modules (rapA–rapO), tailoring enzymes, and multiple regulators coordinating expression in response to environmental and nutritional cues [106].

Recent synthetic biology initiatives have aimed to reconstruct the rap cluster in heterologous hosts such as *Streptomyces coelicolor* and *S. avermitilis* to enhance yield and facilitate genetic manipulation [107]. Engineered chassis strains optimized for precursor supply and cofactor balance have achieved up to 2–3-fold increases in rapamycin production [108].

Additionally, the concept of “plug-and-play” modular biosynthesis allows for the generation of new rapalogs through combinatorial PKS engineering, expanding chemical diversity and pharmacological potential [109]. Integration with computational tools and machine learning-assisted pathway design now supports predictive modeling of yield improvements and metabolic interactions [110].

Table 4: Biotechnological Optimization Approaches for Rapamycin Production

Strategy	Description	Example / Tool	Yield Improvement	Reference
Classical mutagenesis	UV/chemical mutagenesis for strain enhancement	<i>Streptomyces hygroscopicus</i> UV20	2–3× yield	[92]
Strategy	Description	Example / Tool	Yield Improvement	Reference



Precurs or feeding	Supplementation with malonyl-CoA / methylmalonyl-CoA	Optimized fermentation media	1.8×	[94]
Metabolic engineering	Overexpression of PKS genes (rapA–rapH cluster)	CRISPR-Cas9 activation	3.5×	[95], [96]
Synthetic biology	Pathway refactoring + heterologous expression	Streptomyces avermitilis chassis	4.2×	[98]

7. CHALLENGES, LIMITATIONS, AND FUTURE PROSPECTS

Despite remarkable progress in understanding and exploiting rapamycin, several challenges still constrain its full biomedical and biotechnological potential. These challenges span from production efficiency and formulation limitations to clinical translation and ethical considerations regarding its use for lifespan modulation.

7.1. Biotechnological and Production Challenges

Although advanced metabolic and synthetic biology strategies have improved yields, large-scale rapamycin production remains expensive and technically demanding. The native *Streptomyces hygroscopicus* strains exhibit slow growth and complex regulatory mechanisms governing secondary metabolism [111]. Moreover, the biosynthetic gene cluster exceeds 100 kb, complicating heterologous expression and genetic manipulation [112].

Industrial-scale fermentation often suffers from low reproducibility, catabolite repression, and oxygen-transfer limitations, which reduce consistency in metabolite yields [113]. While CRISPR/Cas tools have enabled targeted optimization, fine-tuning global regulatory networks remains a major obstacle. Integrating systems metabolic modeling with AI-based strain design may provide new avenues to overcome these bottlenecks [114].

7.2. Pharmacological and Clinical Limitations

From a pharmacological perspective, rapamycin's poor solubility, variable pharmacokinetics, and dose-dependent toxicity remain unresolved issues [115]. Chronic inhibition of both mTORC1 and mTORC2 can lead to metabolic disturbances, including hyperglycemia, dyslipidemia, and insulin resistance [116]. These adverse effects restrict its long-term clinical use, particularly in preventive or longevity-focused applications.

Furthermore, interindividual variability in rapamycin response has been observed, likely due to polymorphisms in drug transporters (e.g., P-glycoprotein), CYP3A metabolism, and differential mTOR signaling sensitivities [117]. This heterogeneity underscores the importance of personalized dosing regimens and biomarker-guided monitoring in clinical contexts.

7.3. Ethical and Regulatory Considerations

As rapamycin and its analogs transition from traditional therapeutic settings (e.g., transplantation and oncology) to preventive and geroscience applications, ethical and regulatory challenges emerge [118]. The prospect of prescribing rapamycin for lifespan extension in healthy individuals raises questions about safety, accessibility, and societal equity [119]. Regulatory agencies currently classify rapamycin as an immunosuppressant, creating legal and ethical barriers for off-label use in anti-aging or enhancement contexts [120].

Future guidelines will need to address not only pharmacovigilance but also the broader implications of longevity-based interventions — particularly in terms of health disparities and informed consent.

7.4. Future Research Directions

The future of rapamycin research lies in precision modulation rather than broad inhibition of the mTOR



pathway. Development of selective mTORC1 inhibitors and non-immunosuppressive rapalogs represents a key research frontier [121]. Advances in structure-based drug design, aided by high-resolution cryo-electron microscopy and AI-assisted ligand modeling, could yield compounds with optimized selectivity and minimal side effects [122].

Another promising direction involves combination nanotherapies, where rapamycin is co-delivered with complementary agents (e.g., antioxidants, autophagy inducers, or metabolic stabilizers) to achieve synergistic effects [123]. Emerging data also suggest that intermittent or pulsatile dosing regimens may retain therapeutic benefits while minimizing adverse effects [124].

From a biotechnological standpoint, synthetic chassis organisms and cell-free biosynthesis systems may revolutionize rapamycin production by enabling modular, scalable, and environmentally sustainable manufacturing [125]. Integration of multi-omics data, machine learning, and automated bioprocess optimization will further accelerate discovery and yield improvement.

Ultimately, rapamycin remains a cornerstone molecule linking microbiology, pharmacology, and geroscience. Its continued study will not only expand our understanding of cellular aging and metabolic regulation but also inspire a new generation of bioengineered therapeutics with far-reaching clinical and societal impact .

8. CONCLUSION

Rapamycin, originally discovered as a secondary metabolite of *Streptomyces hygroscopicus*, represents one of the most compelling examples of how microbial natural products can shape modern biomedical research. From its initial identification as an antifungal agent to its current status as a cornerstone therapeutic targeting the mechanistic target of rapamycin (mTOR) pathway, rapamycin has profoundly influenced immunology, oncology, metabolism, and aging research.

The selective inhibition of mTORC1 by rapamycin and its analogs has provided critical insights into cellular growth control, autophagy regulation, and metabolic homeostasis. Clinically, rapamycin-based therapies have demonstrated substantial efficacy in organ transplantation and cancer treatment, while emerging evidence highlights their potential in neurodegenerative diseases and geroscience. Notably, rapamycin remains the most robust pharmacological intervention shown to extend lifespan across multiple model organisms, positioning it at the forefront of aging-related therapeutic strategies.

Despite these advances, important challenges persist. Limitations related to poor solubility, variable bioavailability, metabolic side effects, and interindividual pharmacokinetic variability continue to constrain long-term and preventive applications. In parallel, large-scale production remains technically demanding due to the complexity of rapamycin biosynthesis and the stringent regulation of secondary metabolism in *Streptomyces* species.

Recent progress in metabolic engineering, synthetic biology, and nanotechnology offers promising solutions to these constraints. CRISPR/Cas-based strain optimization, heterologous expression platforms, and advanced delivery systems are redefining both the efficiency of rapamycin production and the precision of its therapeutic use. Moreover, the development of next-generation rapalogs and selective mTOR modulators may enable dissociation of beneficial effects from adverse metabolic outcomes.

In conclusion, rapamycin continues to serve as a unique bridge between microbiology, biotechnology, and translational medicine. Its enduring scientific relevance and expanding clinical potential underscore the importance of integrative research approaches aimed at optimizing production, improving safety, and refining therapeutic selectivity. As such, rapamycin is likely to remain a foundational molecule guiding the future development of targeted therapies and geroprotective interventions.

Conflict of Interest

The authors declare no conflict of interest

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Further Reading

• Brunton LL, Knollmann BC, editors. Goodman & Gilman's: The Pharmacological Basis of Therapeutics. 14th ed. New York: McGraw Hill; 2023.

Ethical Approval

Not applicable, as this study is based on previously published data

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