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Review Article

Exploring the frontiers of penicillin pharmaceuticals: a comprehensive review of advancements and innovations

Assyl Boshkayeva[1], Abay Massakbayev[1], Galiya Sayakova[2], Serzhan Mombekov[3], Rustam Yussupov[4], Tanagul Arystanova[5], Aigul Tazhiyeva[6], Galiya Ibadullayeva[7], Nazym Pushkarskaya[1], Meruyert Tleubayeva[8], Gulbarshyn Aitzhanova[9], Farida Kayupova[10], Kaldanay Kozhanova[3*]

[1]Department of Pharmaceutical and Toxicological Chemistry, Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. [2]Department of Pharmacognosy with Botany course, Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. [3]Department of Engineering Disciplines and Good Practices, Asfendiyarov Kazakh National Medical University, Kazakhstan. [4]Department of microbiology, virology, Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. [5]Department of Pharmaceutical Disciplines, NpJSC Astana Medical University, Astana, Kazakhstan. [6]Department of Science, Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. [7]Department of Pharmaceutical Technology, Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. [8]Department of Organization and Management and Economics of Pharmacy and Clinical Pharmacy, Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. [9]Department of Normal Physiology and Biophysics, Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan. [10]Candidate of Pharmaceutical Science, Kazakh-Russian Medical University, Irgeli village, Karasay district, Almaty region, Kazakhstan.

Correspondence: Kaldanay Kozhanova, Department of Engineering Disciplines and Good Practices, Asfendiyarov Kazakh National Medical University, Kazakhstan. kaldanay_k@mail.ru

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ABSTRACT

Penicillin and its derivatives have remained a cornerstone in combating bacterial infections for decades. However, growing concerns about bacterial resistance and the limitations of conventional penicillin production methods underscore the need for innovative approaches. The aim of this review is to address the challenge of developing penicillin and related compounds by proposing a novel approach to improving the quality of medicines to meet healthcare demand. The discovery and optimization of microbial sources for penicillin production pose potential solutions to the growing demand for novel antibiotics. High-throughput screening techniques, genomics, and bioinformatics tools enable the identification and characterization of novel penicillin-producing microorganisms. The optimization of enzyme production and purification processes further enhances the scalability and cost-effectiveness of penicillin biosynthesis. However, despite significant progress, several gaps persist in large-scale, sustainable penicillin production and effective manufacturing processes. To address these gaps, this review provides a comprehensive overview of recent advancements and emerging trends in the development and manufacturing of penicillin. With an interdisciplinary lens, we point out new approaches, including biocatalysis, green chemistry principles, and advanced processes. Integrating these strategies would develop the next-generation penicillin derivatives with better efficiency, reduced environmental footprint, and enhanced resistance profiles. This extraordinary combination of research identifies current gaps in penicillin drug development and charts a path toward sustainable solutions for future antibiotic needs.

Keywords: Pharmaceuticals, Antibiotics, Penicillin synthesis, Green manufacturing, Computational design

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Introduction

Since its introduction, penicillin and its derivatives have remained among the most essential antibiotics for combating bacterial pathogens, forming the cornerstone of antimicrobial therapy. Initially derived from Penicillium molds, penicillin later evolved through the development of semisynthetic analogs with improved pharmacokinetic and antibacterial properties [1-4]. This trajectory represents a continuous effort to innovate in

response to emerging microbial threats [5]. **Figure 1** presents a timeline of significant milestones in penicillin's discovery, development, and application, alongside key scientific and technological advances.

A critical aspect of penicillin biosynthesis and modification involves penicillin acylases (PAs), which play a central role in the enzymatic production of semisynthetic penicillins. Enzymes such as penicillin G acylase (PGA) and penicillin V acylase (PVA) catalyze the hydrolysis and subsequent acylation of 6-aminopenicillanic acid (6-APA) or its analogs, yielding antibiotics with enhanced activity spectra and stability. Enzymatic synthesis offers several advantages over chemical methods, including higher specificity, selectivity, and environmental sustainability [6-9].

Despite the longstanding success of penicillin-based antibiotics, current challenges include the growing prevalence of antibiotic resistance and the need for greener, more sustainable manufacturing processes [10-15]. The emergence of multidrug-resistant bacterial strains has intensified the demand for novel antimicrobial agents and strategies to circumvent resistance mechanisms [16]. Conventional chemical synthesis of penicillins is often associated with environmental concerns, including the generation of hazardous waste and high energy consumption [17]. Accordingly, sustainable biocatalytic approaches, aligned with the principles of green chemistry, offer a promising alternative [18-21]. These include enzymatic catalysis, biotransformations, and the use of renewable feedstocks to reduce environmental impact and improve process efficiency [22, 23].

This review focuses on the role of penicillin acylases in penicillin biosynthesis and modification. It encompasses structural and mechanistic insights, synthetic methodologies, recent advances in enzyme engineering, and computational modeling. By synthesizing current knowledge and identifying existing gaps, this review aims to provide valuable guidance for researchers, pharmaceutical scientists, and clinicians involved in the development of next-generation penicillin-based therapeutics. Emphasizing a multidisciplinary approach that integrates experimental and computational strategies, the review addresses key challenges in antibiotic development and explores innovative solutions to combat antibiotic resistance.

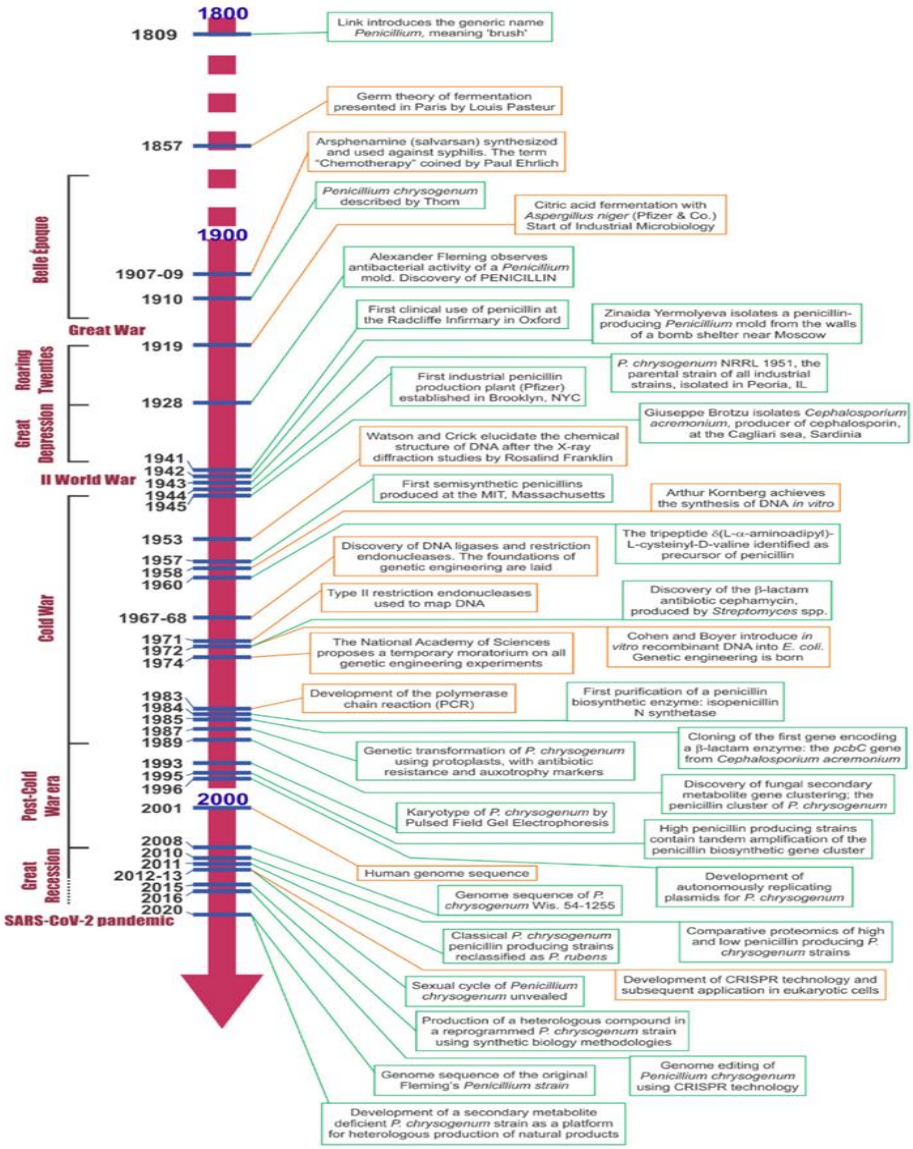


Figure 1. Timeline highlighting key milestones in the discovery, application, and research of penicillin (green squares), along with relevant scientific and technical developments that influenced the penicillin field, focusing on genetic engineering (brown squares) [24].

Materials and Methods

This review is based on a systematic analysis of published scientific data on the development of penicillin and its derivatives, microbial production, and modern manufacturing technologies. The research materials include recent peer-reviewed scientific articles and review papers indexed in international scientific databases.

Results and Discussion

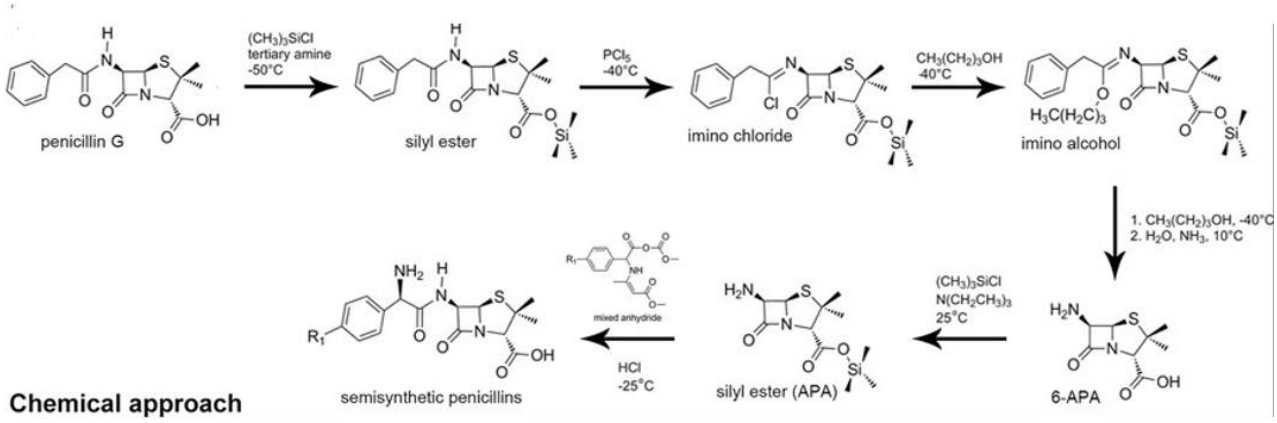
Synthesis strategies for novel penicillin compounds

Considerable effort has been devoted to chemically synthesizing semisynthetic penicillins with enhanced antimicrobial activity, pharmacological efficacy, and reduced toxicity. These approaches primarily involve structural modifications of the penicillin core, particularly at the side chain, via acylation, esterification, nitration, and other functional-group transformations **(Figure 2a)**. Cheptea *et al*. reported the synthesis of sodium 1,2,4-triazole-5-α-thioacetate derivatives via the reaction of monochloroacetates with mercaptotriazoles, followed by their coupling with 6-APA. The intermediates were further activated with pivaloyl chloride to form mixed anhydrides, which readily reacted with 6-APA to yield novel penicillin derivatives. The nature of the substituents at position four of the triazole ring significantly influenced antimicrobial activity, with p-methoxyphenyl-substituted analogs exhibiting broader spectra than p-tolyl counterparts [25-29]. Similarly, Sunel *et al*. synthesized penicillins by coupling 6-APA with various amino acid residues using mixed anhydrides, leading to products with notable diastereoselectivity and bioactivity [30]. Basu *et al*. further expanded this approach by developing dipenicillins and dicephalosporins based on asparagic acid, highlighting potential synergetic actions [31]. Liu *et al*. introduced a novel route involving β-lactam ring modifications to yield six thiazolidine amide derivatives (2-a to 2-f) and five 8-hydroxypenillic acid analogs via ring rearrangement and esterification. Select compounds exhibited promising biological activities beyond antimicrobial effects, including modulation of ion channels and protein–protein interactions, underscoring the broad pharmacological potential of modified penicillin scaffolds [32]. Advancements in synthetic strategies for penicillin compounds have been driven by the need for higher efficiency, selectivity, and sustainability. Organocatalysis, particularly using chiral amines and proline derivatives, has enabled the asymmetric synthesis of optically active penicillin intermediates with high enantioselectivity [33]. Transition metal-catalyzed cross-coupling reactions (e.g., palladium, nickel, copper) have expanded the chemical diversity of penicillin derivatives through the formation of C–C and C–X bonds [34]. Photochemical and electrochemical methods have been applied to facilitate selective functionalization steps, often using visible or UV light to modify penicillin scaffolds [35].

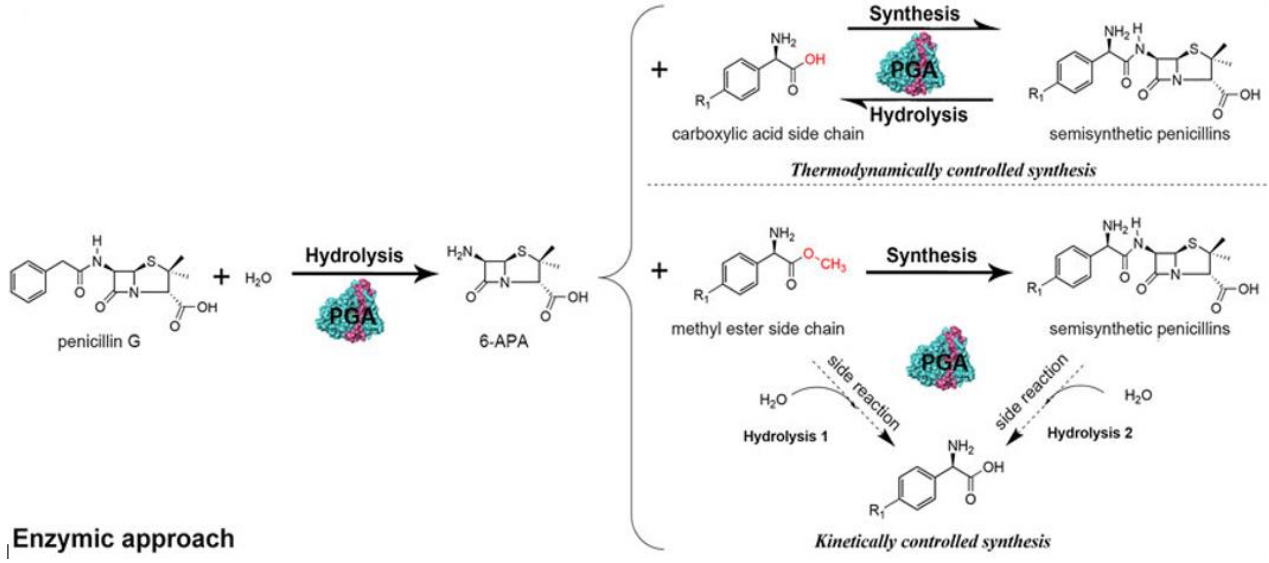
The pursuit of environmentally sustainable antibiotic production has led to significant interest in enzyme-catalyzed and biocatalytic synthesis methods **(Figure 2b)**. Enzymes such as PAs, amidases, and β-lactamases offer advantages over traditional chemical synthesis by operating under milder conditions, producing fewer byproducts, and enhancing reaction specificity [36, 37]. Water-based reaction systems, ionic liquids, and mechanochemical techniques have been explored to minimize organic solvent usage [38]. The use of fermentation-derived sugars as feedstocks significantly reduces reliance on petrochemicals and associated emissions. For instance, yields of penicillin from xylose and glucose-based media reached up to 70%, demonstrating both economic and environmental benefits [39].

Continuous-flow chemistry and microreactor technologies have further optimized synthesis processes. Gutmann *et al*. achieved a 90% yield of penicillin G under controlled microreactor conditions (50°C, 8 bar, 15 min RT), with only 2% byproduct formation [40]. In silico design and process analytical technologies enable enhanced reaction monitoring and control, thereby increasing efficiency and sustainability [41].

Flow chemistry, when coupled with inline analytical monitoring, has proven to be both scalable and safe. High-throughput screening and automation further accelerate the optimization of reaction conditions, enabling rapid discovery and development of novel penicillin compounds in accordance with green chemistry principles [42-46].



a)



b)

Figure 2. a) Traditional chemical synthesis of β-lactam antibiotics. b) Enzymatic synthesis of β-lactam antibiotics by penicillin G acylase (PGA). Initially, PGA deacylates penicillin G to produce the key antibiotic nucleus, 6-aminopenicillanic acid (6-APA). Subsequently, PGA catalyzes the condensation of 6-APA with an acyl donor under thermodynamic or kinetic control, forming various semisynthetic penicillins [36].

Microbial sources and screening strategies

The identification of novel microbial sources capable of penicillin production remains a key strategy for enhancing yields and discovering new analogs. While Penicillium chrysogenum remains the principal industrial strain, alternative species such as P. nalgiovense and Talaromyces pinophilus have been explored for their biosynthetic potential [47, 48].

Metagenomic approaches allow for the discovery of previously uncharacterized biosynthetic gene clusters from environmental samples [49]. High-throughput screening platforms, such as those developed by Thykaer *et al*. [50], have enabled rapid evaluation of microbial libraries, leading to significant enhancements in penicillin V production **(Figure 3)**.

Experimental design methods, including Plackett-Burman and response surface methodology (RSM), have been applied to optimize strain selection, fermentation parameters, and downstream processing [51].

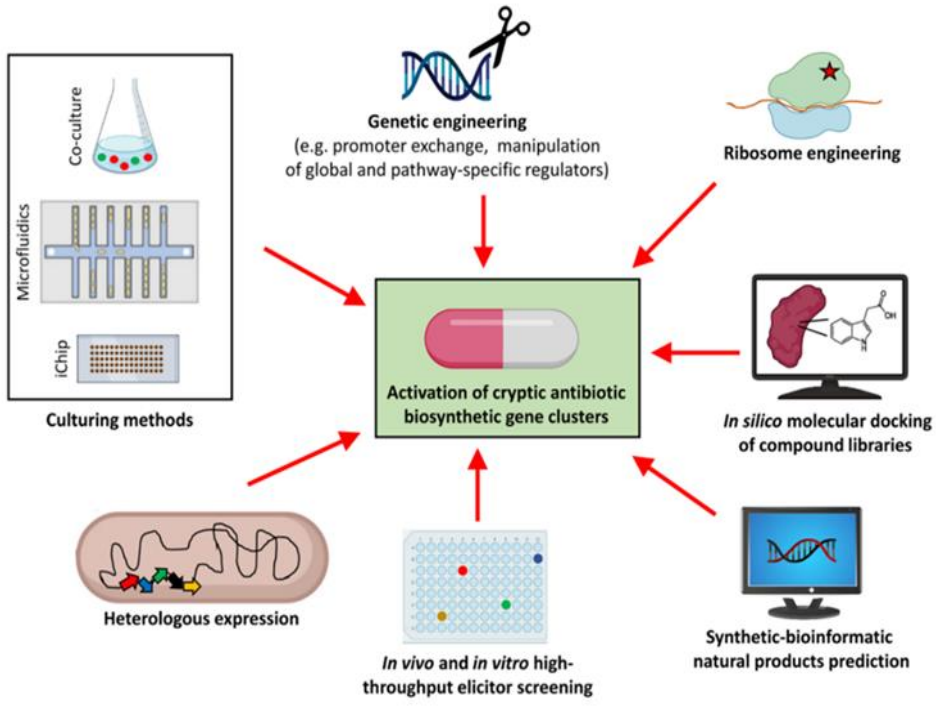


Figure 3. High-throughput screening platforms and genomic approaches used to identify novel penicillin-producing strains and optimize production conditions [52].

Enzyme Engineering for Enhanced Catalytic Performance

Optimization of enzymes involved in penicillin biosynthesis, including IPNS, PAs, and PCL, is vital for improving production efficiency. Rajendran *et al*. employed CLEA technology for immobilizing B. badius PA, achieving 100% enzyme retention over 20 cycles and complete conversion of penicillin G to 6-APA within 60 minutes [53]. Directed evolution strategies have produced thermostable and highly active PA variants, such as PA8.0, which demonstrated a 2.2-fold improvement in penicillin G yield [54]. Downstream purification methods, including ion-exchange, hydrophobic interaction, and gel filtration chromatography, have been refined to improve enzyme recovery and purity [55].

Enzyme engineering is central to improving the catalytic properties of biocatalysts involved in penicillin synthesis. Techniques such as directed evolution and rational design have been successfully applied to enhance substrate specificity, activity, and stability [56, 57]. Mutagenesis, informed by structural and mechanistic insights, has enabled modifications to key enzymes such as IPNS and AT, allowing the semisynthetic production of penicillins with modified side chains [58]. Immobilization strategies and fusion with solubility-enhancing tags further improve enzyme stability, folding, and expression [59]. Enzyme immobilization on supports such as glyoxyl-agarose enhances thermal stability and reusability, contributing to cost-effective production. Integrated bioprocessing strategies combining fermentation, enzyme purification, and semi-synthesis further streamline penicillin production [60].

Computational techniques, including molecular dynamics simulations and machine learning, have facilitated the rational design of enzyme variants with improved properties **(Figure 4)** [61]. Computational tools are increasingly used to inform the rational design of enzymes involved in penicillin biosynthesis. Homology modeling and molecular dynamics simulations enable the prediction of enzyme conformations, active-site architecture, and residue dynamics [62]. Molecular docking and QSAR modeling enable the evaluation of enzyme-substrate and enzyme-inhibitor interactions, aiding in the design of more efficient and selective biocatalysts [63, 64]. Machine learning algorithms and deep neural networks have emerged as powerful tools for structure-function prediction, enabling virtual screening of large sequence libraries [65]. Metagenomic mining using in silico screening has expanded the pool of potential biocatalysts, identifying novel enzymes with unique biosynthetic capabilities [66]. The integration of computational and experimental approaches facilitates iterative design–test cycles, accelerating the development of improved penicillin derivatives.

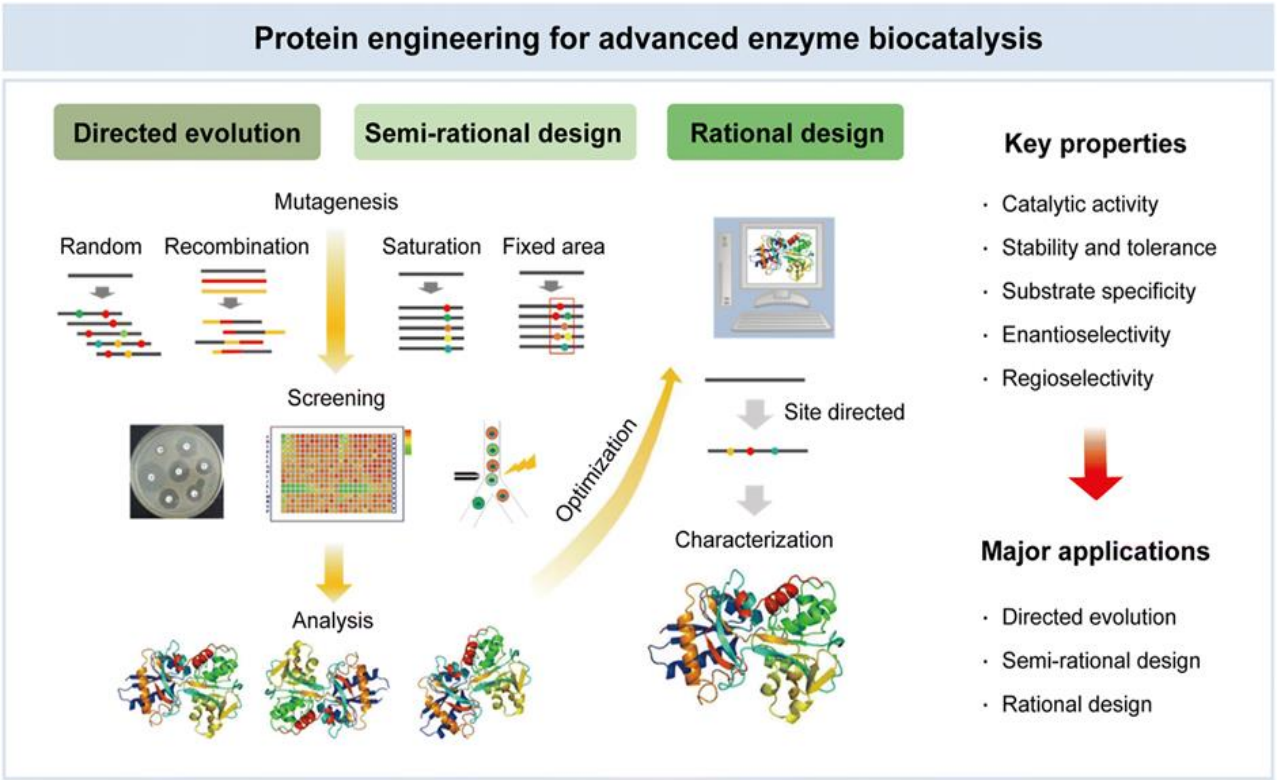


Figure 4. Optimization of enzyme biocatalysis for pharmaceutical applications through protein engineering [61].

Development and quality control measures for new penicillin derivatives

The discovery and development of novel penicillin derivatives rely heavily on advanced characterization techniques that enable detailed structural, physicochemical, and functional analysis. Nuclear Magnetic Resonance (NMR) spectroscopy remains a cornerstone technique, providing critical information on molecular structure, stereochemistry, and conformational dynamics. Specifically, 1H NMR and 13C NMR have been employed to determine proton and carbon environments,

elucidate substitution patterns, and confirm the structural integrity of penicillin molecules [67, 68].

Mass spectrometry (MS), particularly high-resolution (HRMS) and tandem MS/MS, offers precise molecular weight determination, elemental composition analysis, and detection of structural impurities. These techniques are essential for confirming synthetic success and evaluating compound purity [69-75].

Vibrational spectroscopy techniques, such as infrared (IR) and Raman spectroscopy, are routinely used to identify functional groups and study molecular vibrations. These methods help reveal the presence and nature of substituents, intramolecular interactions, and secondary structural features. When used in tandem with NMR and MS, they provide comprehensive structure-property correlations [76-80].

X-ray crystallography, offering atomic-level resolution, has been instrumental in revealing three-dimensional structures, molecular geometry, and packing interactions in crystalline penicillin derivatives. Such insights are critical for structure-based drug design and rational optimization of pharmacological properties [81-84].

Thermal analysis techniques, such as Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA), are used to assess thermal stability, melting behavior, and phase transitions. These are particularly relevant in evaluating the impact of formulation and processing conditions on compound stability [85].

Chromatographic techniques, including High-Performance Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC), remain standard tools for assessing purity, monitoring reaction progress, and performing quality control during synthesis and fermentation processes. These methods have been essential in improving purification workflows and ensuring batch-to-batch consistency [86, 87].

Together, these analytical tools offer a robust framework for the comprehensive characterization of penicillin derivatives, facilitating their optimization for clinical and industrial applications.

The development, manufacturing, and commercialization of penicillin compounds are governed by stringent regulatory frameworks to ensure product quality, safety, and efficacy. Adherence to these regulations is critical throughout the product lifecycle, from early development through post-marketing surveillance. Regulatory bodies such as the FDA, EMA, and WHO mandate compliance with current Good Manufacturing Practices (cGMP), which establish a comprehensive system encompassing facility design, equipment qualification, process validation, personnel training, documentation, and environmental control [88].

Quality control (QC) forms a foundational component of regulatory compliance. It involves a suite of validated analytical methods to verify that penicillin products meet predefined specifications related to identity, potency, purity, and stability [89]. Analytical method validation ensures the reliability, accuracy, and reproducibility of these tests, enabling manufacturers to monitor critical quality attributes effectively [90].

Stability studies are another key aspect of QC, designed to determine a compound's shelf life and appropriate storage conditions. These studies involve exposing the drug product to controlled variations in temperature, humidity, and light, assessing physical and chemical stability over time. Degradation patterns revealed by such studies inform packaging, labeling, and transport recommendations, all of which are crucial to maintaining therapeutic efficacy [91].

Beyond product-specific quality considerations, manufacturers must comply with health, safety, and environmental (HSE) regulations. These include responsible handling of hazardous substances, proper use of protective equipment, emissions control, and sustainable waste management. Implementation of environmentally conscious manufacturing practices is increasingly emphasized as part of pharmaceutical stewardship and corporate social responsibility.

Safety and efficacy assessment in preclinical studies

Prior to clinical evaluation, all novel penicillin derivatives must undergo rigorous preclinical testing to assess their safety, pharmacological properties, and antimicrobial efficacy [92, 93]. These assessments follow a systematic progression encompassing target identification, compound optimization, and both in vitro and in vivo evaluations **(Figure 5)**. Initial in vitro assessments typically involve cell-based assays and biochemical screens to evaluate cytotoxicity, genotoxicity, and off-target effects [94]. These tests provide essential insight into the safety profile and mechanism of action at the molecular level. In vivo studies are then conducted in appropriate animal models, commonly rodents and non-rodents, to evaluate systemic safety, pharmacokinetics, and antimicrobial efficacy under physiological conditions [95]. Toxicological evaluations monitor signs of acute or chronic toxicity, organ damage, behavioral alterations, and mortality. These data are critical for establishing safe dosage ranges [96].

Pharmacokinetic profiling involves the characterization of absorption, distribution, metabolism, and excretion (ADME) parameters, offering insights into bioavailability, half-life, tissue distribution, and clearance pathways. These studies are vital for identifying potential drug accumulation or rapid elimination, which can significantly influence therapeutic outcomes. Antimicrobial efficacy is assessed using relevant infection models such as systemic, localized, or strain-specific infections, including models for multidrug-resistant bacteria [97]. These studies evaluate the therapeutic performance of the compound in reducing bacterial load and improving clinical symptoms in comparison to standard treatments [88].

All studies are conducted under strict adherence to Good Laboratory Practice (GLP) and regulatory frameworks to ensure data reliability and compliance [98]. Comprehensive documentation of protocols, results, and adverse events supports regulatory submissions and facilitates the transition to clinical

trial phases [99]. According to the requirements of Good Manu-facturing Practice (GMP), the production process be-gins with sanitisation of the room, equipment, and processed clothing to prevent microbial contamination of production [100, 101].

Overall, the integration of advanced characterization with systematic preclinical evaluation lays the foundation for translating novel penicillin derivatives into clinically viable antimicrobial agents.

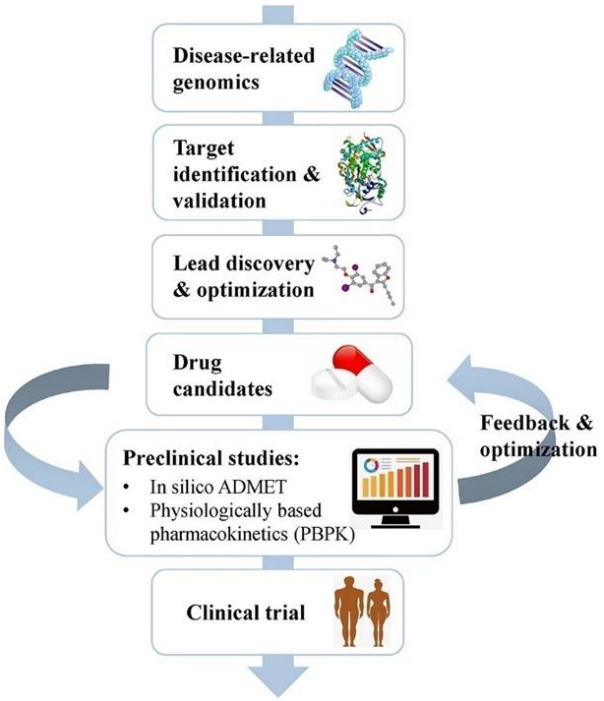


Figure 5. Illustration showing the primary components of preclinical investigation. Preclinical studies predominantly encompass in silico simulation, pivotal in guiding the identification and refinement of potential future antibiotics [88].

Conclusion

Despite being one of the oldest antibiotic classes, penicillin continues to play a vital role in modern antimicrobial therapy, particularly in the context of rising antimicrobial resistance (AMR). The economic viability of penicillin production hinges on multiple factors, including raw material availability, process efficiency, scalability, and market dynamics. Advances in fermentation technology, strain engineering, and semisynthetic production have significantly reduced manufacturing costs and improved yields [102].

The global penicillin market is poised for continued growth, fueled by increasing incidence of infectious diseases, expanding healthcare access, and renewed interest in β-lactam antibiotics. According to market projections, the penicillin market is expected to grow from USD 10.71 billion in 2024 to USD 12.55 billion by 2029, reflecting a compound annual growth rate (CAGR) of 3.21%. Broad-spectrum penicillins remain in high demand due to their clinical efficacy and favorable safety profiles.

In response to AMR, pharmaceutical companies are investing in research and development to expand the spectrum and efficacy of existing antibiotics. Semisynthetic modifications of penicillin molecules have yielded analogs with improved β-lactamase resistance and enhanced pharmacological properties [103]. Combination therapies pairing penicillins with other antibiotics or β-lactamase inhibitors have emerged as a strategy to overcome resistance and improve clinical outcomes via synergistic effects [104]. In parallel, novel applications of penicillin derivatives are being explored in non-antimicrobial contexts, including oncology and immunomodulation. Preliminary studies suggest that structural analogs of penicillin may possess antiproliferative or immune-modulatory properties, opening avenues for drug repurposing and therapeutic innovation.

Moving forward, a multidisciplinary approach integrating medicinal chemistry, synthetic biology, computational modeling, and regulatory science will be essential for the sustainable development of next-generation penicillin-based therapeutics. Emphasis on green manufacturing, regulatory alignment, and market adaptability will ensure that penicillin remains a cornerstone of infectious disease management in an era of evolving microbial threats.

Overall, while the significance of penicillin and its derivatives will most likely remain pivotal to the field of medicine in the future, their economic effects, market deployment, and future are determined by research efforts, breakthroughs in technology, and resistance to antimicrobials that will continue to develop over time.

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