“Natural Products as Inspiration for Novel Antibiotics: Chemical Diversity and Mechanism of Action”

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Abstract

Antibiotic resistance is a pressing global concern, highlighting the need for new and effective antibiotics. Natural products have long been a valuable source of inspiration for the creation of novel anti-microbial agents. This abstract explores the chemical diversity and mechanism of action of natural products as potential antibiotic sources.

Natural products derived from plants, animals, and microorganisms exhibit a wide range of chemical structures and biological activities, offering a vast array of potential antibiotic compounds. Exploring diverse environments, such as rainforests, marine ecosystems, and extreme habitats, has uncovered a rich collection of bioactive natural products.

The mechanism of action of natural product antibiotics is often complex, targeting crucial bacterial processes like cell wall synthesis, protein synthesis, nucleic acid synthesis, and membrane integrity. This interference with essential bacterial functions results in robust anti-microbial activity against various pathogens.

Numerous natural compounds have demonstrated promising antibacterial activity against drug-resistant strains, such as *Methicillin-resistant Staphylococcus aureus* (MRSA), multidrug-resistant Gram-negative bacteria, and ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). Due to their intricate chemical structures, iconic drugs like penicillin, vancomycin, and tetracycline have revolutionised medicine and served as models for the creation of new antibiotics.

Utilizing natural products as models for antibiotic development comes with challenges such as sourcing, isolation, structural elucidation, and optimization of pharmacokinetic properties. Technological advances like genome mining, synthetic biology, and combinatorial chemistry have facilitated the discovery and development of antibiotics derived from natural products.

This book chapter explores the use of natural products as a source of inspiration for the development of novel antibiotics and understanding their chemical diversity and mechanism of action can develop effective anti-microbial agents to combat the threat of antibiotic resistance.

Keywords: *Antibiotic resistance, MRSA, ESKAPE, Genome mining, Combinatorial chemistry, Anti-microbial agents*.

A. Introduction to natural products as a source of inspiration for novel antibiotics

The development of new antibiotics is the aspect of the issue that is most frequently mentioned in publications and conversations. New medication developments are constantly modifying multiple diseases. The discussions over new antibiotics, however, need to centre on the overlooked basic idea that antibiotics are special because they are the only pharmaceuticals that exhibit transmissible loss of potency over time[1].
The term "antibiotics" means "against life" and is a strange title for a class of medications that has prevented millions of deaths over the past 50 years. They fight for microbes, which have been considered a threat to humans for a very long time[2].

Antibiotics made from natural sources are preferred in this therapeutic scenario:

Since Alexander Fleming's 1928 discovery of penicillin, natural compounds have been an important source of antibiotics[3,4]. The primary source for novel medications in modern medicine is natural compounds obtained from microorganisms and plants[4]. The modern era of antibiotics came with the discovery of penicillin from the Penicilliummould, which allowed physicians to cure previously life-threatening diseases[4]. Since then, researchers have looked at more natural compounds that might be use as antibiotics.
The traditional method of discovering new bioactive compounds from natural sources involves a variety of steps, including extraction, fractionation or isolation, chemical characterization, and, finally, the execution of biological assays of the isolated or fractionated natural products. These steps are obtained from biological material using ethnological knowledge[5].

Natural products are secondary metabolites produced by various organisms, such as plants, animals, and microorganisms. They have a wide range of chemical structures and biological activities, including antimicrobial, immunosuppressive, anticancer, and anti-inflammatory effects. Many natural products have been developed as drugs or have inspired the synthesis of novel antibiotics with new mechanisms of action. However, the discovery of new antibiotics from natural sources has faced challenges such as the rediscovery of known compounds, the low yield of active ingredients, and the emergence of resistant bacteria. Therefore, there is a need for innovative strategies to explore the diversity and potential of natural products as a source of novel antibiotics.

A.1. Some of the strategies that have been employed or proposed include:

- Analyzing bacterial genome sequences using computational techniques to find new antibiotic congeners: As an example of applying this method to modify the genome of a soil bacteria produced the novel antibiotic macolacin[6].
- Using microbial cells as a source of physiologically active compounds: In order to increase the production, variety, and modification of natural products, this entails modifying the biosynthetic pathways of natural products in host species or transferring them to heterologous hosts[7]
- Using organic compounds having antibacterial characteristics as complements or substitutes for synthetic antibiotics[8]. Garlic, honey, ginger, echinacea, goldenseal, clove, and oregano are some examples of these compounds[8,9]. These chemicals could be advantageous due to their accessibility, no negative side effects, and immune system modulation[8].
- These strategies illustrate how natural products can serve as a source of inspiration for novel antibiotics and how they can be exploited to overcome the limitations of current antibiotic discovery. Natural products may offer new solutions for the treatment of infectious diseases and the prevention of antibiotic resistance.
- The comparison between natural products and synthetic antibiotics in terms of efficacy and safety is not straightforward, as it depends on many factors, such as the type, source, dose, and quality of the natural product, the type, spectrum, and mechanism of action of the synthetic antibiotic, the type and severity of the infection, the susceptibility and resistance of the bacteria, and the individual characteristics and health status of the patient.

However, some general points can be made based on the available evidence:

- Natural products may be superior to synthetic antibiotics in several ways, such as being easily accessible, having less side effects, altering immunological function, and having a variety of targets and mechanisms of action that may lower the chance of the emergence of resistance[10, 11].
- In addition to having quicker and more predictable effects, higher potency and specificity, well-defined chemical structures and pharmacokinetics, and being subject to strict quality control and regulatory standards, synthetic antibiotics may have advantages over natural antibiotics in other areas[12, 13].
- Synthetic antibiotics and natural products may occasionally have comparable or complimentary actions, such as improving each other's efficacy, lowering each other's toxicity, or exerting additive or

synergistic effects against certain diseases[11, 12].

Therefore, the efficacy and safety of natural products and synthetic antibiotics may vary depending on the context and the outcome of interest. More research is needed to compare and optimize the use of natural products and synthetic antibiotics for different types of infections.

A.2. Natural product isolation and characterization techniques

The methods of getting pure substances from natural sources, such as plants, animals, or microbes, and figuring out their chemical structures and biological functions are known as natural product isolation and characterization. Depending on the kind, quantity, and quality of the natural source, the complexity and polarity of the natural product, the objective and scope of the study, there are many approaches for isolating and characterizing natural products. Several of the popular methods include:

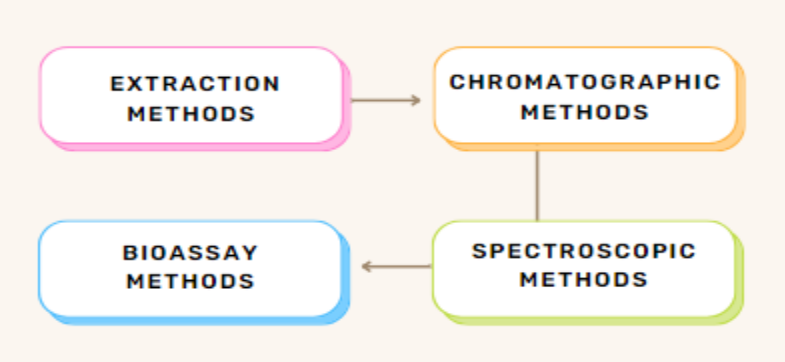

Fig 1: Natural product isolation and characterization techniques

- **Extraction methods**, which are used to separate the natural product from the raw material by using a suitable solvent or a combination of solvents. Extraction methods include solvent extraction, distillation, pressing, sublimation, ultrasound-assisted extraction, microwave-assisted extraction, accelerated solvent extraction, supercritical fluid extraction, extraction with ionic liquids, and extraction on solid phases[14,15,16].

- **Chromatographic methods**, which use a stationary phase and a mobile phase to separate the natural product from other extract constituents. Chromatographic methods include planar chromatography (such as thin-layer chromatography or high-performance thin-layer chromatography), column chromatography (such as low-pressure column chromatography, flash chromatography, medium-pressure liquid chromatography, or high-performance liquid chromatography), countercurrent chromatography, chiral chromatography, gas chromatography, and hyphenated techniques (such as liquid chromatography-mass spectrometry or gas chromatography-mass spectrometry)[15,17].

- **Spectroscopic methods**, which make use of electromagnetic radiation or magnetic fields to identify the chemical structure and functional groups of the natural product. Spectroscopic methods include ultraviolet-visible spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry, near-infrared spectroscopy, and X-ray crystallography[18].

- **Bioassay methods**, which use biological systems or living beings to identify the biological activity and mechanism of action of natural products. Bioassay methods include microbiological assays (such as antibacterial, antifungal, antiviral, or anti-parasitic assays), enzymatic assays (such as inhibition or activation assays), cellular assays (such as cytotoxicity, proliferation, differentiation, or apoptosis assays), molecular assays (such as gene expression, protein expression, or DNA interaction assays), and animal assays (such as pharmacological or toxicological assays)[14,15].

A.3. Chemical diversity of natural products and its importance in antibiotic discovery

The chemical variety of natural products and their significance in antibiotic discovery is an exciting subject. Compounds produced by living organisms such as plants, fungi, bacteria, and animals are known as natural products[16]. They feature a diverse set of biological activity and chemical structures, making them excellent sources of novel medications, particularly antibiotics. Antibiotics are compounds that have the ability

to kill or prevent the growth of bacteria, which cause a variety of infectious diseases[17]. Bacteria, on the other hand, can develop resistance to antibiotics, rendering them ineffective or useless[18]. Many common infections may become untreatable as a result of this, posing a severe threat to public health and medicine. Antibiotic resistance can be combated by developing new antibiotics with unique modes of action or targets[19]. Since the discovery of penicillin in 1928, natural materials have been the primary source of antibiotics. Many natural agents have distinct modes of action that are not present in synthetic compounds, and they can overcome or postpone the development of resistance.[20]

However, in recent years, natural product discovery has faced numerous challenges, including rediscovery of known compounds, difficulty in isolating and identifying new compounds, low yield and availability of natural sources, and a lack of investment and interest from pharmaceutical companies.[21]

To address these issues, researchers have created new methodologies and technology to investigate the chemical variety of natural compounds and their potential as antibiotic leads. Among these strategies are:

- Investigating fresh sources of natural products, such as uncultured microorganisms, marine creatures, extremophiles, symbionts, and endophytes..
- The use of synthetic biology techniques to alter biosynthetic pathways produces novel substances, or increase yields.
- Search for cryptic or silent biosynthetic gene clusters in genomes that encode for new natural resources.
- Modifying natural compounds or developing analogues with better qualities using medicinal chemistry.
- Screening for adjuvants that enhance the activity or overcome the resistance of existing antibiotics.
- Targeting virulence factors or host-pathogen interactions instead of bacterial growth.

These strategies aim to harness the chemical diversity of natural products and their evolutionary advantages in antibiotic discovery. By doing so, they hope to find new solutions to the global health crisis caused by antibiotic resistance[21]

B. Advanced techniques for natural product discovery and screening (e.g., metagenomics, high-throughput screening)

There are several advanced techniques for natural product discovery and screening, including metagenomics and high-throughput screening. Metagenomics is a culture-independent technique that has led to the discovery and synthesis of numerous biologically significant compounds like polyketide synthase, Non-ribosomal peptide synthetase, antibiotics, and biocatalyst through its sequence- and function-based screening[22].

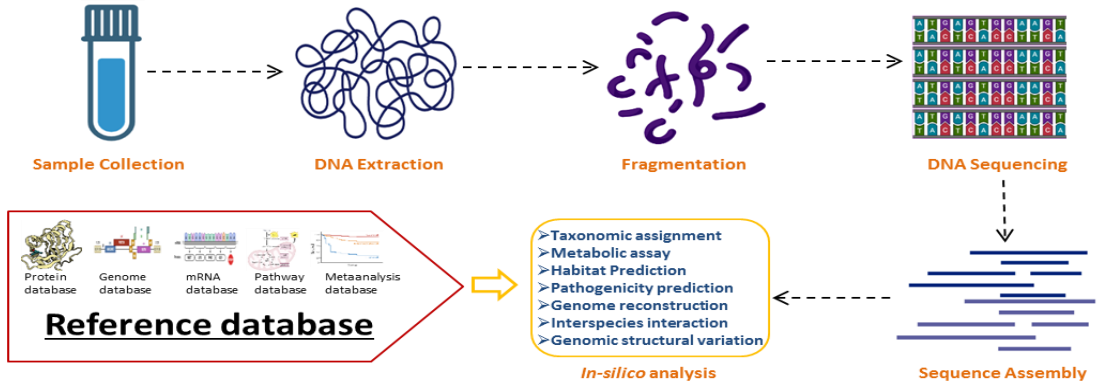

Fig 2: Metagenomics process

High-throughput screening is another important technique for natural product discovery. In this approach, large libraries of natural product extracts or synthetic compounds are screened for bioactivity using automated assays. Hits from these screens can then be further characterized to identify the active compound. High-throughput (HT) workflow for natural product (NP) discovery includes bioassay screening, docking, mode of action (MoA) prediction, HT analytical equipment, metabolomics, genomics, NP databases, in silico computational approaches that support NP dereplication (early identification of known compounds), metabolite profiling, quantitative structure activity relationship (QSAR), and computer assisted structure elucidation (CASE), as well as methods for the determination of secondary metabolites relative and absolute configuration to elucidate their 3D chemical structure[23].

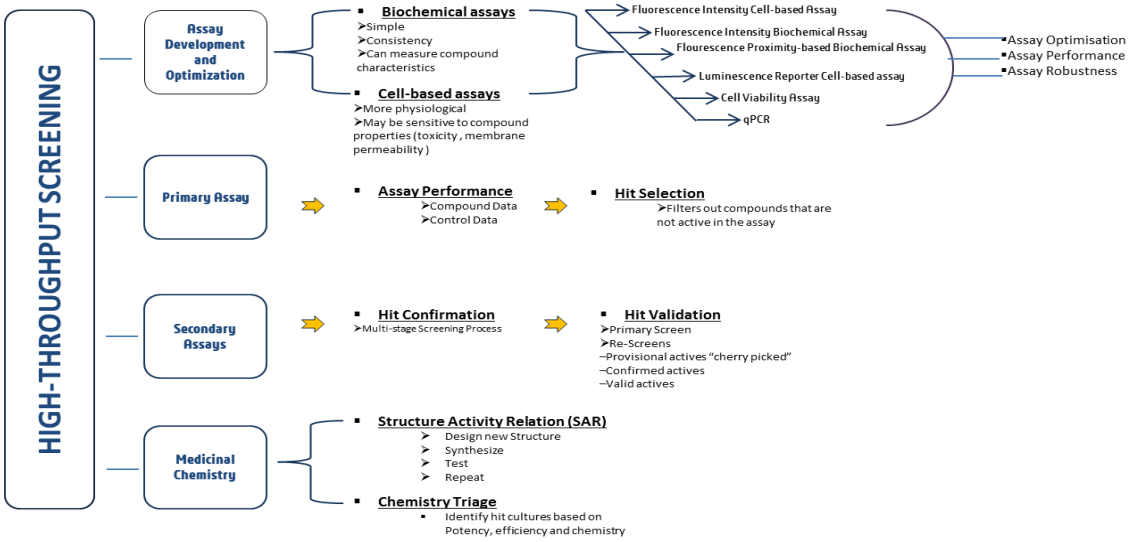

Fig 3: High-throughput Screening Process

Spectroscopic techniques such as ESI-ICRFTMS, FACS-MS, SAR by NMR and STD-NMR are also being used to directly screen natural product and synthetic libraries[24]. Overall, these advanced techniques have greatly expanded our ability to discover new natural products with potential applications in medicine, agriculture, and other fields.

B.1. Case studies of natural products with antibiotic activity

There are many natural products that have been found to have antibiotic activity. For example, plant extracts, essential oils, small antimicrobial peptides of animal origin, bacteriocins and various groups of plant compounds (triterpenoids; alkaloids; phenols; flavonoids) have been shown to have antimicrobial and antiviral activity[25]. Some specific examples include cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs[26]. Essential oils of Citrus sinensis and Citrus latifolia have also shown antibacterial capacity[27].

One of the main reasons for exploring natural products with antimicrobial activity is the ever-expanding plasmid-transmitted antibiotic resistance genes and the presence of diseases (mainly respiratory and neurological) that are not covered by natural or plant-derived substances[28].

(One example of a plant-derived substance that has been used as an antibiotic is **Albicidin**. Albicidin is a new antibiotic produced by the plant pathogen **Xanthomonas albilineans**, which is responsible for causing sugar cane's destructive leaf scald disease. It has been identified as one of the most promising new antibiotics in decades due to its unique way of killing harmful bacteria[29].

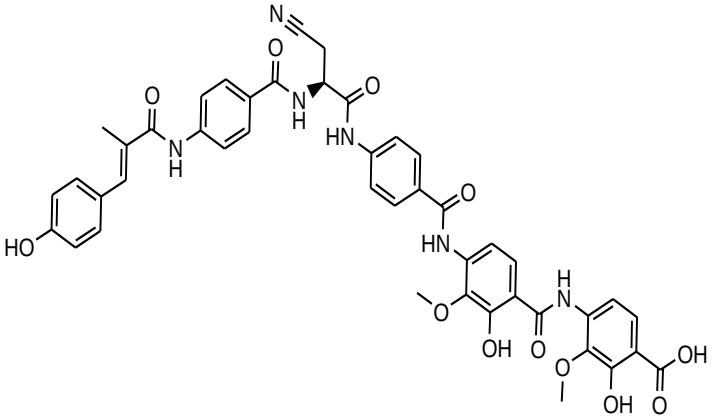

Fig 4: Structure of Albicidin

B.2. Mechanisms of action of natural product antibiotics

Natural product antibiotics can act in several ways to inhibit the growth or kill bacteria. Some act by directly inhibiting the growth or killing the bacteria, while others act as potentiators that augment or transform other agents. Some natural product antibiotics can also act as immunomodulators to host cells or block pathogen virulence[30].

For example, a large number of antibiotics work by inhibiting bacterial cell wall synthesis; these agents are referred to generally as β-lactam antibiotics[31]. However, the exact antimicrobial mechanisms of action of specific natural compounds against the target microorganisms are still unknown.

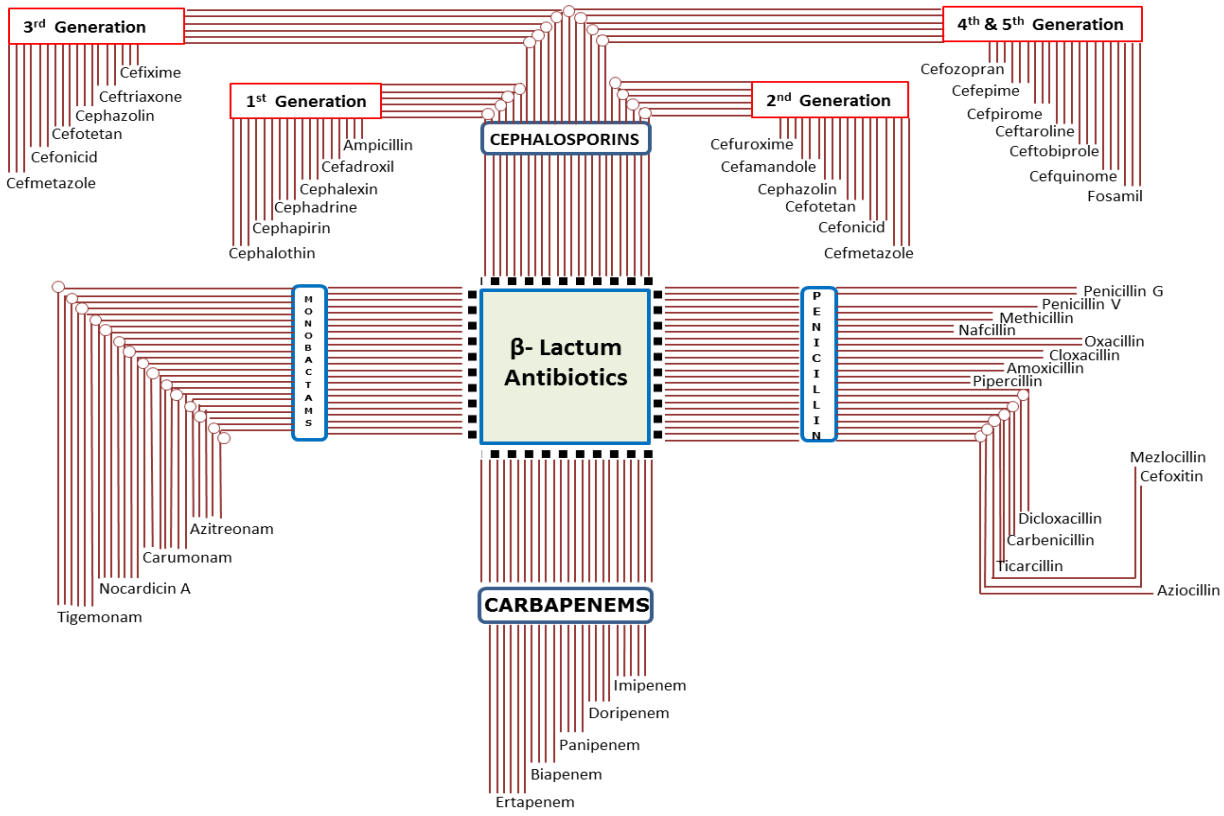

Fig 5: Classification of β-Lactum Antibiotics

B.3. Modes of action of natural product antibiotics (e.g., cell wall synthesis inhibition, protein synthesis inhibition)

Natural product antibiotics can act in several ways to inhibit the growth or kill bacteria. Some common modes of action include:

1. **Inhibition of cell wall synthesis:** Several different classes of antibacterials block steps in the biosynthesis of peptidoglycan, making cells more susceptible to osmotic lysis. Antibacterials that target cell wall biosynthesis are bactericidal in their action. Because human cells do not make peptidoglycan, this mode of action is an excellent example of selective toxicity. Antibiotics that inhibit the cell wall biosynthesis of bacteria include the penicillins, cephalosporins, vancomycin, and bacitracin[32].

2. **Inhibition of protein synthesis:** Some antibiotics target the bacterial ribosome, which is responsible for protein synthesis. These antibiotics can bind to either the 30S or 50S ribosomal subunit and interfere with the formation of peptide bonds between amino acids, thus preventing the synthesis of new proteins. Examples of antibiotics that inhibit protein synthesis include aminoglycosides, tetracyclines, macrolides, lincosamides, chloramphenicol, and oxazolidinones[33].

3. **Disruption of membranes:** Some antibiotics can disrupt the bacterial cell membrane by binding to lipopolysaccharide (LPS) or other membrane components, leading to leakage of cellular contents and cell death. Examples of antibiotics that disrupt membranes include polymyxin B, colistin, and daptomycin[34].

4. **Inhibition of nucleic acid synthesis:** Some antibiotics can inhibit the synthesis of nucleic acids (DNA or RNA) by targeting enzymes involved in these processes. For example, rifamycin can inhibit RNA synthesis by binding to bacterial RNA polymerase, while fluoroquinolones can inhibit DNA synthesis by targeting DNA gyrase or topoisomerase IV[35].

5. **Antimetabolites:** Some antibiotics can act as antimetabolites by inhibiting enzymes involved in key metabolic pathways. For example, sulfonamides and trimethoprim can inhibit folic acid synthesis by targeting different enzymes in this pathway[36].

C. Resistance mechanism against natural product antibiotics:

Resistance mechanism against natural product antibiotics is a topic of interest for many researchers, as it is related to the development of new and effective antimicrobial agents. Natural products are compounds derived from natural sources, such as plants, animals, fungi, bacteria, and minerals. They have been used as potent therapeutics against pathogenic bacteria for centuries, and some of them are the basis of modern antibiotics, such as penicillin, tetracycline, and vancomycin[37].

Resistance mechanisms against natural product antibiotics are the ways that bacteria can evade or overcome the effects of natural compounds that have antimicrobial activity. Natural products are substances derived from living organisms, such as plants, animals, fungi, or bacteria. They have been used as sources of antibiotics since ancient times, but they often face challenges such as low solubility, poor bioavailability, or high toxicity. Therefore, researchers have tried to modify their structures to improve their pharmacological profiles and overcome these limitations[38].

C.1. Some of the common resistance mechanisms against natural product antibiotics are:

1. **Efflux pumps:** These are membrane proteins that actively transport natural products out of the bacterial cells, reducing their intracellular concentration and effectiveness. For example, some bacteria can use efflux pumps to expel tetracycline's, macrolides, and quinolones[39].
2. **Enzymatic degradation:** Some bacteria can produce enzymes that degrade or modify natural products, rendering them inactive or less potent. For example, some bacteria can produce beta-lactamases that hydrolyze the beta-lactam ring of penicillin's and cephalosporins[39].
3. **Target modification:** Some bacteria can alter the structure or expression of their cellular targets that natural products bind to, reducing their affinity or accessibility. For example, some bacteria can modify their ribosomes to prevent binding of aminoglycosides, macrolides, and chloramphenicol[40].
4. **Biofilm formation:** Some bacteria can form biofilms, which are communities of cells embedded in a matrix of extracellular polymeric substances. Biofilms can protect bacteria from natural products by acting as a physical barrier, reducing diffusion, or enhancing resistance gene transfer[40].

Therefore, it is important to discover new natural products with novel modes of action, or to modify existing ones to overcome bacterial resistance. Some strategies that have been proposed include combining natural products with conventional antibiotics, using prebiotics, probiotics, synbiotics, bacteriophages, nanoparticles, or bacteriocins to enhance the antimicrobial activity of natural products, or applying new technologies such as -omics, network pharmacology and informatics to identify and characterize new natural products[41].

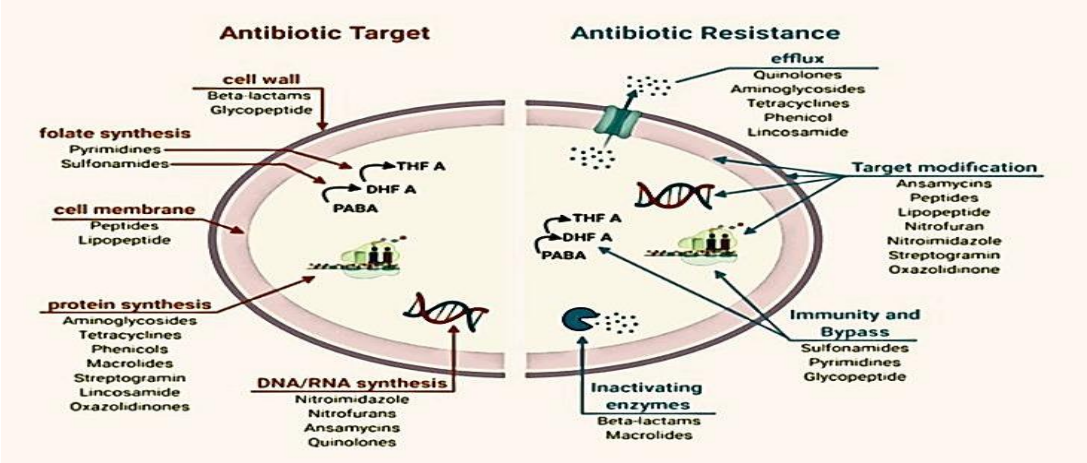

Fig 6: Resistance mechanism of antibiotics

C.2. Strategies to overcome antibiotic resistance in natural products:

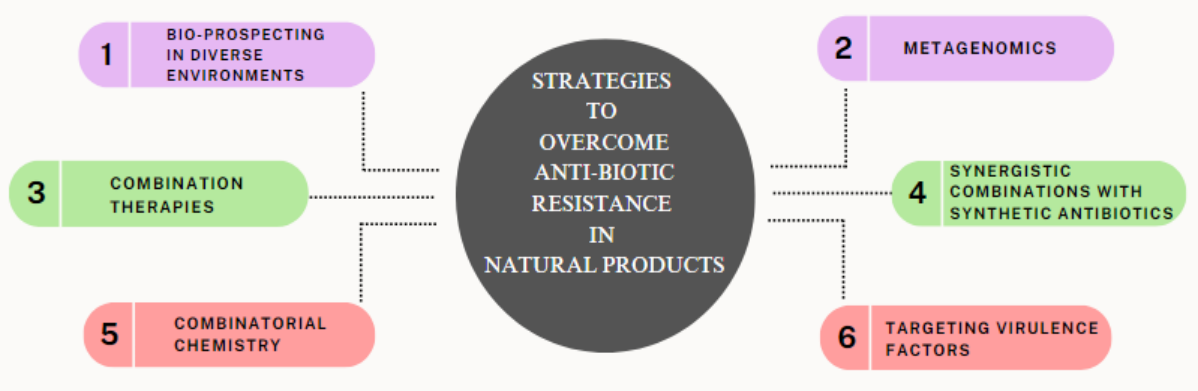

Fig 7: Strategies to leverage natural products in the fight against antibiotic resistance

Antibiotic resistance is a serious global health issue, and there is a growing need for novel strategies to combat it. Natural products have been a valuable source of antibiotics and can play a significant role in addressing antibiotic resistance[42].

1. **Bio-prospecting in diverse environments:** Explore diverse ecosystems, such as rainforests, marine environments, and soil, to discover new natural products with antibiotic properties. There may be undiscovered microorganisms or plants that produce novel antibiotic compounds[43].

2. **Metagenomics:** Utilize metagenomics to study the genetic material directly extracted from environmental samples. This approach allows researchers to access the genetic potential of entire microbial communities, increasing the chances of discovering new antibiotic-producing genes[43].

3. **Combination therapies:** Combine different natural products with distinct modes of action to create more potent and effective treatment regimens. By using multiple compounds, the development of resistance to one specific antibiotic becomes less likely[44].

4. **Synergistic combinations with synthetic antibiotics:** Explore the synergy between natural products and existing synthetic antibiotics. Combining natural compounds with conventional antibiotics can enhance their efficacy and potentially reduce the development of resistance[44].

5. **Combinatorial chemistry:** Employ combinatorial chemistry techniques to modify the chemical structures of existing natural products and create analogs with improved efficacy and reduced resistance potential[45].

6. **Targeting virulence factors:** Rather than directly killing bacteria, focus on natural products that can inhibit bacterial virulence factors. This approach may help attenuate the pathogenicity of bacteria without promoting resistance[45].

C.3. <u>Structural modification of natural products to enhance antibiotic activity:</u>

Structural modification of natural products is a strategy to enhance their antibiotic activity by altering their chemical properties and biological effects. Natural products are compounds derived from living organisms, such as plants, animals, fungi, or bacteria. They have been used as sources of antibiotics since ancient times, but they often have drawbacks such as low solubility, poor bioavailability, or high toxicity. By modifying their structures, researchers can improve their pharmacological profiles and overcome these limitations[46]

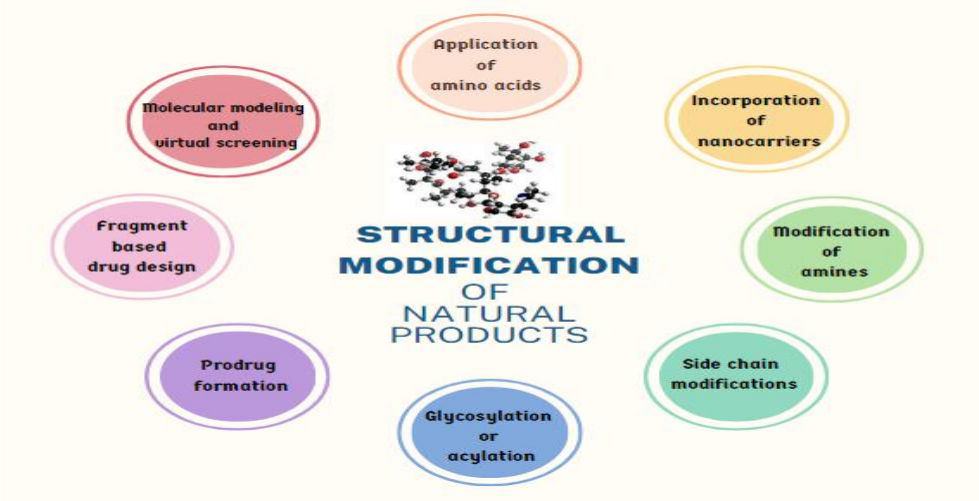

Fig 8: Some examples of structural modification of natural products

1. **Application of amino acids:** Amino acids are organic molecules that contain an amino group and a carboxyl group. They are the building blocks of proteins and have various physiological functions. By introducing amino acids into natural products, researchers can enhance their solubility, stability, activity, and specificity. For instance, amino acids can be used to modify alkaloids, phenols, flavonoids, and triterpenoids, which are common classes of natural products with antimicrobial activity[46].
2. **Incorporation of nanocarriers:** Nanocarriers are tiny particles that can deliver drugs to specific targets in the body. They can improve the stability, bioavailability, cellular uptake, and pharmacokinetic profile of natural products. They can also reduce the toxicity and side effects of natural products by protecting them from degradation or elimination. Some examples of nanocarriers used for natural products are liposomes, micro-emulsions, nanocapsules, solid lipid nanoparticles, polymeric micelles, dendrimers, etc[46].
3. **Modification of amines:** Amines are organic compounds that contain a nitrogen atom bonded to one or more carbon atoms. They are often found in natural products with antimicrobial activity, such as alkaloids and peptides. By modifying the structure of the amines, researchers can change their polarity, charge, hydrophobicity, and steric hindrance. These factors can affect the interaction between the natural products and the microbial targets. For example, researchers have modified the amines of marine natural products such as halichondrins and didemnins to enhance their antimicrobial activity[47].
4. **Side chain modifications:** Changing the side chains of natural products can improve their pharmacological properties. This could involve modifying the length, branching, or functional groups of the side chains to optimize interactions with the target pathogen[48].
5. **Glycosylation or acylation:** Adding sugar or acyl groups to the natural product can improve its solubility, bioavailability, and stability. This modification can also alter the pharmacokinetics of the compound, leading to improved efficacy[49].
6. **Prodrug formation:** Designing prodrugs involves modifying the natural product to create a biologically inactive precursor that can be converted into the active antibiotic once inside the body. Prodrugs can enhance absorption, distribution, and target specificity[49].
7. **Fragment-based drug design:** Break down the natural product into smaller fragments and use them as starting points for rational drug design. This approach allows researchers to optimize specific regions of the molecule for improved activity[50].
8. **Molecular modeling and virtual screening:** Use computational methods to predict how modifications will affect the natural product's interactions with its target. This approach can guide the design of more potent derivatives[50].

These are some of the techniques used to alter the structure of natural products in order to improve their antibiotic activity. Researchers can identify novel and effective medications to tackle multidrug-resistant diseases this way.

D. Synthetic biology approaches to natural product antibiotic production:

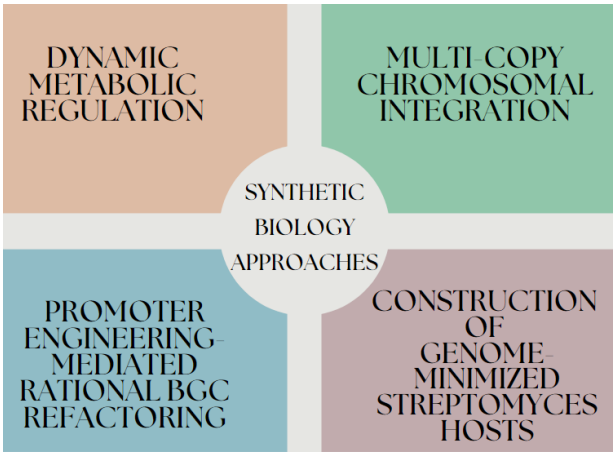

Fig 9: Synthetic biology approaches to natural product antibiotic production

Synthetic biology is a field of science that combines engineering principles with biology to design and construct new biological systems or modify existing ones. One of the applications of synthetic biology is to optimize the production of natural product antibiotics, which are compounds derived from living organisms that have antimicrobial activity. Natural product antibiotics have been used as sources of drugs for treating infections since ancient times, but they often face challenges such as low yield, poor solubility, or high toxicity. Therefore, researchers have tried to modify their structures or biosynthesis pathways to improve their pharmacological profiles and overcome these limitations[51].

1. **Dynamic metabolic regulation:** This approach involves using metabolite-responsive promoters or biosensors to control the expression of biosynthetic genes in response to environmental or metabolic cues. This can enhance the production of natural products by avoiding feedback inhibition, improving precursor availability, or synchronizing biosynthesis with growth phases[51].

2. **Multi-copy chromosomal integration:** This approach involves inserting multiple copies of a target biosynthetic gene cluster (BGC) into the chromosome of a host organism, such as yeast or actinobacteria. This can increase the production of natural products by amplifying the gene dosage, improving gene stability, or enhancing gene expression[51].

3. **Promoter engineering-mediated rational BGC refactoring:** This approach involves replacing the native promoters of a BGC with synthetic promoters that have different strengths or characteristics. This can optimize the production of natural products by fine-tuning the expression levels, timing, or coordination of biosynthetic genes[51].

4. **Construction of genome-minimized Streptomyces hosts:** This approach involves deleting non-essential genes from the genome of Streptomyces, which are actinobacteria that produce many natural product antibiotics. This can improve the production of natural products by reducing metabolic burden, increasing genetic stability, or facilitating genetic manipulation[51].

These synthetic biology approaches can significantly accelerate the discovery and development of natural product antibiotics, leading to more efficient and sustainable production processes. Additionally, the ability to engineer microbes and biosynthetic pathways opens up the possibility of producing new variants of existing antibiotics or entirely novel compounds, potentially overcoming existing resistance mechanisms[52].

D.1. <u>Biosynthetic pathways of natural product antibiotics:</u>

Infectious disease pharmacotherapy has relied on natural products and structural mimics. The pharmaceutical industry stopped pursuing natural products in the 1990s due to technical impediments to screening, isolation, characterization, and optimisation. Recent improvements in analytical techniques, genome mining and engineering, and microbial culturing are tackling such issues and creating new options. Thus, compounds from natural products (NPs) as pharmacological leads are reviving, especially for antimicrobial resistance[53,56].

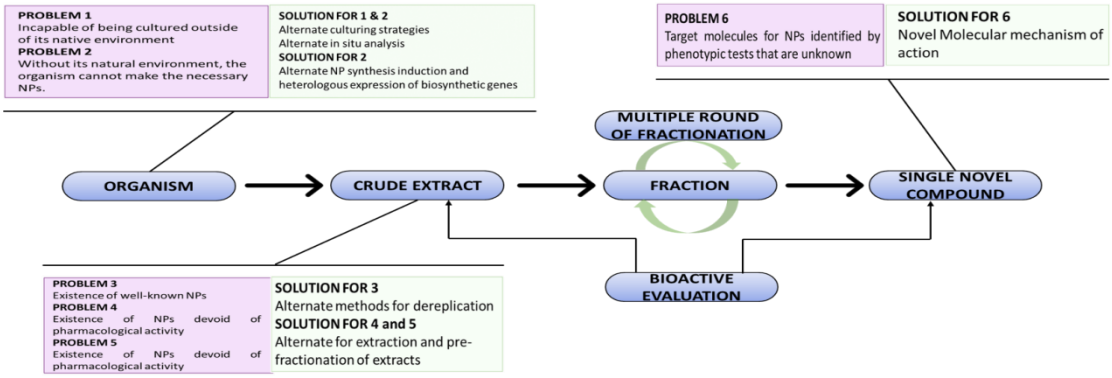

Fig 10.: Purple boxes represent process steps, red boxes reveal critical restrictions, and green boxes show modern Natural Products (NPs) based drug development advancements that overcome these restrictions. Extraction, isolation, pre-formulation proceeded with multiple polar solvents which polarify crude extracts and maximise NP diversity for biological extraction. Further, repeated bioactivity-guided fractionation finds therapeutic bioactive components in a crude extract. Many potential source organisms cannot grow outside their native habitat which stops producing relevant NPs which is a key limitation of this approach. Also, this approach cannot find novel NPs which can be overcome by the technique of new culturing, *in-situ* analysis, NP synthesis induction, and heterologous biosynthetic gene expression. At the Crude extract steps, problem of filter out the drug-like Natural Products from non-drug NPs or insufficient NPs for characterization can be fixed by the application of the process such as dereplication, extraction, and pre-fractionation. Finally, at the last stage, Phenotypic assays uncover bioactive chemicals, but affected molecular targets identification takes time and efforts. Accelerated elucidation of molecular pathways can resolve these constraints.[53]

Infections kill most people in impoverished nations. New infectious pathogens and antibiotic resistance are the main causes. Bacteria have gotten smarter and resistant to antibiotics due to clinical overuse of antibiotics. Microbial infection treatment is plagued by antibiotic resistance. Bacteria use antibiotic inactivation, target alteration, altered permeability, and metabolic pathway "bypass" to resist biochemically. Phenotypes and genomic analyses of bacterial antibiotic resistance are useful. Understanding antibiotic resistance pathways will aid antibiotic use in many situations[54,57].

Microorganisms produce bioactive compounds called natural antibiotics that kills or inhibits other bacteria. Biosynthetic gene clusters (BGCs) encode enzymes and regulators that assemble and modify complex chemical structures[55,56].

Examples of biosynthetic pathways for some legacy and new natural product antibiotics:

- **Penicillin:** Enzymes with several regulatory domains that function in nonribosomal peptide biosynthesis. The amino acids L-cysteine, L-valine, and L-α-aminoadipic acid are condensed by a nonribosomal peptide synthetase (NRPS) to create the tripeptide δ- (L- α-aminoadipyl)-L-cysteinyl-D-valine (ACV). An enzyme cyclase produces cyclization in ACV to form the β-lactam ring, which is then converted by an acyltransferase and a peroxidase enzyme to yield penicillin[57].

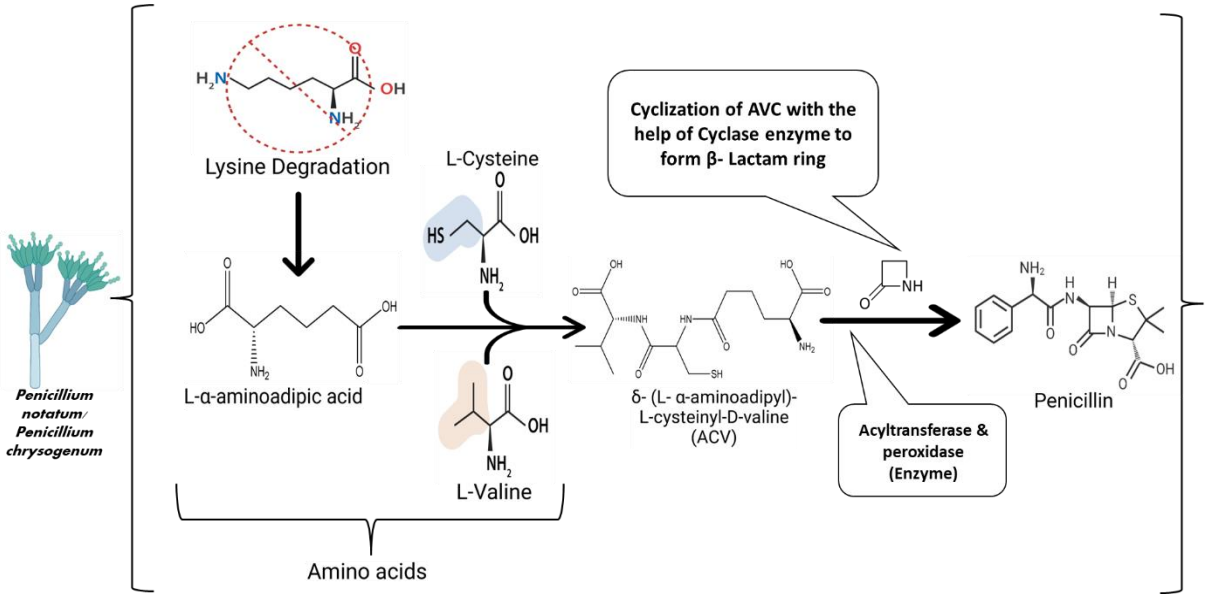

Fig 11: Depicting the biosynthesis of Penicillin antibiotic from *Penicillium notatum/ P. chrysogenum.*

- **Erythromycin:** Multi-domain biosynthetic enzymes for polyketide biosynthesis. The pathway involves the sequential addition of two-carbon units from malonyl-CoA by a polyketide synthase (PKS) to form a linear polyketide chain. The chain is then cyclized by a cyclase to form the macrolactone ring, which is further modified by glycosyltransferases, methyltransferases, and hydroxylases to yield erythromycin[58]

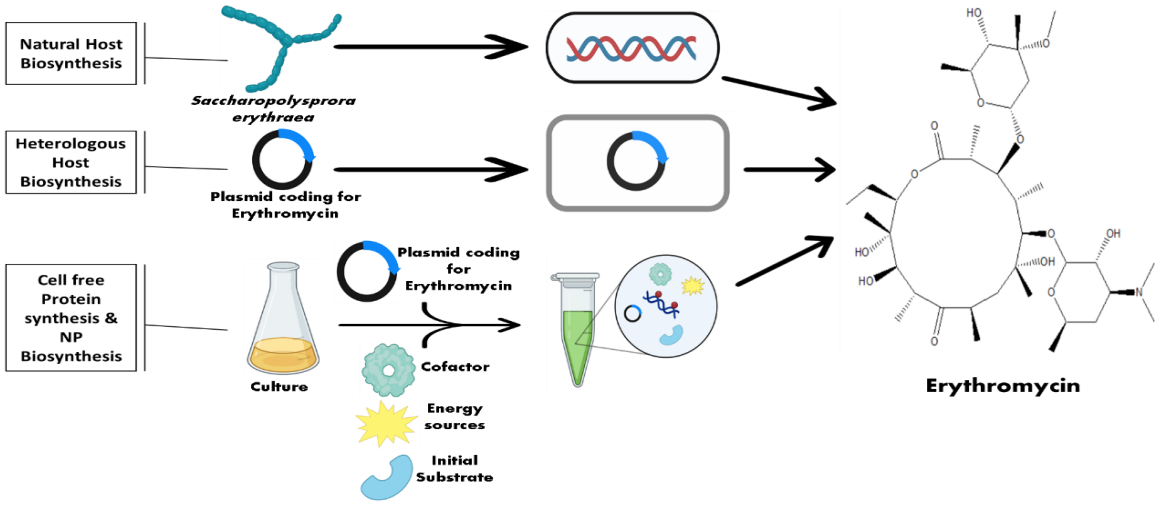

Fig 12: Depicting the biosynthetic pathway of Erythromycin via Natural host, Heterologous host and Cell free protein synthesis methods.

- **Bicyclomycin:** Single-domain biosynthetic enzyme for oxidative cyclization. In the first step, Bcm A construct the cyclic iso leucyl (cIL) backbone from the two linear peptide precursors (L-isoleucyl tRNA & L-leucyl tRNA) followed by the subsequent modification of the leucine half of the cIL by Bcm C, Bcm G, etc catalyzing hydroxylation at C_8 and C_9 respectively. Oxidases Bcm B, D, E, & F are likely responsible for the remaining transformations needed to generate Bicyclomycin[59,60].

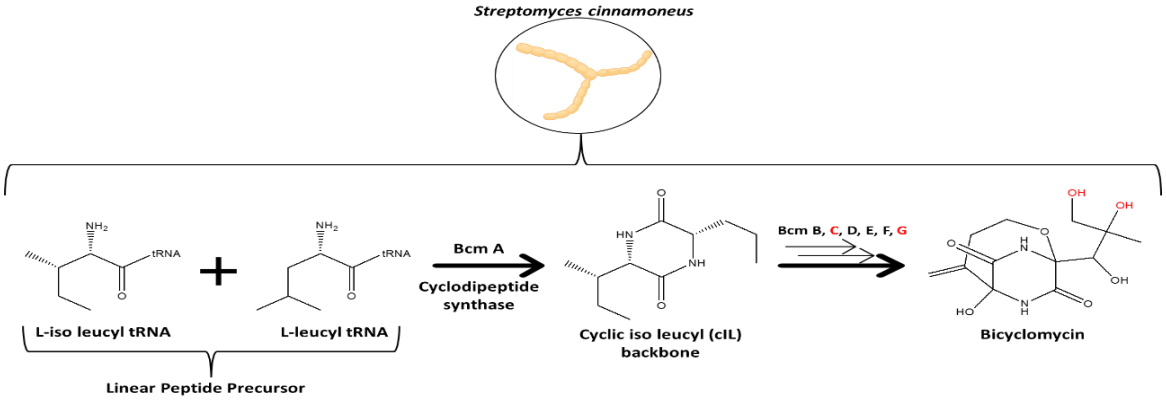

Fig 13: Biosynthetic pathways depicting the formation for Bicyclomycin from *Streptomyces cinnamoneus*.

- **Puromycin (Aminonucleoside antibiotics):** Multi-domain biosynthetic enzyme for nucleoside modification. The pathway involves the methylation of adenosine[61]. The Rationale to produce the initial steps of the puromycin biosynthetic pathway from S. alboniger[62] considers the finding that Adenosine Triphosphate (ATP) is a direct precursor of the 3'-amino-3'-deoxy-Adenosine moiety of puromycin. In this Adenosine Triphosphate is converted to 3'-amino-3'-deoxy-Adenosine-triphosphate with the help of Pur 10 (puromycin biosynthesis protein) followed by Pur 7 (puromycin biosynthesis protein) and Pur 4 (puromycin biosynthesis protein) to produced 3'-amino-3'-deoxy-Adenosine-monophosphate. 3'-amino-3'-deoxy-Adenosine-monophosphate on reaction with L-tyrosine, Pur 6, Pac (puromycin N-acetyltransferase), Pur 5 (N-methyltransferase), Pur 3 (puromycin biosynthesis protein), dmpM (O-demethylpuromycin O-methyltransferase [code- 2.1.1.38]) to produce N-Acetylpuromycin further combining with napH (N-acetylpuromycin N-acetylhydrolase) to produce Puromycin[63,64].

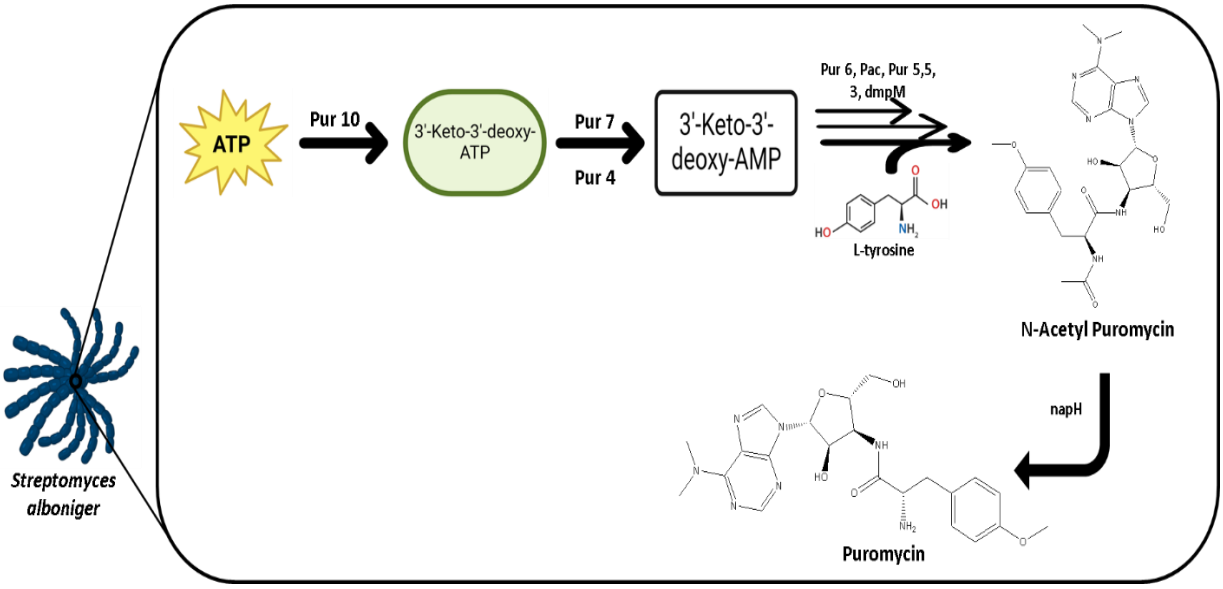

Fig 14: Puromycin biosynthesis from *Streptomyces alboniger*.

D.2. <u>Strategies for optimizing production of natural product antibiotics.</u>

Natural antibiotic substances are in peril. Modern natural product research is great for antibacterial activity and drug discovery. Natural antibiotics are effective, selective, and can penetrate bacterial cells physicochemically. Legacy antibiotics increase efficacy and avoid resistance. Novel antibiotic studies cannot match legacy medications efficacy and safety. A resistant bacterium medication with acceptable host toxicity may never see enough patients to determine its therapeutic index. This global issue requires natural goods. Antibiotic development is hampered by stringent criteria. Most natural product antibiotics were identified on the Waksman Platform using straightforward culture-based tests. In this way, effective antibiotics were discovered.

Antibiotics need multiple solutions. First, we need antibiotic chemical scaffolds without pathogen resistance pathways. Secondly, Protect our old legacy drugs from the danger of pathogen resistance. Finally, we need non-traditional anti-infectives alternative which can be produced by Natural products antibiotics. Of the over 28,000 natural product antibiotics developed over the past few decades, less than 1% are currently in clinical use. Many of the other 99.9% will never be medications due to intractable issues with efficacy, toxicity, stability, etc. One option is to reconsider the safety of some legacy chemicals. Antivirulence and biofilm inhibitors could be used clinically if they were found to block the activities of bacterial toxins, secretion systems, adhesins, impaired nutrient acquisition, and other molecular requirements for infection or biofilm formation. Short-term antibiotic combos may also help various antibiotic alternatives[65].

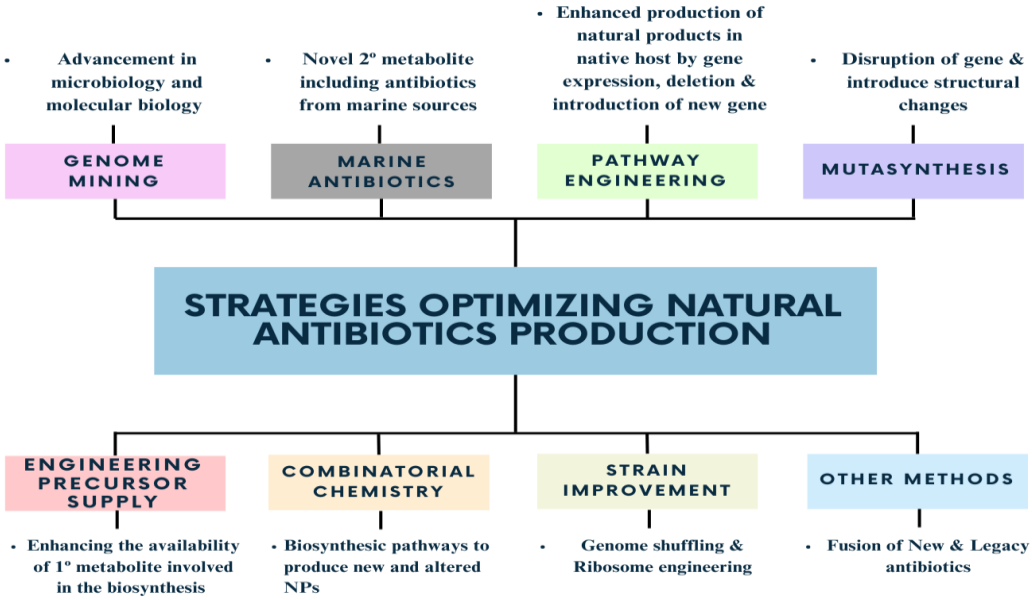

Fig 15: This image depicts the strategies for optimization of natural antibiotics production.

Few more strategies for optimizing the production of natural antibiotics can be-

- **Exploring new sources of antibiotic-producing organisms, such as marine microbes, and applying bioprocess intensification methods to improve their yield and quality.**

- **Strain Improvement-**

DNA shuffling and genome recombination are used in whole genome shuffling to increase the viability of microorganisms. Two different *Streptomyces fradiae* strains, for instance, showed as much as a nine-fold uptick in synthesis of the antibiotic tylosin. A mutant strain of *S. cellulosum* GSUV3-205 with 130-fold increase was created by genome shuffling and protoplasts fusion in order to improve epothilone synthesis[66].

Another strategy for boosting the titer and output of secondary metabolite synthesis is ribosome engineering. Secondary metabolite biosynthetic gene clusters (BGC) have been shown to be transcriptionally activated by RNA polymerase β subunit (rpoB) mutations in S. griseus, S. coelicolor, and S. erythraea. A recombinant Streptomyces viridochromogenes strain showed a 37-fold increase in avilamycin production, and an H437R mutant of rpoB from S. erythraea boosted erythromycin production[66].

- **Combinatorial Biosynthesis-**

Combinatorial biosynthesis is a form of genetic engineering that alters biosynthetic pathways to generate novel and modified forms of natural product structures. This method utilises designed enzymes and pathways to make synthetic versions of natural products without being selective about their substrates. To do this, scientists use mutagenesis and selection techniques to alter enzyme function and then design new molecules with the desired functionality based on predictions about how the molecules' structures will affect their behaviours. By altering the activity of key enzymes involved in the biosynthesis of natural products, directed evolution can increase the diversity of natural products by creating novel and more effective analogues[66].

- **Engineering Precursor Supply**

Precursor supply engineering is the process of enhancing the availability of primary metabolites or molecules involved in the biosynthesis of natural products. This can be achieved by manipulating pathways or enzymes involved with the precursor supply. Malonyl-CoA and methylmalonyl-CoA are commonly used precursors for polyketides, while aromatic amino acids and other metabolites derived from the shikimate pathway are also used. Precursor supply engineering has been successfully applied to produce most major classes of natural products, with applications to heterologous producing strains and native producers. It can also be employed to increase recombinant protein production by reducing unwanted by-products, such as acetate formation[66].

- **Mutasynthesis**

Gene disruption and mutasynthesis can generate novel natural product analogs. Disruption of a gene, such as a tailoring enzyme, introduces structural changes. Two analogs were produced by targeting gene disruption in *Streptomyces species*. Mutasynthesis involves coupling gene inactivation with precursor feeding to generate new structural analogs. Precursor feeding is useful due to the substrate-promiscuity of the biosynthetic enzyme. Mutasynthesis can generate new analogs for various compounds, such as Cahuitamycin D, which has two-fold enhanced biofilm inhibitory activity. This approach has also been applied to generate nonbenzoquinone analogs of Hsp90 inhibitor geldanamycin, which has anti-proliferative activity on tumor cells[66].

- **Pathway engineering**

Metabolic pathway engineering can enhance the production of natural products in native hosts through repetitive gene expression, gene deletion, and introduction of new genes. For example, Overexpression of genes can increase actinorhodin production, while deletion of genes can eliminate competing pathways that may siphon off important precursors or intermediates. Negative regulation by pathway-specific repressors can improve secondary metabolite pathways. Deleting non-essential genes and directing cellular resources toward essential pathways can improve cellular efficiency and streamline biochemical production. Similar approaches have been employed to improve the secretion capability and productivity of biologics, such as engineering the protein trafficking pathway, increasing the copy number of genes associated with protein secretion, and enhancing the secretion of protease-sensitive human growth hormones (hGH)[66].

- **Marine Antibiotics**

Marine biotechnology is gaining interest due to the potential of the vast genetic diversity found in marine life to harbour new chemicals. Novel secondary metabolites, including antibiotics, from marine bacteria are attracting attention due to the growing demand for new antibiotics. However, there is little advancement in the production of the secondary metabolites and the production has been classified in two phase- trophophase (growth phase) and iodophase (production phase). All this need choice of carbon source which greatly influences secondary metabolism and antibiotic production. Fast-growing cells (such as marine bacteria) generally have secondary metabolism "switched off" until their growth rate slows, via feed-back inhibition. For example, slowly utilized substrates such as galactose have improved antibiotic yields from fungi, exhibiting a less well-defined separation of trophophases and iodophases[67].

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- **Employing synthetic biology approaches to modify known scaffolds, generate new compounds, and improve biosynthetic pathways.**

- **Genome mining and metagenome mining-**

Advancements in microbiology and molecular biology techniques have enabled the cultivation of microbes that were previously difficult to access. Next-generation sequencing (NGS) provides unprecedented access to the genomic details of these organisms, revealing a remarkable quantity and genetic diversity of natural products that can be synthesized by microbes. For example, Actinomycetes encode 20-40 natural-product biosynthetic gene clusters, while fungi encode even more. These advances enable rapid triage for novelty, yielding new antibiotic scaffolds like telomycins targeting bacterial membrane components. Strategies to activate these clusters include deletion or overexpression of regulatory genes, chemical perturbations, physical stress, and selection of mutants. Metagenomic strategies, which collect total DNA from sources, are being employed to create new antimicrobial compounds like turbomycins, glycopeptide variants, and colicins[68].

- **Other methods**

Combining antibiotics with adjuvants, non-antibiotic compounds that overcome resistance and enhance drug activity or blocking pathogen virulence as orthogonal strategies to traditional antibiotics[67,68].

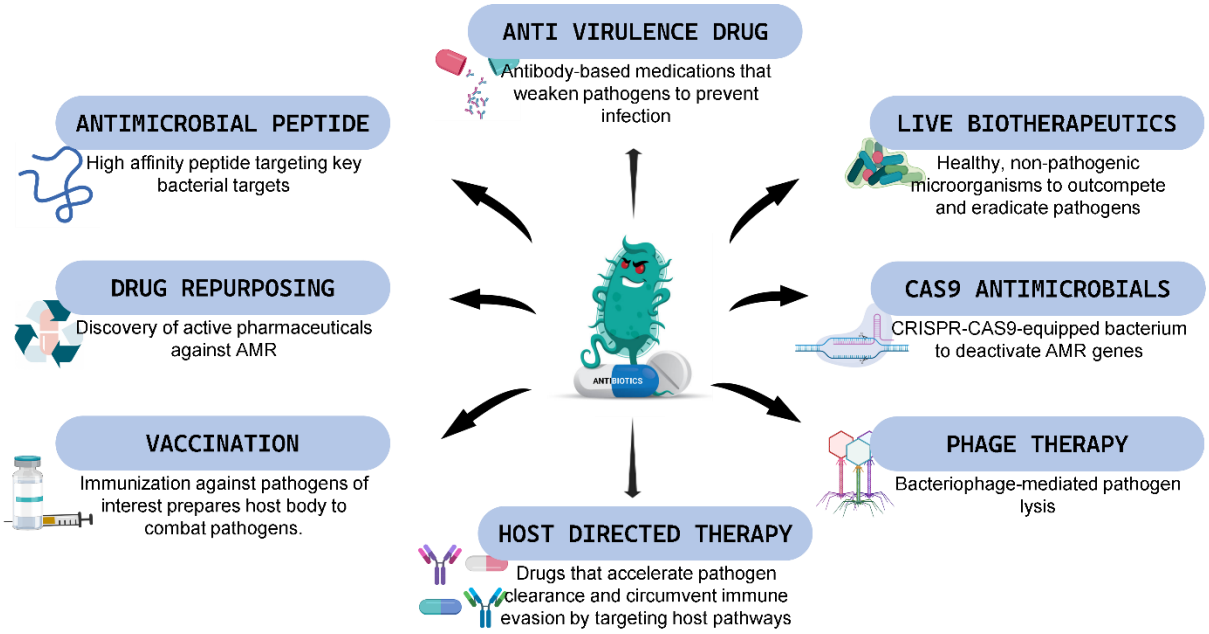

Fig 16: This image depicts the strategies for overcoming antibiotics resistance.

D.3. <u>Combination therapies involving natural product antibiotics.</u>

When multiple drugs or therapies are used together, rather than individually, the results can be more beneficial which is known as combination therapy. It is effective against a wide range of illnesses, including bacterial & viral infections, cancer, diabetes, etc[69].

Antibiotic combination therapy is used to cure resistant bacteria, treat infections with several causes, increase the effectiveness of individual antibiotics, and expand the range of bacteria that can be killed[69-72]. The following are examples of antibiotic combinations:

- ✓ The beta lactam amoxicillin and the suicide inhibitor clavulanic acid found in amoxicillin/clavulanic acid enable it to resist degradation by beta lactamase[69,70].
- ✓ Trimethoprim/sulfamethoxazole, which prevents the manufacture of folic acid in bacteria by blocking two separate but consecutive stages[69,70].
- ✓ Synergistic effects against gram-negative and gram-positive bacteria can be achieved by combining aminoglycosides with other medications like beta lactams, glycopeptides, or fluoroquinolones.[15]

To combat the rise of drug-resistant bacteria, natural product antibiotic combination therapies hold great promise. A research article found that patients with life-threatening illnesses caused by drug-resistant bacteria benefited greatly from combination therapy. Also, the synergy of natural extracts and conventional antibiotics against bacterial and fungal diseases may be the best method[71].

Antibiotics derived from natural sources include a wide variety of plant chemicals (triterpenoids; alkaloids; phenols; flavonoids) that have antibacterial and antifungal action achieved this property by inhibiting bacterial or fungal enzymes, disturbing biofilms, influencing the human immunological response, or interfering with microorganisms replication, these natural chemicals can boost the anti-microbial action of conventional antibiotics[72,73].

However, there are also some challenges and limitations in using combination therapies involving natural product antibiotics, such as the variability of natural sources, the lack of standardization and quality control, the potential toxicity and adverse effects, the pharmacokinetic interactions, and the regulatory issues. Therefore, more research is needed to optimize the formulation, dosage, delivery, and safety of these combination therapies[72].

One possible way to improve the stability, bioavailability, cellular uptake/internalization, pharmacokinetic profile and reduce toxicity of natural product antibiotics is to use nanocarriers, such as liposomes, microemulsions, nanocapsules, solid lipid nanoparticles, polymeric micelles, dendrimers, and many more. Some recent studies have focused on the incorporation of natural substances with antimicrobial activity into polymeric nanoparticles, niosomes and silver nanoparticles (which have been shown to have intrinsic antimicrobial activity)[74]. Another possible way to enhance the efficacy of combination therapies involving natural product antibiotics is to use rational design based on molecular docking and pharmacophore modeling to identify the optimal combinations and targets. For example, an article has shown the application of molecular docking to

screen for potential synergistic combinations of aminoglycoside antibiotics with other drugs against bacterial resistance mechanisms[75].

Challenges and prospects in natural product-inspired antibiotic discovery

Natural products are compounds produced by living organisms that have biological activity. They are a rich source of **antibiotics**, which are drugs that kill or inhibit the growth of bacteria[65,76-78]. Many of the antibiotics in clinical use today are natural products or their derivatives. **Antibiotic discovery** is the process of finding new antibiotics from natural or synthetic sources. It is an urgent need because of the rise of **multidrug-resistant bacteria**, which are bacteria that can survive exposure to multiple antibiotics. These bacteria pose a serious threat to human health and can cause infections that are difficult or impossible to treat [65,76-78]. **Natural product-inspired antibiotic discovery** is a strategy that uses natural products as leads or models for developing new antibiotics. It involves screening natural products for antibacterial activity, identifying their structures and biosynthetic pathways, modifying their chemical properties and biological targets, and optimizing their production and delivery[76,79].

- **Some of the challenges in natural product-inspired antibiotic discovery include:**
  - The **rediscovery** of known compounds, which wastes time and resources.
  - The **activation** of silent or cryptic biosynthetic gene clusters, which encode novel natural products but are not expressed under normal conditions[78,80].
  - The **yield** and **purification** of natural products, which are often low and complex due to the diversity and complexity of microbial cultures[78,80].
  - The **economic** and **regulatory** issues, which discourage investment and innovation in antibiotic development due to the high cost, low return, and short lifespan of antibiotic[78].

- **Some of the prospects or opportunities in natural product-inspired antibiotic discovery include:**
  - The **genomic** and **metagenomic** data, which provide insights into the diversity and potential of natural product biosynthesis in cultured and uncultured microorganisms[78,80].
  - The **synthetic biology** and **metabolic engineering** approaches, which enable the manipulation and optimization of natural product biosynthesis and the generation of novel analogs with improved properties[76,79].
  - The **alternative producers**, such as symbiotic or uncultivated microorganisms, which may harbour unique or underexplored natural products with novel structures and activities[80].
  - The **adjuvants** or **inhibitors of resistance mechanisms**, which can enhance the efficacy and durability of natural products by overcoming bacterial resistance or enhancing drug activity[81].

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