# Antibiotic conjugates and natural products as next-generation strategies against antimicrobial resistance

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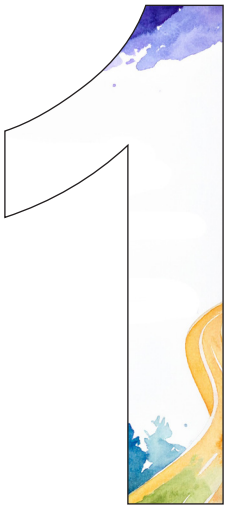
# Strategies to Overcome Antibiotic Resistance: Drug Combinations, Conjugates, and Natural Products



## Abstract

Antibiotic resistance has become a critical global health issue, threatening the efficacy of current antimicrobial effectiveness. A major aim of the research summarized in this thesis is the pursuit of new antibacterial agents to counteract resistance. This introductory chapter provides an overview of the mechanisms of antibiotic resistance and focuses on innovative counter-strategies, including the use of antibacterial drug combinations, the development of multifunctional antibiotic conjugates, and the discovery of novel antibacterial natural products. Emphasis is placed on the design principles of antibiotic conjugates, their application in overcoming resistance mechanisms, and how natural product scaffolds continue to make significant contributions to the antibacterial drug discovery landscape.

## 1. Introduction

The discovery of antibiotics in the early 20th century marked a turning point in medical history, transforming once-deadly bacterial infections into manageable conditions and saving countless lives. From the introduction of penicillin to the development of a wide range of antimicrobial agents, antibiotics have become indispensable tools in modern medicine. They are used not only to treat infections but also to prevent them during surgeries, in immunocompromised patients, and in various medical procedures.[1-3] However, the widespread and often indiscriminate use of antibiotics has accelerated the emergence of many antibiotic-resistant bacteria that now pose a serious global threat to public health.[4]

Antimicrobial resistance (AMR) undermines the effectiveness of antibiotics, leading to longer hospital stays, increased healthcare costs, treatment failures, and elevated mortality rates.[5,6] Resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and carbapenem-resistant *Enterobacteriaceae* (CRE) exemplify the urgent need for novel antimicrobial agents and innovative treatment strategies.[7-10] The pipeline for new antibiotics has been dwindling over the past decades, and few new classes have reached the market in recent years, exacerbating the crisis.[11,12]

In this context, the discovery and development of new antibiotics and alternative therapeutic approaches have become an urgent global priority. The research described in this thesis aims to address this need and this introductory chapter provides a comprehensive overview of recent advances in antibiotic development, with a particular focus on three promising strategies: combination therapy, antibiotic hybrids, and natural-derived products. Each of these approaches offers unique opportunities to combat resistant bacteria, either by enhancing the efficacy of existing antibiotics, circumventing resistance mechanisms, or by exploiting novel modes of action.[13-18]

To set the stage for this discussion, we will first explore the fundamental mechanisms by which antibiotics act on bacterial cells, followed by a detailed examination of the various resistance mechanisms employed by bacteria to evade these drugs. This will provide the necessary background for understanding how modern therapeutic strategies are being designed to outmaneuver resistance and restore the effectiveness of antibacterial treatments.

## 2. Mechanisms of Antibiotic Action and Resistance

Understanding how antibiotics exert their effects and how bacteria counteract them is essential for guiding the development of new therapeutic strategies. Antibiotics function by targeting vital cellular processes in bacteria, disrupting their survival and proliferation. Common mechanisms of action include inhibition of cell wall synthesis, disruption of cell membrane integrity, interference with DNA replication, inhibition of RNA transcription, and obstruction of protein synthesis (**Figure 1**).[19] For example, β-lactam antibiotics such as penicillin block the cross-linking of peptidoglycan in bacterial cell walls, leading to cell lysis.[20] Polymyxins interact with lipopolysaccharides and phospholipids in the bacterial membrane, causing leakage of cellular contents.[21] Fluoroquinolones inhibit DNA gyrase and topoisomerase IV, enzymes essential for DNA replication, while rifampicin binds to RNA polymerase, halting transcription.[22,23] Aminoglycosides, tetracyclines, and macrolides interfere with ribosomal function, impairing protein synthesis and ultimately leading to bacterial death.[24]

In response to the pressure presented by antibiotics, bacteria have evolved diverse resistance mechanisms that can neutralize or evade antibiotic activity. One major strategy is the alteration of drug entry pathways, such as the downregulation or modification of porin channels in Gram-negative bacteria, which limits antibiotic penetration.[25] Another common defense is enzymatic inactivation, exemplified by β-lactamases that hydrolyze the β-lactam ring of penicillins and cephalosporins.[26] Bacteria can also alter or mutate the molecular targets of antibiotics, reducing drug binding affinity. An example of this phenomenon can be seen in MRSA strains with altered penicillin-binding proteins or in fluoroquinolone-resistant bacteria with mutated DNA gyrase.[27,28] Active efflux pumps can expel antibiotics from the bacterial cell before they reach their target.[29] Additionally, horizontal gene transfer via plasmids, transposons, and bacteriophages facilitates the rapid spread of resistance traits between species, while biofilm formation provides a physical and biochemical shield against antimicrobial penetration (**Figure 1**).[30]

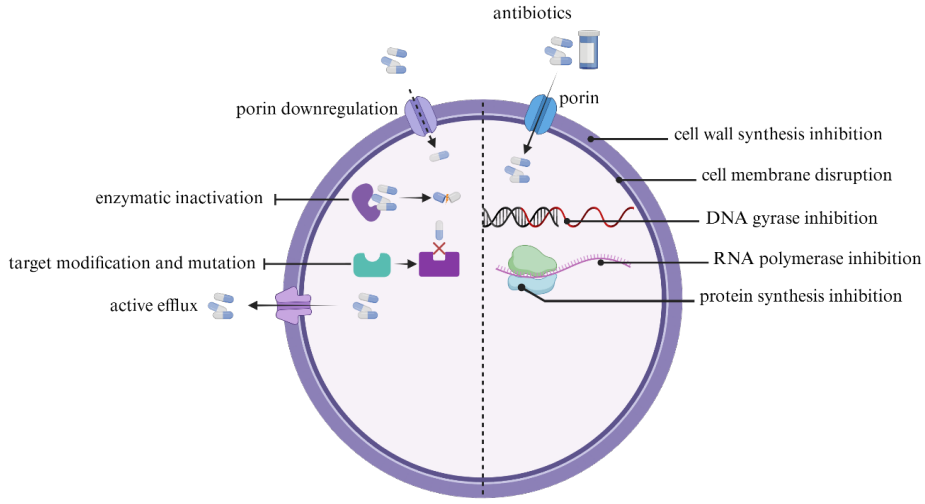



**Figure 1.** Antibiotics mechanism and antibiotic resistance

The interplay between antibiotic mechanisms and bacterial resistance is a dynamic evolutionary arms race. Each new class of antibiotic is inevitably followed by the emergence of resistance, often within years of clinical introduction.[31,32] This cycle underscores the necessity of developing novel drugs and therapeutic approaches that can circumvent or suppress these adaptive bacterial defenses.[33] By examining these mechanisms together, we can gain a clearer perspective on both the vulnerabilities of bacterial pathogens and the sophisticated strategies they employ for survival, enabling more targeted innovation in the fight against antimicrobial resistance.

## 3. Therapeutic Approaches to Overcome Antimicrobial Resistance

This section focuses on three major strategies currently implemented in the clinic or under investigation: combination therapy, antibiotic hybrids, and natural products. Each of these approaches offers distinct advantages and also poses unique challenges, but together, they represent promising avenues for reviving the clinical utility of antibiotics and expanding the arsenal against resistant pathogens.[16,34,35]

Combination therapy involves the simultaneous use of two or more agents to achieve synergistic effects, prevent resistance development, and broaden antimicrobial spectra.[36,37]

Antibiotic hybrids are structurally integrated compounds that combine pharmacophores from different antibiotic classes or mechanisms, designed to exert dual or enhanced antibacterial effects.[15,38] Meanwhile, natural product antibacterials, including both traditional antibiotics and newly identified bioactive compounds from microbial or plant sources, continue to provide a rich and largely untapped source of antimicrobial agents.[39]

The following subsections will explore each of these therapeutic strategies in greater detail, discussing their underlying principles, representative examples, challenges encountered during development or clinical application, and prospects for future use in the fight against AMR.

### 3.1 Combination Strategies

Combination therapy represents an important approach to combat AMR and involves using two or more antimicrobial agents simultaneously to enhance treatment efficacy as well as suppressing the emergence of resistant strains.[40,41] This strategy is particularly vital in the context of infections caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacteria, where traditional monotherapies often fail.[42]

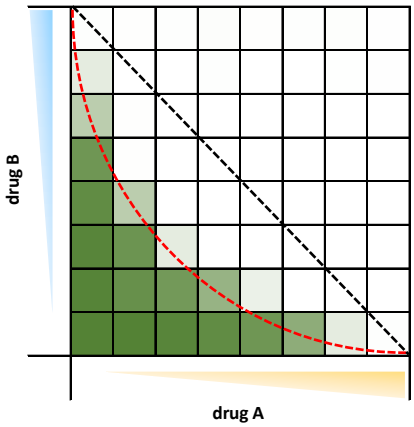



**Figure 2.** Schematic of a hypothetical checkerboard assay, in which two antibiotics (blue and orange) are arranged in concentration gradients along the x- and y-axes, respectively. Increasing concentrations of each antibiotic reduce bacterial growth, indicated by a shift from dark green to light green squares. The resulting inhibition front (red dashed line) displays a hyperbolic shape, consistent with a synergistic interaction, in contrast to the expected additive effect (black dashed line).

The rationale for combination therapy is multifaceted. Using antibiotics with distinct mechanisms of action can produce synergistic effects, where the combined antibacterial

activity surpasses the sum of the individual agents. Such synergy can improve bacterial killing, lower the effective dose of each drug, and reduce treatment-related toxicity.[43] In addition, combining antibiotics can slow or prevent the emergence of resistance, as pathogens must acquire multiple resistance mechanisms simultaneously to overcome all agents in the regimen. Synergy is commonly assessed using the so-called "checkerboard assay", in which two antibiotics are tested across orthogonal concentration gradients to evaluate their combined effects on bacterial growth.[44] A hyperbolic inhibition front, as illustrated in **Figure 2** (red dashed line), is indicative of synergy, whereas a linear boundary suggests an additive or independent interaction (black dashed line).

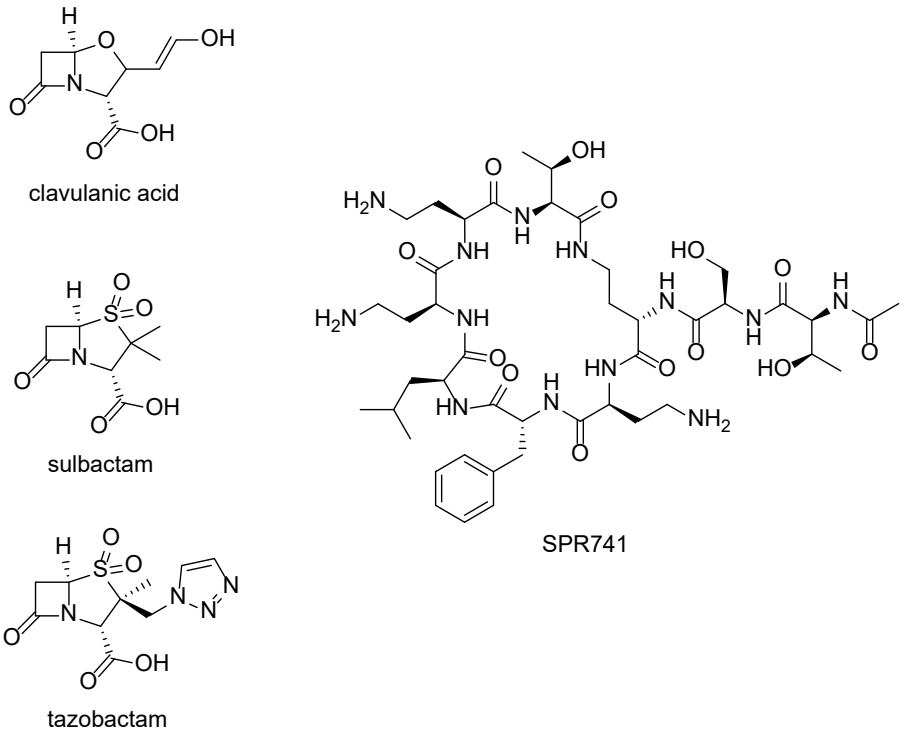



**Figure 3.** Chemical structures of some β-lactamase inhibitors and SPR741

One of the most well-known applications of combination therapy is the use of β-lactam antibiotics with β-lactamase inhibitors.[45,46] β-lactam antibiotics, such as penicillins and cephalosporins, target bacterial cell wall synthesis but are susceptible to degradation by β-lactamase enzymes produced by resistant bacteria. By co-administering a β-lactamase inhibitor like clavulanic acid, sulbactam, or tazobactam (**Figure 3**), this enzyme is neutralized, allowing the antibiotic to retain its activity.[47,48] This principle has led to the development of

several widely used combinations, including amoxicillin-clavulanate, piperacillin-tazobactam, and ceftolozane-tazobactam, which are effective against a broad range of Gram-positive and Gram-negative pathogens.

Another promising avenue involves the combination of antibiotics with non-antibiotic adjuvants that enhance bacterial membrane permeability or disrupt resistance mechanisms.[49] One notable example is SPR741, a polymyxin-derived adjuvant that increases the outer membrane permeability of Gram-negative bacteria, allowing otherwise ineffective antibiotics to reach their intracellular targets (**Figure 3**). SPR741 has been studied in combination with rifampicin, erythromycin, and other agents against pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*, showing enhanced efficacy *in vitro* and in animal models.[50,51]

Combination therapy can also involve the use of two or more antibiotics that act on different targets within the bacterial cell.[52] For instance, the pairing of colistin with carbapenems has been explored for treating carbapenem-resistant Enterobacteriaceae, while vancomycin combined with gentamicin is used for treating enterococcal infections.[53] These combinations aim to exploit the strengths of each antibiotic while compensating for their individual limitations.

Despite its advantages, combination therapy is not without challenges. The potential for antagonistic interactions between drugs is a significant concern, as certain combinations may reduce overall efficacy.[43] Furthermore, the increased risk of toxicity due to additive side effects requires careful dose optimization and monitoring.[54,55] The selection of appropriate drug combinations also depends on the pathogen's susceptibility profile, site of infection, and the pharmacokinetic and pharmacodynamic properties of the agents involved.[56] Another limitation is the lack of robust clinical data for many combination regimens. While *in vitro* studies and animal models can provide valuable insights, translating these findings into clinical practice requires well-designed clinical trials.[57] Additionally, the cost and complexity of combination therapy may limit its use in resource-constrained settings.

Despite these concerns, combination therapy remains a cornerstone strategy in the fight against AMR. By leveraging synergistic interactions and circumventing resistance mechanisms, combination regimens can restore the efficacy of existing antibiotics and improve clinical outcomes. Continued research, clinical validation, and integration with novel drug development efforts will be essential to fully realize the potential of this approach in addressing the global antibiotic resistance crisis.[58,59]

### 3.2 Antibiotic conjugates

Antibiotic hybrids represent a promising strategy in antimicrobial development aimed at overcoming resistance and expanding antibacterial coverage. This approach involves the covalent linkage of two distinct moieties—either two antibiotics or an antibiotic combined with a resistance-targeting agent—into a single hybrid molecule. Such hybrids can exert dual or complementary mechanisms of action, potentially enhancing bactericidal effects while reducing the risk of resistance emergence.[60,61]

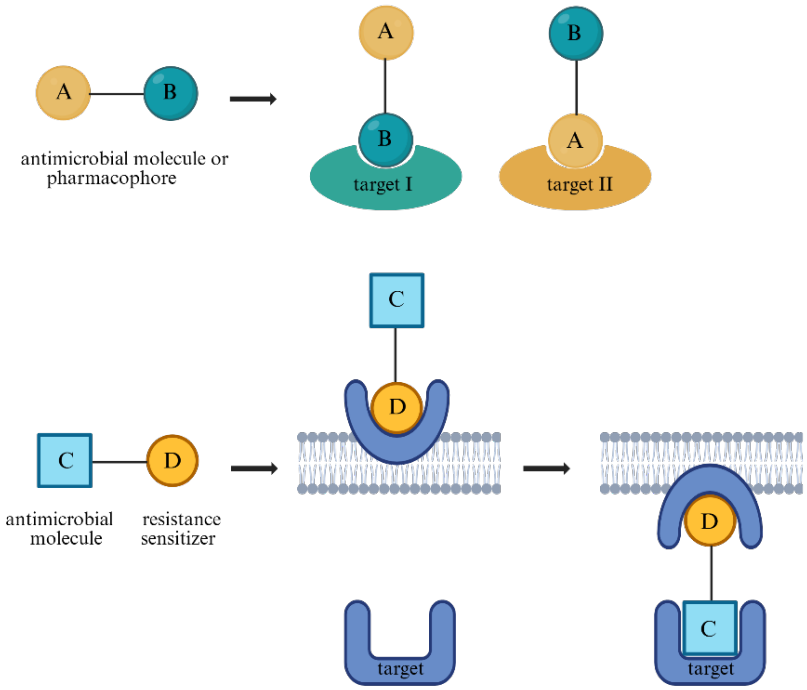



**Figure 4.** Antibiotic hybrid strategies. (A, B) Hybrids formed by covalent linkage of two antimicrobial molecules or pharmacophores. (C, D) Hybrids formed by covalent linkage of an antimicrobial molecule with a resistance-sensitizing molecule.

Building on the principles of combination therapy, antibiotic hybrids offer the advantage of ensuring simultaneous delivery of both active components within a single molecular entity.[62] This can lead to synergistic activity at the infection site and improved pharmacokinetic and pharmacodynamic profiles, optimizing efficacy and minimizing toxicity.[63-65] Moreover, by combining functionalities in one compound, hybrids may better evade bacterial defense mechanisms such as efflux pumps and enzymatic degradation.[66] **Figure 4** illustrates general

antibiotic hybrid design strategies and **Figure 5** provides the structures of some specific examples of hybrid antibiotics, highlighting the covalent linkage of either two antimicrobial pharmacophores or an antimicrobial molecule paired with a resistance-sensitizing partner.

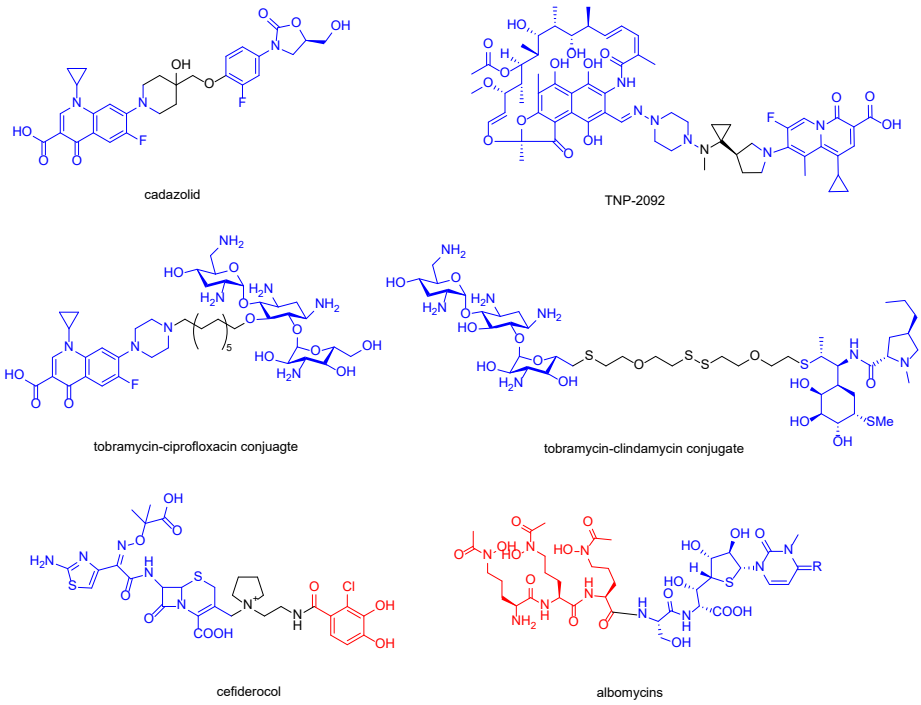



**Figure 5.** Examples of antibiotic conjugates, with the antibiotic shown in blue and the siderophore shown in red.

A prominent class of antibiotics often used in hybrids is are the fluoroquinolones. Fluoroquinolones, such as ciprofloxacin and moxifloxacin, act by inhibiting bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication and transcription. Researchers have developed fluoroquinolone hybrids by linking them to other bioactive agents, such as oxazolidinones or aminoglycosides, to create dual-function compounds that target both DNA synthesis and protein synthesis.[67,68] This dual-target approach has shown promise in preclinical models against multidrug-resistant (MDR) bacterial strains, particularly Gram-positive pathogens.[69,70]

Aminoglycoside antibiotics have also been used to generate various hybrids. For example, tobramycin, an aminoglycoside antibiotic that inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit, has been chemically modified by attachment of additional

antimicrobial moieties. Some of these hybrids exhibit enhanced membrane permeability and activity against biofilm-forming bacteria such as *Pseudomonas aeruginosa*, a common pathogen in cystic fibrosis and chronic wound infections.[71-73] By improving uptake and retention in bacterial cells, the tobramycin hybrids show improved therapeutic potential compared to the parent compound.

Another innovative hybrid strategy is exemplified by the Trojan horse mechanism, such as that employed by the clinically-used cefiderocol. This siderophore-cephalosporin conjugate exploits bacterial iron uptake systems to gain entry into cells.[74] By mimicking iron-chelating molecules, cefiderocol is actively transported through the outer membrane of Gram-negative bacteria via iron transporters, bypassing permeability barriers. Once inside, enzymatic cleavage releases the active antibiotic, which then inhibits cell wall synthesis in the same manner as traditional β-lactams.[75-77] This mechanism not only enhances penetration into Gram-negative pathogens but also reduces susceptibility to efflux pumps and β-lactamases, offering a potent weapon against carbapenem-resistant organisms. Interestingly, some natural product antibiotics also employ a Trojan horse mechanism such as the bacterial serinyl tRNA synthetase inhbitor albomycin produced by strains of *Streptomycetes.*

Hybrid antibiotics, while promising, face several key challenges. A primary issue is their often high molecular weight, which can hinder penetration through the outer and inner membranes of Gram-negative bacteria, particularly by limiting passage through nonselective porin channels. This permeability barrier reduces their effectiveness against these pathogens.[78] Chemical synthesis is another challenge, as linking two distinct pharmacophores without disrupting their individual biological activities requires careful design of the connecting linker. The linker must maintain stability and avoid eliciting a toxic reaction, while preserving the essential pharmacophoric regions of each component. Additionally, optimizing the pharmacokinetic and pharmacodynamic properties of hybrids is complex. Differences in solubility, metabolism, and tissue distribution between the combined moieties can impact efficacy and safety. Finally, although hybrid antibiotics aim to reduce the emergence of resistance by targeting multiple mechanisms simultaneously, bacteria's adaptability means resistance can still develop. This necessitates ongoing surveillance and resistance monitoring as these agents move into clinical use.

In conclusion, antibiotic hybrids represent a compelling strategy in the fight against antimicrobial resistance. By merging the mechanisms of existing agents into a single molecule,

these compounds offer the potential for enhanced efficacy, reduced resistance, and improved pharmacological properties. While scientific and regulatory challenges remain, the success of some modern hybrid agents such as cefiderocol provides a strong foundation for future innovation. Furthermore, integrating hybrid antibiotics with targeted delivery systems, such as nanoparticles or liposomes, may enhance their therapeutic index and reduce off-target effects. As research continues to refine this approach, antibiotic hybrids are poised to become a valuable addition to the antimicrobial arsenal, particularly against MDR and XDR pathogens.

### 3.3 Natural Products in Antibiotic Development

Natural products have been central to antibiotic discovery, yielding many of the most effective agents in clinical use, including penicillin, vancomycin, and rifampicin.[35,79] Produced primarily by soil-dwelling bacteria, fungi, and certain plants as chemical defenses, these compounds possess diverse structures and unique mechanisms of action that are often difficult to replicate synthetically.[80] During the "golden era" of antibiotic discovery (1940s–1960s), microorganisms such as *Streptomyces* were found to produce numerous breakthrough drugs, although the pace of innovation slowed in later decades due to repeated rediscovery of known molecules.[31]

Advances in genomics, metagenomics, and synthetic biology have since renewed interest, uncovering the vast, untapped biosynthetic potential of both culturable and unculturable microbes.[81] From 1981 to 2019, analyses of approved small-molecule drugs show that over two-thirds were either natural products, derivatives, or synthetic agents are inspired by natural scaffolds. In the antibacterial field, this influence is even greater—more than 70% of approved agents in that period originated from natural products or their modified forms.[39] This enduring dominance reflects their unparalleled structural diversity, evolutionary fine-tuning for biological activity, and capacity to overcome resistance mechanisms.[16] Even as synthetic chemistry continues to advance, natural product scaffolds remain indispensable for creating new antibacterial agents and sustaining innovation in the fight against antimicrobial resistance. **Figure 6** provides an overview of some clinically importamt natural product antibiotics.

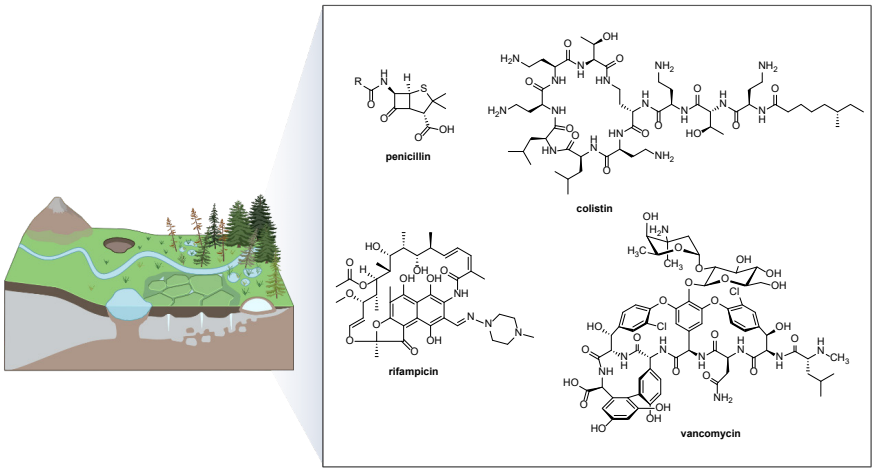



**Figure 6.** Examples of antibiotics derived from natural products

Vancomycin, originally isolated from culture of *Amycolatopsis orientalis*, is a glycopeptide antibiotic that functions by inhibiting bacterial cell wall synthesis. It achieves this by binding tightly to the d-Ala-d-Ala terminus of peptidoglycan precursors, thereby preventing the polymerization and cross-linking necessary for maintaining bacterial cell wall integrity. This mechanism makes vancomycin particularly effective against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*.[82,83]

Rifamycins, produced by *Streptomyces rifamycinica*, act by inhibiting bacterial RNA synthesis. They bind specifically to the β-subunit of RNA polymerase, thereby blocking the elongation of RNA chains. This mechanism makes them particularly effective against *Mycobacterium tuberculosis*, and rifampicin remains a cornerstone of tuberculosis treatment. Rifamycins are also used in the management of leprosy and certain staphylococcal infections.[23,84]

Polymyxins, including polymyxin B and polymyxin E (colistin), are cyclic lipopeptides derived from *Bacillus polymyxa*. These antibiotics interact with the outer membrane of Gram-negative bacteria by binding to lipopolysaccharides (LPS), disrupting membrane integrity, and ultimately leading to cell lysis. Because of their effectiveness against multidrug-resistant Gram-negative pathogens, such as carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*, polymyxins have been reintroduced in recent years as a last-resort therapy.[85,86]

Several other natural-derived antibiotics demonstrate the continued relevance and diversity

of this class. Daptomycin, a cyclic lipopeptide from *Streptomyces roseosporus*, acts by causing calcium-dependent depolarization of the bacterial membrane, effectively killing Gram-positive organisms.[87] Fidaxomicin, a macrocyclic antibiotic used for *Clostridium difficile* infections, targets bacterial RNA polymerase but in a different manner than rifamycins, offering a narrow-spectrum approach that spares normal gut flora.[88] Other examples include thuricin and lantibiotics, which are bacteriocins produced by Gram-positive bacteria. These agents often exhibit narrow-spectrum activity and have limited impact on beneficial microbiota, making them attractive for targeted therapies.[89,90]

Despite their potential, natural products pose several challenges in the drug development process. One major hurdle is the frequent rediscovery of previously known compounds, which has historically slowed progress in natural product screening.[35] In addition, the structural complexity of many natural compounds can complicate efforts to synthesize or modify them, thereby limiting the ability to improve pharmacological properties.[91] Another challenge lies in their toxicity and pharmacokinetic profiles. Some natural-derived antibiotics have poor solubility, limited bioavailability, or toxic side effects that hinder clinical application.[92] Furthermore, the regulatory pathways for these complex molecules are often more demanding, requiring extensive characterization and validation.[39]

To overcome these limitations, researchers are employing a variety of modern approaches. Genome mining enables the identification of previously silent biosynthetic gene clusters that may produce novel compounds.[93] Metagenomics allows access to the vast diversity of uncultured microbes, while synthetic biology techniques make it possible to engineer microbial strains to produce or enhance desirable molecules. Chemoenzymatic synthesis also offers a way to generate complex natural products through a combination of biological and chemical processes.

## 4.Future Perspectives and Outlook

The escalating threat of antimicrobial resistance demands sustained and coordinated innovation across multiple fronts of antibiotic development. The three strategies discussed above—combination therapy, antibiotic hybrids, and natural product–driven discovery—each presents complementary avenues for innovation, and their continued advancement will be essential to staying ahead in the race against resistant bacteria.

Combination therapy will benefit from deeper integration of pharmacokinetic/pharmacodynamic modeling, rapid diagnostics, and AI-driven synergy prediction tools.[94,95] Future efforts should also explore adjuvant-based regimens, where non-antibiotic compounds enhance penetration, inhibit resistance mechanisms, or disrupt biofilms, thereby restoring the potency of existing antibiotics. Such approaches are especially promising for Gram-negative pathogens, where permeability barriers and efflux pumps severely limit treatment options.[96]

Antibiotic hybrids are emerging as a particularly versatile strategy, capable of delivering dual mechanisms within a single molecule and overcoming multiple resistance barriers simultaneously.[15] Several hybrids—such as cadazolid, CBR-2092 (TNP-2092), and MCB3837—are progressing through clinical trials, and the siderophore-cephalosporin conjugate cefiderocol has already been approved.[76,97-99] The success of cefiderocol is encouraging, demonstrating that rationally designed hybrids can retain potency against some of the most recalcitrant bacterial threats.[100] We anticipate a growing number of hybrid agents entering clinical development, both as stand-alone antibiotics and as adjuncts to existing therapies.

Natural products will remain a cornerstone of antibiotic innovation, but future discovery must rely less on traditional culture-based screening and more on genome mining, metagenomics, and synthetic biology to access the vast biosynthetic potential of both culturable and unculturable microorganisms.[101] Coupled with structure-guided semi-synthetic modification, these tools can optimize pharmacological properties while preserving the unique activity of natural scaffolds.[102] The integration of AI-based structure–activity prediction and high-throughput biosynthetic engineering is likely to accelerate the translation of new natural products into clinically viable agents.[103]

Beyond these specific approaches, a forward-looking strategy against AMR will necessitate the integration of diagnostics, stewardship, and surveillance into the drug development pipeline. Rapid, point-of-care diagnostics will allow for more targeted therapy, minimizing unnecessary antibiotic use and slowing resistance evolution. Global coordination—through shared resistance databases, harmonized clinical trial frameworks, and equitable drug access policies—will be critical to ensuring that emerging therapies achieve widespread and sustained impact. Ultimately, the fight against AMR will hinge not on a single breakthrough, but on the sustained, synergistic application of diverse strategies. By combining scientific innovation with policy commitment and equitable implementation, the coming decades may witness a revitalization of the antibiotic arsenal and a restoration of the balance in our ongoing evolutionary struggle against bacterial pathogens.

## 5.Scope of the Thesis

The chapters of this thesis describing original experiment work (**Chapters 2-5**) are broadly concerned with the developed and discovery of novel antibacterials. **Chapter 2** describs investigations into the design, synthesis, and antibacterial evaluation of carbapenem/metallo-β-lactamase (MBL) inhibitor conjugates as a strategy to restore carbapenem activity against MBL-producing pathogens. MBLs hydrolyze carbapenems through zinc-dependent catalysis and represent a critical resistance mechanism for which no clinically approved inhibitors currently exist. In this work, selected carbapenem scaffolds are covalently linked to MBL-inhibiting moieties to generate hybrids with both β-lactam antibacterial activity and MBL inhibiting activity. The conjugates' antibacterial potency is evaluated against MBL-producing and non-MBL-producing strains, while enzyme inhibition assays quantify activity against purified MBL enzymes. Stability studies are conducted to assess hydrolytic resilience in relevant biological media. These findings aim to advance the development of antibiotic–inhibitor conjugates as a therapeutic approach to overcome clinically relevant carbapenem resistance.

**Chapter 3** explores the design, synthesis, and antibacterial evaluation of rifampicin–siderophore conjugates as a targeted strategy to improve the efficacy of rifampicin and address emerging resistance. The concept builds on the "Trojan horse" approach, in which siderophores, naturally produced by bacteria for iron acquisition, are exploited to actively transport covalently linked antibiotics into bacterial cells via specific uptake pathways. In this study, carefully selected siderophore moieties are covalently attached to the rifampicin scaffold through linkers optimized to maintain both iron-binding and antibacterial properties. Their antibacterial activity is assessed against both rifampicin-susceptible and resistant pathogens, with additional evaluation of cellular uptake efficiency. Structure–activity relationships are examined to understand the influence of siderophore type and linker design on biological performance. The findings provide insight into the potential of siderophore–antibiotic conjugates to enhance drug delivery and combat resistant bacterial infections.

**Chapter 4** explores the rediscovery and characterization of poriolide and isoporiolide, two plant-derived natural products isolated from *Leucothoe zeblid*, focusing on their dual antibacterial and anticancer activities. Utilizing a broad screening of 2240 plant extracts, this research investigates antibacterial effects against multiple bacterial strains, including drug-resistant species, and evaluates anticancer potency across diverse human cancer cell lines. The work includes bioassay-guided fractionation, compound isolation, structural identification,

and mechanistic studies of poriolide's anticancer activity, particularly its ability to induce cell cycle arrest. Our results highlight the challenges in natural product drug discovery, such as compound yield and toxicity, and underscores the potential of integrating antibacterial and anticancer screening for multifunctional therapeutic agent development. This work aims to provide foundational data supporting further preclinical investigation of poriolide as a promising candidate for anticancer drug development.

**Chapter 5** further investigates poriolide, a natural product with potent and selective anticancer activity, as a novel payload for antibody–drug conjugates (ADCs). The work starts with an investigation into the importance of its hydroxyl groups for biological activity and progresses with the identification of the 1-OH group as a suitable site for chemical modification and linker attachment. Selective functionalization strategies were developed to enable precise linker attachment. Two model prodrug derivatives incorporating self-immolative spacers were synthesized and evaluated for controlled, pH-responsive drug release. Building on these results, ADC-compatible linker–drug conjugates using cleavable (Val-Cit–PABC) and non-cleavable linkers were designed and synthesized to facilitate targeted intracellular payload delivery. The work establishes a synthetic framework for future antibody conjugation and supports poriolide's promise as a potent, selective ADC payload.

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