Comprehensive Overview of Penicillin

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Abstract

Penicillin is a β-lactam antibiotic produced by certain Penicillium species. It was the first antibiotic found and first mass produced for wide clinical use as a medicine by Alexander Fleming in 1928. Initial preparations of penicillin were unstable, very difficult to purify and not suitable for large scale clinical use. It was the work of Howard Florey, Ernst Chain and others in the late 1930s and early 1940s on the extraction and purification of penic.

Penicillin and its derivatives cause bacterial cell lysis by inhibiting bacterial cell wall synthesis by binding to penicillin-binding proteins. The β-lactam ring is the structure which is responsible for the antibacterial activity as well as the chemical instability of penicillins. As for many other antibioticsase inhibitors led to an extended spectrum of action as well as to an increased resistance to the bacterial β, the development of semisynthetic penicillins as well as of β-lactam and ampicillin, are among the most prescribed antibiotics worldwide and are still of major interest for the-lactamases. Today, penicillin and its derivatives, e.g. amoxicillin treatment of respiratory tract, skin, urinary tract and also of systemic infections. This review will give an overview on the history of discovery, the structure as well as the biosynthesis of penicillins and on their mechanism of action. Furthermore, it will describe the production of these drugs on an industrial scale, the resistance to penicillins, their pharmacokinetics as well as their clinical application. Finally, an outlook on the current use and future perspectives of penicillins will be given.

In view of the persistence of penicillin and its derivatives (amoxicillin and ampicillin for instance) as among the most widely used antibiotics world-wide, for treatment of most kinds of bacterial infections including respiratory tract infections, of most skin and soft tissue infections, many urinary tract infections, and of systemic (bloodstream) infections, this review provides a fully comprehensive description of all the main aspects, concerning the history of its discovery, the chemical structure of the molecule and of semisynthetic analogs, the biological synthesis of the molecule, the mechanism of action by which the molecule kills bacteria, details of the commercial production of the molecule, reasons for bacterial resistance, pharmacology and clinical use of penicillin and of its many semisynthetic derivatives.

Keywords

Penicillium chrysogenum; β-lactamase; Thiazolidine ring; Ampicillin; Amoxicillin; Fermentation; Clavulanic Acid

Introduction

Penicillin refers to a group of broad-spectrum beta-lactam antibiotics originally derived from and produced by certain species of the fungus Penicillium. It was the first antibiotic to be discovered and was the first to be widely used. Its discovery marked a revolution in the field of medicine and has greatly contributed to the field of antibiotic treatment [1].

Penicillin consists of a conserved bicyclic core structure of 6-aminopenicillanic acid that is a highly strained beta-lactam ring fused with a thiazolidine ring, giving antibacterial activity [2]. During the chemical synthesis process of penicillin, it is often cleaved at crucial steps and purified using pH adjustments and crystallization. The beta-lactam ring is covalently attached to the penicillin-binding proteins (PBPs). The PBPs are involved in bacterial cell wall biosynthesis, resulting in cell wall defects and bacterial lysis. The side chain attached to the beta-lactam ring of penicillin allows for modification of penicillin derivatives to improve their stability, spectrum of activity, and pharmacokinetics [3]. Penicillin is used as a medication to treat bacterial infections, such as those caused by susceptible Gram-positive bacteria, and is also used in respiratory, skin, and systemic infections [4].

The discovery of penicillin by Alexander Fleming was reported in 1928. He observed that a Penicillium mold culture had an inhibitory effect on the growth of Staphylococcus bacteria. The crude product was however, difficult to purify and to stabilize. The crude penicillin preparations were very chemically heterogeneous and unstable, no matter what the storage conditions, and they were not always suitable for therapeutic use in man or animals.

In the 1930s, the molecular structure of penicillin was not well understood, and various proposed structures were based on its behavior in the laboratory. Many models were proposed, such as a peptide structure as well as a variety of cyclic thioether structures. None of the proposed models could adequately explain the instability of penicillin under certain conditions nor fully utilize the antibacterial potential of the molecule. Consequently, the true structure of penicillin was not determined, and derivatives that could more fully utilize the activity of the molecule were not developed [5].

The discovery of penicillin’s structure in the late 1930s and early 1940s was made possible by Florey, Chain, and others' improvement of methods for culturing, extracting, and purifying penicillin to the point where they could obtain clinically useful amounts of a pure and stable form of penicillin. This, together with improvements in the fermentation and chemical processes during WWII, allowed the large-scale production of penicillin, making it available as a routine clinical medication. Further work on the beta-lactam ring allowed derived structures of the natural penicillins as well as synthetic penicillins to be synthesised, which provided more stable analogues with broader spectra of activity and better resistance to degradation by bacterial beta-lactamases. Penicillin quickly became a base compound from which many synthetic derivatives could be developed leading to a revolution in the field of antibiotic research [6].

Summary and Impact Penicillin Discovery, Structure, and Pharmacology: Past, Present and Future Penicillin has played a significant role in the field of antibiotic science, as the first and by all means, the most successful antimicrobial drug. This review deals with every aspect of penicillin including its discovery and history, its structure, biosynthesis, modes of action, total synthesis and manufacture, pharmacokinetics and metabolism, as well as second-generation semisynthetic derivatives and their analogs. The present work provides a completely integrated view of penicillin and its role in antibiotic science, highlighting its continued and lasting impact as a paradigm in the field of antibiotics and illustrating how penicillin can serve as a benchmark for the design and development of new drugs and thereby contribute to the future of antimicrobial therapeutics.

Despite the development of newer antimicrobial agents, penicillin and its semisynthetic derivatives continue to remain essential components of modern clinical practice. Antibiotics such as amoxicillin and ampicillin are still widely prescribed for respiratory tract infections, skin infections, urinary tract infections, and bacterial pneumonia. However, the increasing emergence of antimicrobial resistance and β-lactamase-producing bacteria has also driven continued research into modified penicillin derivatives and β-lactamase inhibitors. Therefore, penicillin remains both a historical breakthrough and an ongoing subject of pharmaceutical and clinical research.

Methods

A literature review was conducted using Google Scholar, PubMed, and the National Institutes of Health (NIH) to examine the biological significance, synthesis, fermentation, and pharmaceutical relevance of penicillin and related β-lactam antibiotics. Initial searches focused on "β-lactamase", "Thiazolidine ring" to evaluate structural knowledge of penicillin. The specific type of penicillin was then narrowed using keywords such as "ampicillin" and "Amoxicillin". Further searches included "Fermentation" and "Clavulanic Acid" for an understanding of natural penicillin production and β-lactamase inhibitors. Peer-reviewed articles were selected to support the analysis of enzymatic pathways, chemical mechanisms, modification, and clinical relevance.

Results and Discussion

1. Biological and medicinal significance and activity

1.1. Structure of beta-lactam

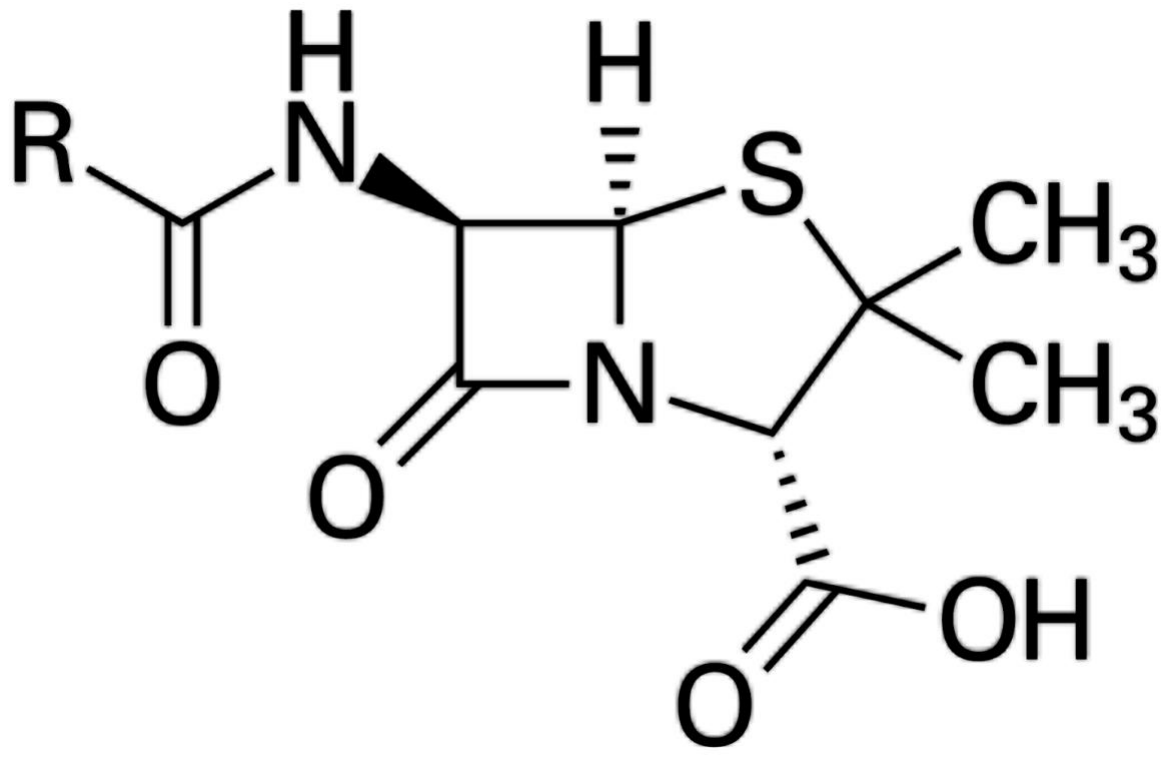


Figure 1. Structure of penicillin

β-lactam antibiotics are a class of antibiotics that are used in clinical practice in both oral and parenteral forms [7]. The β-lactam ring is a core chemical structure of a four-membered ring found in major classes of antibiotics that fight bacterial infection by inhibiting bacterial cell wall synthesis, making them bactericidal. The carbon on the β-lactam ring connected to an amine group has sp3 hybridization, which normally has a bond angle of 109.5 degrees. However, due to the rectangular four-membered ring, the bond angle has been restricted to 90 degrees, as shown in the figure above. The angle strain raises the energy and weakens the bonds, making the molecule very reactive.

1.2 Biological significance and activity

Paul Ehrillich, a German physician established the theory of selective toxicity which is the fundamental principle that makes the antibacterial agents work. Paul has defined antibacterial agents as “substances with an exclusive affinity for bacteria acting deleteriously or lethally on these alone, while at the same time, they possess no affinity for the normal constituents of the body…”. The selective toxicity of β-lactams, for example, is considered to be due to their affinity to penicillin-binding proteins (PBPs) and inhibition of biosynthesis of bacterial cell walls [8]. Penicillin has the unique ability to target the synthesis of peptidoglycan which is a complex polymer that forms the rigid bacterial cell wall. As penicillin inhibits the PBP that builds the essential bacterial component, it leads to cell lysis from osmotic pressure acting as a bactericidal. However, the fact that human cells are not made up of peptidoglycan makes penicillin deadly to bacteria while generally safe for the human body.

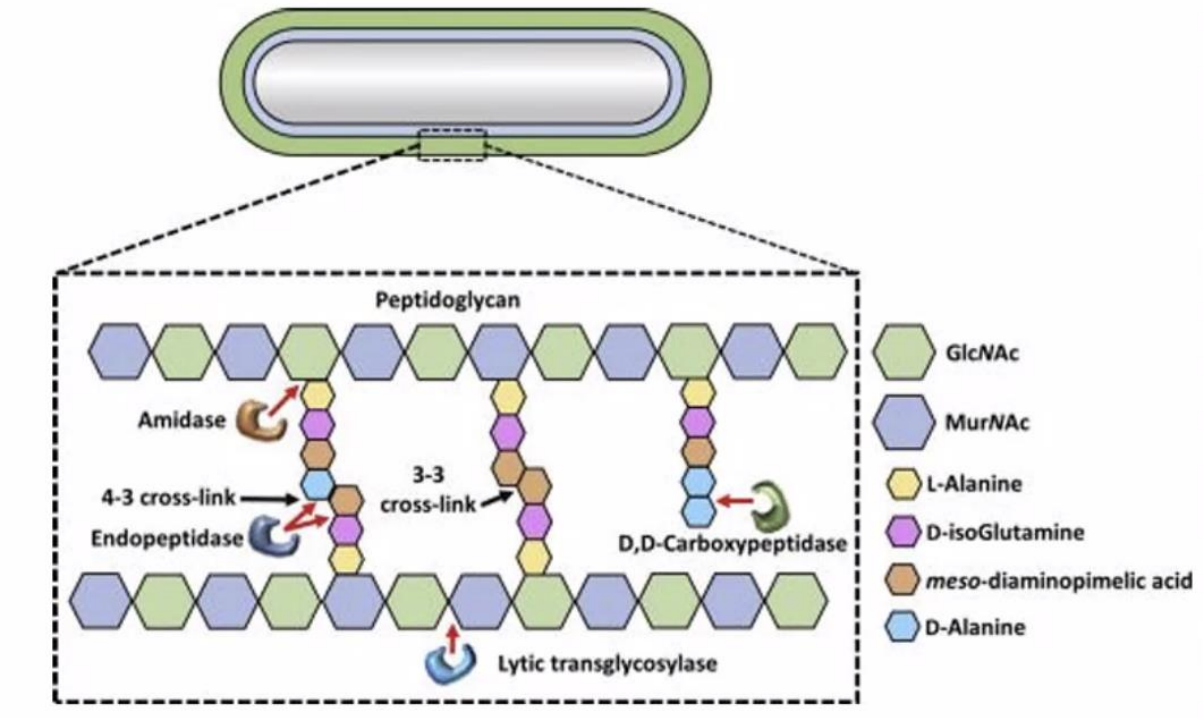


Figure 2. Biological context of penicillin actions

In making and maintaining the cell wall, bacteria have this polymer peptidoglycan which is composed of carbohydrates and short chains of amino acids. The catalyst for this reaction is PBP such as the enzyme DD-transpeptidase which carries out the cross-linking of the cell wall as well as the trimming of

peptidoglycan. The penicillin's beta-lactam ring binds to DD-transpeptidase to irreversibly inactivate cell wall synthesis of bacteria.

1.3 Medicinal significance and activity

After its discovery in 1928, penicillin has been widely used across the world in the field of medicine as the first true antibiotic. The discovery and development of penicillin marked a turning point in the medical field, facilitating the treatment of lethal infections into treatable diseases. Penicillin is mostly used with the treatment of pneumonia, syphilis and meningitis commonly caused by bacteria where penicillin is used to inhibit the synthesis of the bacterial cell wall ultimately killing the bacteria that cause these infections.

Furthermore, penicillin controlled the infections, enabling modern surgery, cancer chemotherapy, and organ transplantation. Before the discovery of penicillin, even clean operations often led to deadly postoperative infections. However, now, with the use of penicillin, it prevents and treats surgical site infections, specifically common bacteria like *Staphylococcus* and *Streptococcus*. The effectiveness and safety of the use of penicillin has been significant in medical value. Penicillin has a high therapeutic index, meaning that the toxic dose is much higher than the effective dose. It further implies that a drug has a wide margin of safety between the dose that produces a therapeutic effect and the dose that causes toxicity, making it relatively safe for clinical use. Therefore, with a low dose, penicillin is effective and well-tolerated. Moreover, with the widespread use of penicillin, it is inexpensive and affordable, which remains relatively inexpensive compared with many newer antibiotics [9].

Taking in consideration of current clinical guidelines, penicillin and its derivatives are the first-line treatments for multiple bacterial infections. For example, natural penicillins of penicillin G and V are still recommended by various guidelines such as the Infectious Diseases Society of America known as IDSA for treating streptococcal pharyngitis, syphilis, and pneumonia mainly caused by *Streptococcal pneumoniae*. More derivatives of penicillin includes amoxicillin and ampicillin which are currently recommended for conditions like otitis media, urinary infections, and others caused by both gram-positive and negative bacteria. They are prescribed to minimize resistance development but also cure infections from bacteria quickly within a short period of time.

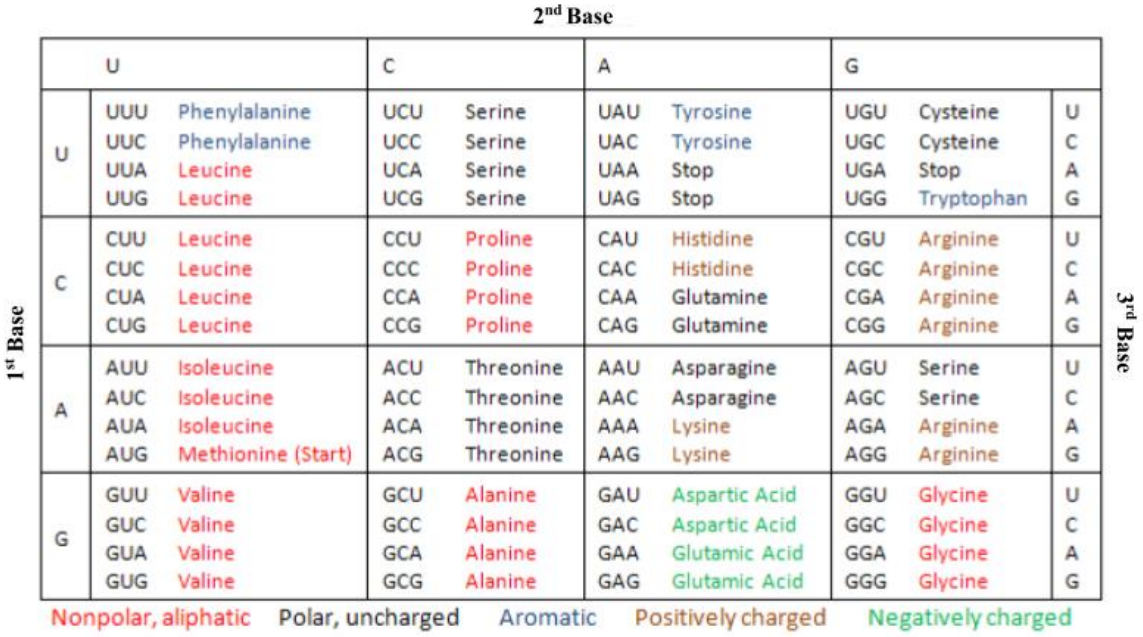
2. Biological mechanism of action

2.1 Role of penicillin as a mechanism based inhibitor

Mechanism-based inhibition is a type of inhibition where a competitive inhibitor irreversibly binds to the active site, to permanently inactivate the enzyme. This happens when the beta-lactam ring is opened and becomes activated, forming a covalent bond with the threonine amino acids located in the active site of the transpeptidase enzyme. The binding is irreversible and permanently disallows the transpeptidase enzyme from functioning. The permanent inactivation of transpeptidase leads to cell lysis under osmotic pressure. As the transpeptidase is inactivated, the enzymes that create new layers of peptidoglycan are inactivated while enzymes that break down old layers continue to function. Therefore, continuous breakdown of the old peptidoglycan layer while inhibition of cell wall synthesis causes the cell wall to become thinner which results in cell lysis when water is taken up.

2.2 Process by which bacteria become resistant to penicillin

2.2.1 Base substitution mutation



1st Base	2nd Base: U		2nd Base: C		2nd Base: A		2nd Base: G		3rd Base
U	UUU	Phenylalanine	UCU	Serine	UAU	Tyrosine	UGU	Cysteine	U
	UUC	Phenylalanine	UCC	Serine	UAC	Tyrosine	UGC	Cysteine	C
	UUA	Leucine	UCA	Serine	UAA	Stop	UGA	Stop	A
	UUG	Leucine	UCG	Serine	UAG	Stop	UGG	Tryptophan	G
C	CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine	U
	CUC	Leucine	CCC	Proline	CAC	Histidine	CGC	Arginine	C
	CUA	Leucine	CCA	Proline	CAA	Glutamine	CGA	Arginine	A
	CUG	Leucine	CCG	Proline	CAG	Glutamine	CGG	Arginine	G
A	AUU	Isoleucine	ACU	Threonine	AAU	Asparagine	AGU	Serine	U
	AUC	Isoleucine	ACC	Threonine	AAC	Asparagine	AGC	Serine	C
	AUA	Isoleucine	ACA	Threonine	AAA	Lysine	AGA	Arginine	A
	AUG	Methionine (Start)	ACG	Threonine	AAG	Lysine	AGG	Arginine	G
G	GUU	Valine	GCU	Alanine	GAU	Aspartic Acid	GGU	Glycine	U
	GUC	Valine	GCC	Alanine	GAC	Aspartic Acid	GGC	Glycine	C
	GUA	Valine	GCA	Alanine	GAA	Glutamic Acid	GGA	Glycine	A
	GUG	Valine	GCG	Alanine	GAG	Glutamic Acid	GGG	Glycine	G

Nonpolar, aliphatic Polar, uncharged Aromatic Positively charged Negatively charged

Figure 3. Genetic code and RNA Codon Table

One process by which bacteria become resistant to penicillin is by base substitution mutation, a natural process that happens randomly and spontaneously. The base substitution mutation involves change in a particular base to another base therefore, leading to the production of a different codon in the bacterial mRNA. This mutation occurs in the gene that codes for the bacterial enzyme transpeptidase. The codon which originally coded for threonine, is mutated into a different codon that codes for serine. The threonine amino acids in the active site of transpeptidase are replaced by serine where penicillin are no longer able to bind to the active site due to conformational change of the active site.

2.2.2. β-lactamase acylation mechanism

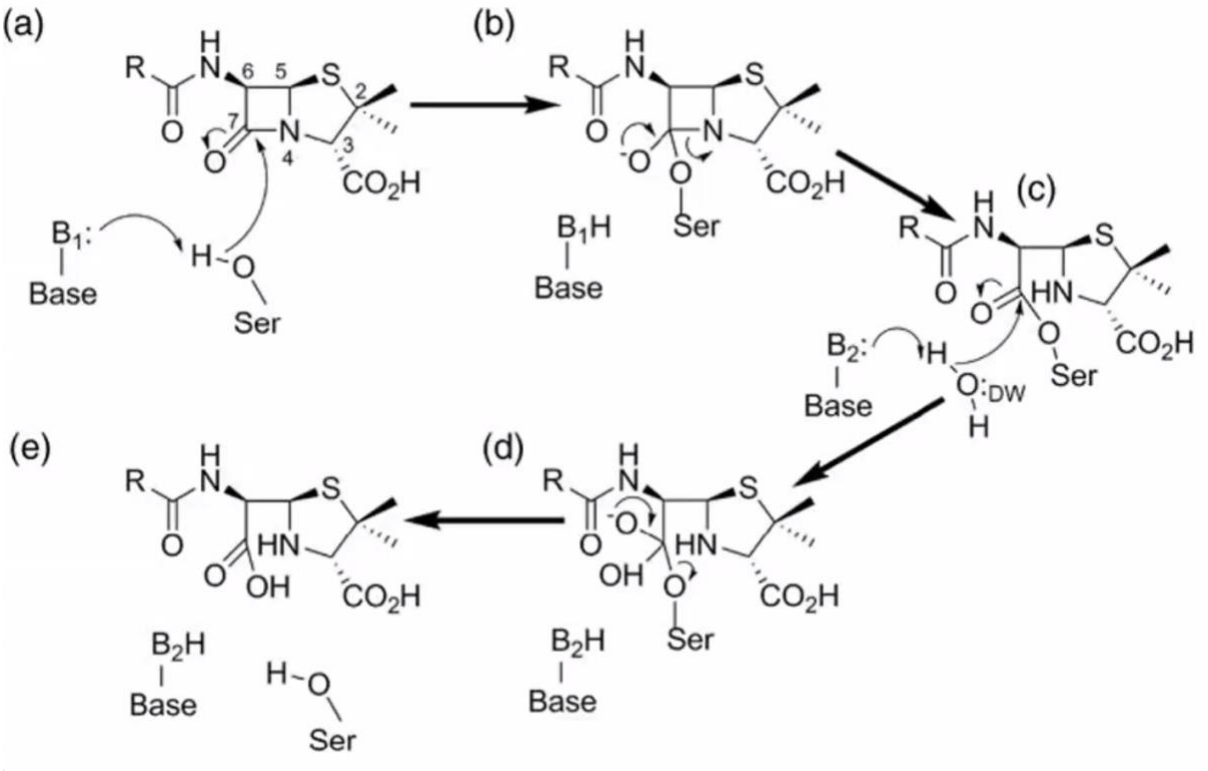


Figure 4. Mechanism overview of serine β-lactamase

Another process by which bacteria become resistant to penicillin is β-lactamase acylation. β-lactamase is a bacterial enzyme that inactivates β-lactam antibiotics, both produced by gram-positive and gram negative bacteria by breaking the core chemical structure of the β-lactam ring.

Figure 4. shows the hydrolysis of generic penicillin substrate due to serine β-lactamase. (a) General base B1 activates Ser for nucleophilic attack on the amide carbonyl carbon (C7) generating covalent acylenzyme (c) via tetrahedral oxyanionic acylation transition state (b). General base B2 activates incoming deacylating water molecule (DW) for nucleophilic attack on the acylenzyme carbonyl liberating penicilloate product (e) via tetrahedral deacylation transition state (d). [10]

Overall, there are two major reactions involved: acylation and hydrolysis. In the acylation reaction, the serine β-lactamase nucleophilic serine residue attacks the substrate β-lactam ring of the penicillin, yielding an ester-linked acyl-enzyme complex. In the hydrolysis reaction, the nucleophilic attack of a water molecule onto the acyl-enzyme complex carbonyl occurs, hydrolysing the antibiotic and releasing the serine.

Therefore, bacteria can become resistant to penicillin by a natural process of base substitutional mutation or through the production of serine β-lactamase. The base substitution mutation involves mutation of the codon that codes for threonine to serine restricting from the binding to the active site. While a mechanism involving serine β-lactamase inactivates the antibiotic via acylation followed by hydrolysis reactions.

3. Biological mechanism & clinical use

3.1 Biological synthesis

Natural penicillins are β-lactam antibiotics biosynthesized by fermenting the fungus *Penicillium chrysogenum,* which includes Benzylpenicillin (Penicillin G) and Phenoxymethylpenicillin (Penicillin V). The specific type of natural penicillin production is determined by the precursor added to the fermentation medium, where phenylacetic acid yields Benzylpenicillin(penicillin G), and phenoxyacetic acid yields Phenoxymethylpenicillin (Penicillin V) [11].

The process starts with the precursors in the cytosol, which are three amino acids: L-α-aminoadipic acid(L-α-AAA), L-cysteine, and L-valine. These amino acids are condensed into a tripeptide δ-(l-α-aminoadipyl)-l-cysteinyl-d-valine (LLD-ACV) by a nonribosomal peptide synthetase(NRPS) δ-(l-α-aminoadipyl)-l-cysteinyl-d-valine synthetase (ACVS) that catalyzes the formation of a linear tripeptide, which is encoded by pcbAB [12,13,14,15]. The tripeptide LLD-ACV is oxidized into isopenicillin N by the enzyme isopenicillin N synthase(INPS), which is encoded by pcbC [13,16]. All penicillins have a bicyclic ring structure, a four-membered β-lactam ring linked to a five-membered thiazolidine ring, which is formed when the tripeptide precursor undergoes oxidative ring closure.

Here, the amide functional group and sulfhydryl group lose hydrogen and form a part of the penicillin core, forming the β-lactam ring, which is responsible for penicillin's antibacterial activity. Finally, the side chain of isopenicillin N, which is α-aminoadipyl, is replaced with a new side chain by phenoxyacetyl-CoA, catalyzed by isopenicillin N acyltransferase(IAT), encoded by penDE [17,18,19,20]. The formation of the side chain phenylacetyl group produces penicillin G, and the side chain phenoxyacetyl group, benzene ring with oxygen atom, produces penicillin V [21].

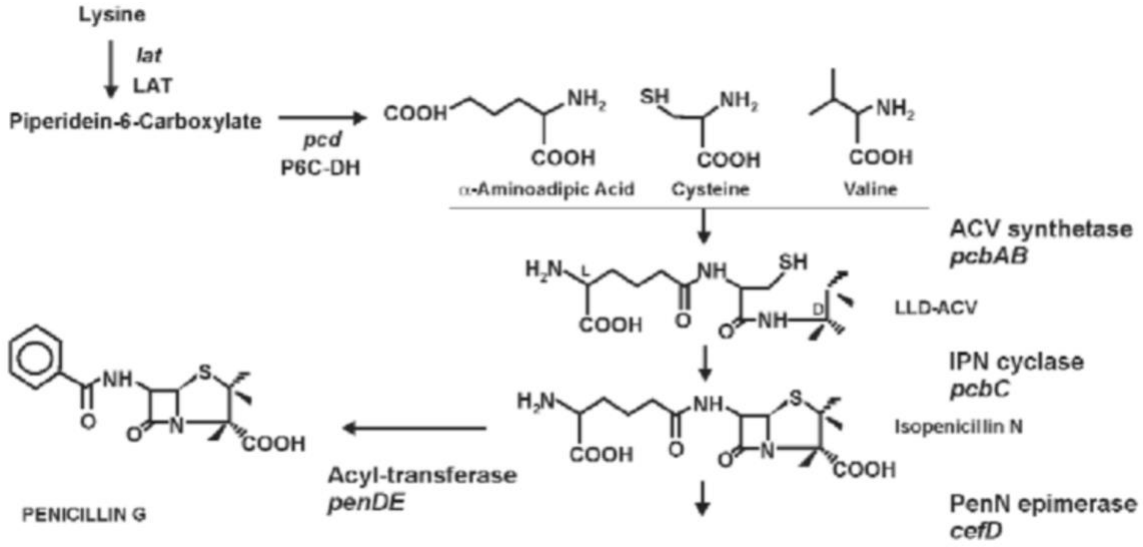


Figure 5. Biosynthetic pathway of penicillin in Penicillium chrysogenum [22]

Lysine is converted to α-aminoadipic acid, combining with cysteine and valine to form ACV tripeptide through ACV synthetase. The tripeptide forms the β-lactam core of penicillin, followed by side-chain replacement to produce penicillin G.

3.2 Active pharmaceutical ingredient (API)

The production of penicillin on the Active Pharmaceutical ingredient(API) involves complex fermentation and chemical purification. The typical stages in the production of API are fermentation, recovery, extraction, purification, crystallization, and quality control.

For the mass production of penicillin, fermentation takes place in stainless steel tank reactors that have a capacity of 30 to 100 thousand gallons. To increase the yield of the Penicillium chrysogenum strain, improvements in the medium composition, large industrial tanks, and sterile air are necessary. In current commercial manufacturing, strains of Penicillium chrysogenum are cultivated in continuously agitated 50,000-gallon stainless steel tanks by submerged fermentation, which results in 40-50 grams of penicillin per liter of culture with recovery yields up to 90% [24]. Recovery and extraction are needed for higher yield and stability. Historically, penicillin recovery relied heavily on conventional solvent extraction using organic solvents such as n-butyl acetate and amyl acetate [25,26]. Although effective, these methods required high solvent consumption and multiple extraction steps [25,26]. However, there are several drawbacks to utilizing conventional solvent extraction, which include high solvent consumption, the need for multiple extraction steps, lower yields, and a risk of emulsification [27]. Many other alternative extraction methods emerged. To address these limitations, researchers later proposed alternative recovery techniques. For example, Edmundowicz et al. developed a non-extractive recovery method. It resulted in an overall yield of approximately 56% by acidifying the fermentation broth without organic solvent extraction [28]. Furthermore, a water-oil emulsion that uses di-n-octylamine as a carrier, a liquid surfactant membrane method from Hano et al., allowed the prompt recovery of penicillin G; however, it had a drawback that required low membrane stability and organic solvents [27**].** Adsorption using hydrophobic resins by de Barros et al. recovered penicillin G. This method provided insights for adsorption-based recovery techniques; however, it faced difficulty in maintaining low temperatures as well as acidic pH levels, which are essential requirements for stability [29]. Following extraction, penicillin is then converted into a salt by separating the penicillin from culture liquors, which creates a concentrated but impure water solution. This step is essential as it reduces the volume by isolating the active penicillin ingredient from a large amount of fermentation broth.
Penicillin is combined with sodium and potassium, forming a penicillin salt concentrate. Water is removed by distillation, leaving the organic solvent [28].

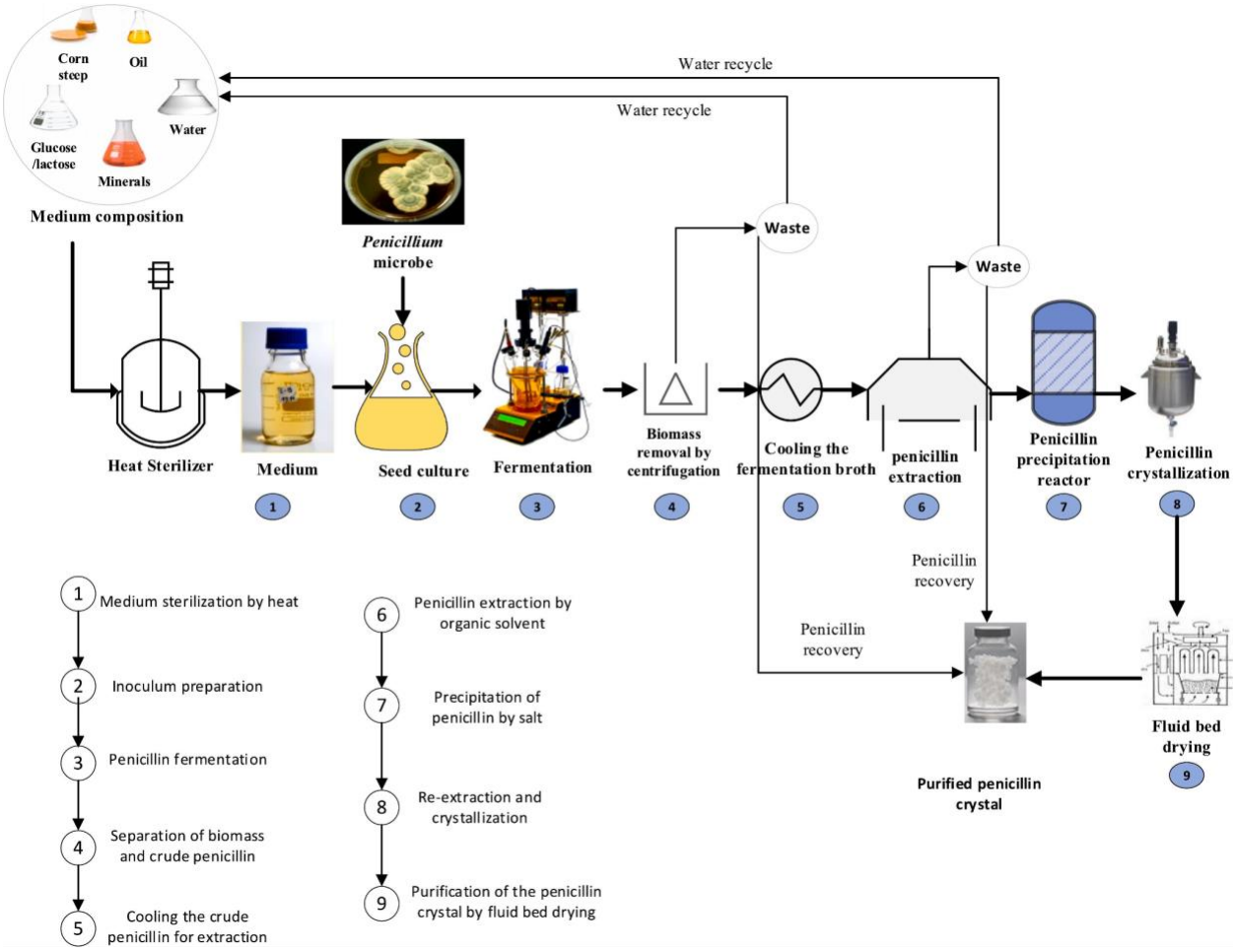


Figure 6. Overview of the current industrial penicillin API production process

API production starts with preparation and heat sterilization of the nutrient medium, with inoculum development and large-scale fermentation using the Penicillium species. After fermentation, biomass is removed by centrifugation and cooled. It is then extracted using an organic solvent and re-extracted for purification. The final steps include crystallization and drying of purified penicillin crystals [25].

Finally, the API is required to undergo quality controls confirmed by Good Manufacturing Practice(GMP). GMP affirms that medical products are appropriately used and controlled to the standards [28] The GMP for each country varies, including the United States' Food and Drug Administration(FDA), European Medicines Agency(EMA) for Europe, China Food and Drug Administration(CFDA) for China, and the World Health Organization(WHO), which sets international standards for pharmaceutical products. As penicillin has a strong allergenic potential, current GMP requirements require that it be produced in a fully separate facility to avoid contamination. Further quality control also includes impurity profiling, potency testing, and stability sampling over time [30].

4. Chemical synthesis

The first total synthesis by John Sheehan in 1957 confirmed penicillin's molecular structure and the possibility of it being produced independently of biological systems. It required the synthesis of the highly strained β-lactam ring and fused thiazolidine ring. This was a 5-step chemical synthesis involving the formation of the thiazolidine ring through condensation, deprotection of the amine, acylation of the side chain, deprotection of the carboxylic acid, and closure of the β-lactam ring [31,32,33,34]. Although Sheehan succeeded in penicillin's total synthesis, it resulted in an overall yield of about 0.7% [34]. This inefficiency prevented total synthesis from being used for industrial production. Still, the total synthesis laid its importance in advancing the field of β-lactam antibiotics by confirming its structure. Furthermore, the modern production of penicillin was achieved through fermentation, followed by semi-synthetic modification to create different penicillin derivatives. For this process, natural penicillin serves as a starting material and is then converted into a core intermediate 6–aminopenicillanic acid that removes the original side chain while retaining the original 6-APA structure [35,36]. Different acyl side chains can then be attached to create new antibiotics, modifying properties such as acid stability, oral bioavailability, and resistance to bacterial enzymes [37].

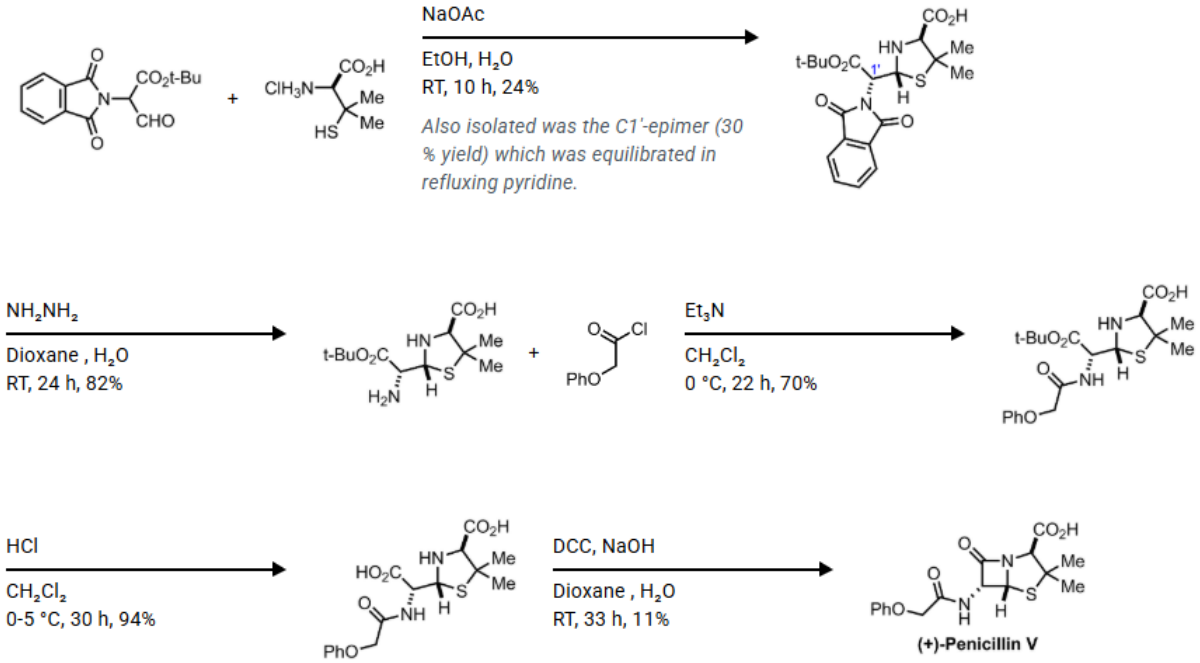


Figure 7. The 5 linear steps to John Sheehan's total synthesis [31]

5. Pharmacokinetics of selected penicillin drugs

When administered orally, Benzylpenicillin demonstrates low bioavailability (<30%) due to degradation by gastric acid. Thus, intravenous or intramuscular administration is the preferred route [38]. Benzylpenicillin gets distributed extensively throughout tissues and body fluids [39]. Benzylpenicillin had an elimination half-life of 0.54 hours when administered intravenously [40]. Approximately 40% of Benzylpenicillin is metabolized in the liver, with penicilloic acid as the primary metabolite. Renal excretion accounts for between 60%–90% of the elimination process [41].

Phenoxymethylpenicillin displays an oral bioavailability of 60%–70%, a huge surge from that of benzylpenicillin, due to its greater acid stability [38]. Following oral administration, phenoxymethylpenicillin shows extensive distribution into tissues and body fluids, like benzylpenicillin [39]. The elimination half-life ranges from 0.5 to 0.8 hours [41]. Its hepatic metabolism is limited, with penicilloic acid as the main metabolite, and renal clearance remains the main route for elimination [42,43].

Oral administration of Ampicillin leads to moderate absorption with a bioavailability of 30-55% [39]. Then, upon administration, ampicillin is broadly distributed in most tissues, body fluids, and organs. The elimination half-life of the drug is approximately 1.33 hours [46]. Its hepatic metabolism is limited to just 20% of a 250-500mg dose to healthy adults [45]. Elimination through the renal system via glomerular filtration and tubular secretion takes place in 12 hours following dosing of a small fraction of a dose (7%) [46,47].

Following oral administration, amoxicillin demonstrates a bioavailability of approximately 95%. The elimination half-life in adults ranges from 1 to 1.5 hours, and the drug shows extensive distribution into tissues and body fluids [48]. Amoxicillin undergoes haptic metabolism through oxidation, hydroxylation, and deamination pathways [49]. Renal excretion is the predominant elimination route, with 60-70% of an orally administered dose excreted unchanged in the urine within 6-8 hours [50].

5. 1 Second and further generation variants of penicillin

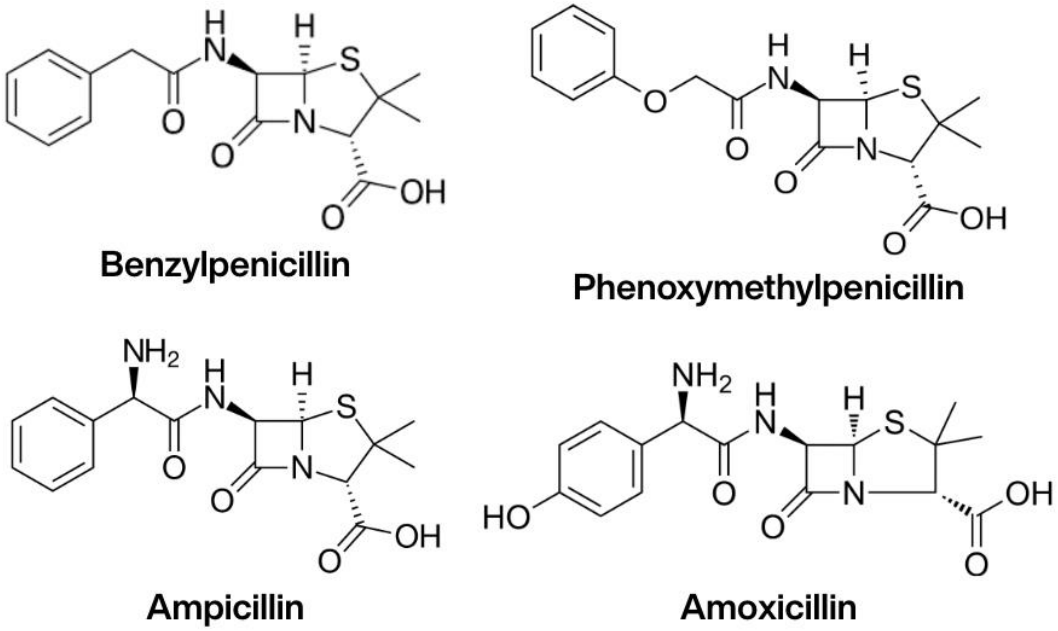


Figure. 8. Structures of common penicillin antibiotics

The development of the second-generation and later penicillin variants emerged as a result of semi-synthesis, driven by the need to overcome the limitations of natural penicillin, including a narrow antibacterial spectrum, poor oral bioavailability, and vulnerability to β-lactamase enzymes. Many of these derivatives still remain widely used in modern clinical practice and are frequently recommended in current treatment guidelines for bacterial infections.

Classified as aminopenicillins, second-generation variants such as ampicillin and amoxicillin are semisynthetic compounds derived from the penicillin parent. The primary structural distinction of ampicillin and amoxicillin (Figure 8) from other penicillins is the presence of an amino group attached to the β-lactam side chain. This amino group substitution was specifically designed to enhance penetration through the outer membrane of Gram-negative bacteria. Consequently, this structural modification broadened the antimicrobial spectrum beyond the predominantly Gram-positive coverage of earlier penicillins to include clinically important Gram-negative pathogens such as *Haemophilus influenzae*, *Escherichia coli*, and *Proteus mirabilis* [51].

Today, amoxicillin remains one of the most commonly prescribed antibiotics worldwide and is currently recommended as treatment for conditions such as tonsillitis, respiratory tract infections caused by Streptococcus species, and community-acquired pneumonia. Ampicillin continues to be used in hospital settings for infections, including bacterial meningitis, genitourinary infections, and septicemia. These derivatives are valued in current clinical practice because of their broad antibacterial spectrum, relatively low toxicity, and effectiveness against susceptible bacterial species.

Particularly, Amoxicillin was structurally modified to enhance oral bioavailability. It works as an amphoteric compound and has a distinctive side chain with a protonated amino group (pKa 2.63) that has strong electron-withdrawing characteristics, making it more acid-resistant than penicillin G [52]. This improved acid stability allows amoxicillin to be administered orally with high absorption, making it commonly used as an oral antibiotic today.

Co-administration of β-lactamase inhibitors with penicillins protects the core structure that gives penicillins their antibacterial activity by preventing β-lactamase enzymes from hydrolyzing the β-lactam ring. In current clinical settings, combinations such as amoxicillin–clavulanic acid and ampicillin–sulbactam are frequently prescribed to treat infections caused by β-lactamase-producing bacteria, including respiratory tract infections and gastrointestinal infections. Typically, these inhibitors are combined as separate substances within the same dosage form during the pharmaceutical formulation process rather than being covalently bound to the penicillin molecule. Sultamicillin, a medication created by chemically bonding ampicillin with the β-lactamase inhibitor sulbactam, is a notable exception.

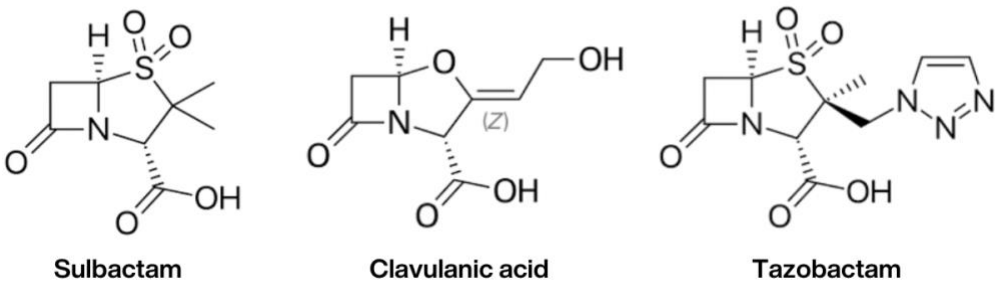


Figure. 9. Structures of β-lactamase Inhibitors

When an enzyme and an inhibitor interact, three different pathways could lead to the formation of a covalent acyl-enzyme intermediate. One approach involves developing a state of a temporarily inactivated enzyme, which deactivates catalytic activity without causing irreversible structural damage. This is similar to reversible inhibition mechanisms, which allow the recovery of enzymatic activity in response to changed environmental conditions.

Alternatively, the acyl-enzyme intermediate might resemble conventional interactions between enzymes and substrates. This process releases the breakdown product and regenerates the unbound, active enzyme by hydrolyzing the inhibitor clavulanic acid. A third possibility is complete and irreversible deactivation of the enzyme, in which the acyl-enzyme complex becomes an irreversibly inactivated species. This pathway is the most clinically desirable outcome for antimicrobial agents, as it achieves suppression of bacterial enzyme function, thereby potentiating antibiotic efficacy [53].

Sulbactam and clavulanic acid had similar inhibitory profiles, meaning they blocked the same types of enzymes to a comparable extent. Both drugs were less effective at inhibiting cephalosporinases, which are beta-lactamases that primarily destroy cephalosporins (a type of beta-lactam antibiotic), than they were at inhibiting penicillinases or broad-spectrum beta-lactamases, which can inactivate many different beta-lactam antibiotics. However, the difference in effectiveness between these enzyme classes was smaller for sulbactam, because sulbactam showed slightly better activity against cephalosporinases than clavulanic acid. Sulbactam and tazobactam both follow the same inhibitory pathway as clavulanic acid [53].

Figure 10. Structures of Second-Generation Variants of Penicillin

An alternative strategy for protecting the β-lactam ring from enzymatic degradation involves steric hindrance through structural modifications. By incorporating bulky substituents adjacent to the β-lactam moiety, these penicillin derivatives create spatial constraints that physically prevent β-lactamase enzyme access to the reactive carbonyl group within the ring. This three-dimensional shielding effect generates an energetically and geometrically unfavorable environment for the enzyme to properly orient and execute nucleophilic attack on the β-lactam bond. Therefore, sterically hindered penicillins maintain their antibacterial activity without the need for co-administration of separate β-lactamase inhibitors, even in the presence of β-lactamase-producing bacteria [53].

Conclusion

Penicillin serves as one of the most transformative discoveries in the history of medicine and the pharmaceutical field until nowadays. Marking the beginning of the discovery by Alexander Fleming to the

enlarged industrial production stage, especially during the World War 2 era, penicillin played and continues to play a crucial role in our society all over the world. The introduction of penicillin has saved thousands and millions of people from infectious diseases that were once untreatable to advancing procedure of surgery and organ transplantation in a safer way.

At a molecular level, penicillin is widely used in medical practices due to its high effectiveness just with the usage of low doses relative to other antibiotics. The β-lactam structure of penicillin plays a significant role in other antibacterial activity and its vulnerability to resistance mechanisms. By inhibiting penicillin-binding proteins (PBPs) and disrupting the building blocks of cell wall (peptidoglycan) at the same time, penicillin specifically targets bacteria with its property of high selective toxicity and high therapeutic index, which allows relatively low doses to be used with the same effectiveness as others.

The further advancement in biosynthesis, fermentation technology, and active pharmaceutical production presents a wide range of usage not only in the medical field but also more. The evolution from natural penicillin G and V to semi-synthetic and finally to second-generation derivations, including ampicillin and amoxicillin stretches and expands the spectrum of activities as antibacterial.

Despite the continuous emergence of other antibiotics from scientists all over the world, penicillin still serves as a historical breakthrough and ongoing innovation with expanding its importance from the fields of Biology, Chemistry, and primarily in the pharmaceutical field against infectious diseases.

Acknowledgements

We thank Professor Stoltz for his guidance and support throughout the project. Chloe Kim, Grace Jang, Chris Kim, Allen Kim, and Eunsuh Park contributed equally to this study.

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