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Challenges and opportunities in discovering new Antibacterial agents

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Abstract

The use of antibacterial agents is essential for effectively treating infectious diseases. While there are numerous drugs commonly used to combat human infections, the discovery and development of new antibacterial agents remain crucial for several reasons. In recent decades, many organisms that typically cause infections in humans have developed increased resistance to existing drugs. This growing resistance has reduced the number of effective antibacterials available for treating specific pathogens. Consequently, there is a need for new antibacterials to combat resistant strains, presenting both challenges and opportunities in this area, which are discussed in this review.

Keywords: Antibacterial resistance, Drug discovery, Bacterial infections, Antibacterials, infectious disease.

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SIGNIFICANCE OF NEW ANTIBACTERIAL AGENTS

The use of antibacterial agents is critical to the successful treatment of infectious diseases. Although there are varieties of drugs that are routinely used to treat infections in humans, there are several reasons why the discovery and development of new antibacterial agents are important. Over the past few decades, there has been an increased development of resistance in organisms that are typical pathogens in humans. These include methicillin/oxacillin-resistant *Staphylococcus aureus*,[1,2] vancomycin resistant and intermediate *Staphylococcus aureus*,[3,4] vancomycin-resistant *Enterococcus*,[2,5] Gram-negative bacilli that produce extended spectrum betalactamases,[6,7] carbapenem-resistant *Klebsiella pneumonia*,[8] and *Pseudomonas* and *Acinetobacter*[9] strains that are resistant to all antibiotics that are typically used for treatment. Figures **1** and **2** show a graphical representation of the rise in resistance for two common pathogenic organisms that are isolated in clinical laboratories.[10]

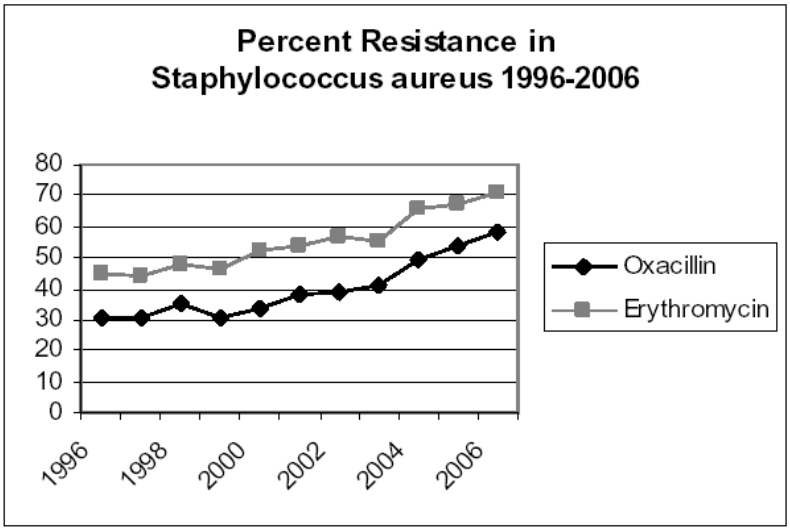


Figure 1: Percent resistance in *Staphylococcus aureus* to selected antibiotics from 1996 to 2006

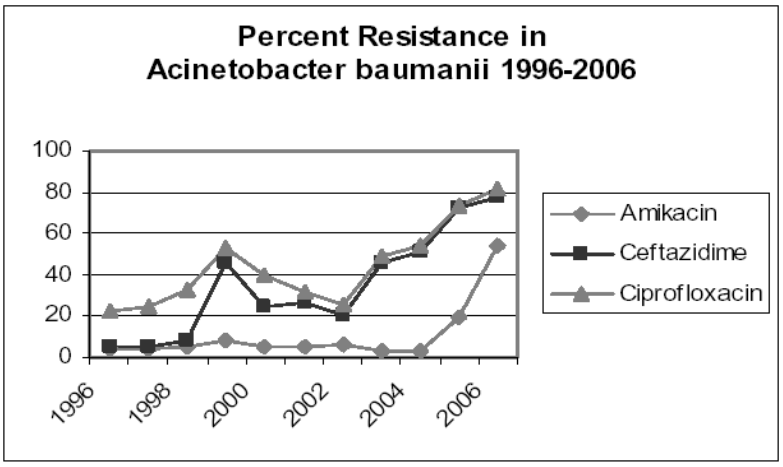


Figure 2: Percent resistance in *Acinetobacter baumanii* to selected antibiotics from 1996 to 2006.

This increased resistance has limited the selection of antibacterials that may be used to treat specific organisms. New antibacterials are therefore needed for resistant strains. Also, there are limited options available to treat infections caused by fungi and mycobacteria. Infections caused by these organisms continue to be a major concern. Chemotherapy for cancer treatment, immunosuppressive drugs for treatment of autoimmune diseases and organ transplant recipients, and infections (such as AIDS) that alter the effectiveness of the host immune system render individuals at high risk for fungal infections and certain mycobacterial infections. Often these infections are caused by environmental organisms that would not typically cause disease in a normal host. There are increased numbers of reports of multi-drug resistant mycobacteria in the United States and throughout the world.[11] Another complicating factor that reduces the effectiveness of treatment of these organisms is non-compliance in completing treatment regimens. Extended length of treatment (sometimes up to one year) and adverse side effects of the drugs used for treatment contribute to the lack of compliance.

Although many infectious diseases have been known for thousands of years, over the past 30 years a number of new infectious diseases have been discovered. Some examples include Lyme disease caused by *Borrelia burgdorferi*, Legionnaires' disease caused by *Legionella pneumophila*, peptic ulcers caused by *Helicobacter pylori* and antibiotic associated diarrhea caused by *Clostridium difficile*. In addition, microorganisms are constantly changing, finding new places to live and new ways to survive, and adapting to new situations. During this process, harmless organisms may turn deadly and deadly strains may move from their normal host to humans. With the continuing discovery of new infectious diseases and the development of new disease processes of existing pathogens (*e.g.*, necrotizing fasciitis caused by *Streptococcus pyogenes*), it is important to continue to find anti-infective agents that can be used to treat suchinfections.[12,13] Development of novel classes of drugs, drugs with fewer side effects, and drugs with shorter lengths of treatment are key drivers in continuing the fight against infectious disease.

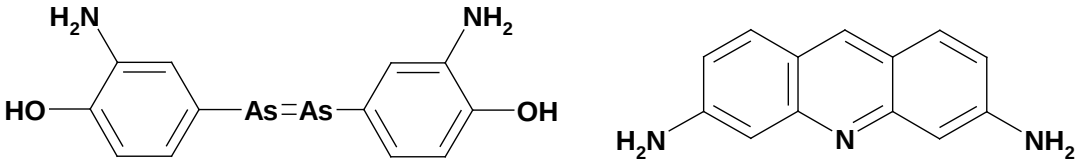
1.2 HISTORY OF ANTIBACTERIAL AGENTS

Bacteria were first identified in the 1670s by Van Leeuwenhoek after his invention of the microscope. However, it was only in the nineteenth century that the link of bacteria with disease was understood. This appreciation followed the elegant experiments carried out by the French scientist Pasteur, who demonstrated that specific bacterial strains caused the fermentation and that these and other microorganisms were far more widespread than previously thought. The possibility that these microorganisms might be responsible for disease began to take hold. It was subsequently suggested that the microorganisms may be responsible for diseases such as tuberculosis, cholera, and typhoid.

Paul Ehrlich is known as the father of chemotherapy – the use of chemicals against infections. According to Ehrlich's concept of chemotherapy, a chemical could directly interfere with the proliferation of microorganisms at concentrations tolerated by the host. This concept was popularly known as the "magic bullet" where the chemical was seen as bullet which could search out and destroy the invading microorganism without adversely affecting the host. The process is based on the selective toxicity where the chemical shows greater toxicity to the target microorganism than to the host cells. Such selectivity can be represented by a chemotherapeutic index, which compares the minimum effective dose of the drug to the maximum dose tolerated by the host.

By 1910, Ehrlich had successfully developed the first example of a purely synthetic antibacterial drug. This was the arsenic containing compound, Salvarsan **1** (Figure **3**). Although the compound was not effective against a wide range of bacterial infections, it did prove effective against the protozoal disease of sleeping sickness (Trypanosomaisis**)** and the spirochete disease of Syphilis. Salvarsan was used until 1945 when it was replaced by Penicillin.

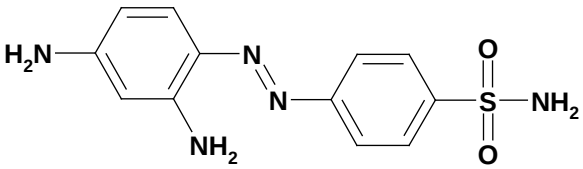
The next major breakthrough came in 1934 with the introduction of Prolavine **2** (Figure **3**). This drug was an aminoacridine derivative and was particularly effective against bacterial infections in deep surface wounds as seen during the Second World War. It was an interesting drug since it targeted bacterial DNA rather than protein.
Despite the success of Prolavine, it was not effective against bacterial infections in the bloodstream and there was still an urgent need for agents which would fight these infections. This need was answered in 1935 with the discovery of a red dye called Prontosil **3** (Figure **4**), which was found to be effective against *Streptococcus* infections.[14]



Salvarsan 1 **Prolavine 2**

Figure 3: Structures of Salvarsan and Prolavine

It was subsequently realized that Prontosil was actually a prodrug for a new class of antibacterial agents, the sulfa drugs (sulfonamides). The discovery of these drugs was a real breakthrough, since they represented the first drugs to be effective against bacterial infections carried in the blood streams. They exclusively ruled the market until Penicillin become available in the early 1940s.



Prontosil 3

Figure 4: Structure of Prontosil

Although Penicillin was discovered in 1928, an effective means of isolating it was only developed by Florey and Chain in 1940. Penicillin revolutionized the fight against bacterial infections and proved more effective than the sulfonamides. Despite the success of Penicillin, it was not effective against all types of infections and the need for antibacterial agents possessing broad spectrum antibacterial activity still remained unmet.

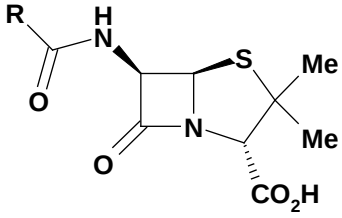


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Figure 5: Structure of Penicillins

Penicillin is an example of a toxic chemical produced by a fungus to kill bacteria. The realization that fungi might be a source for novel antibiotics inspired scientists to carry out a huge investigation of microbial cultures. In 1944, the antibiotic Streptomycin was discovered from a systematic search of soil organisms. This drug extended the range of chemotherapy to *Tubercle bacillus* and a variety of Gram-negative bacteria. This compound was the first example of a series of antibiotics known as the aminoglycoside antibiotics.

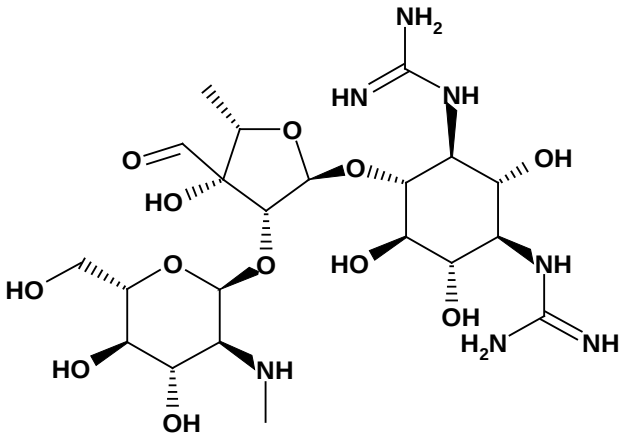

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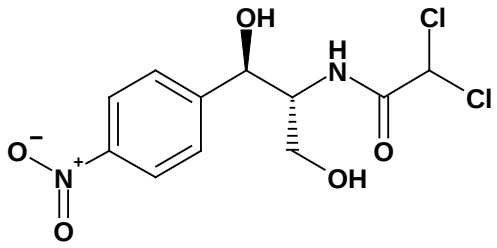
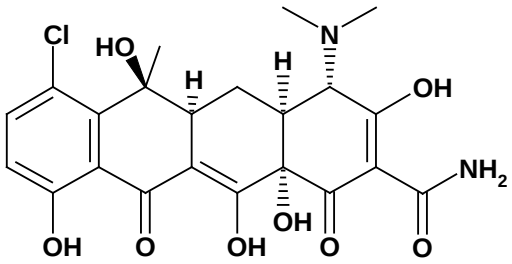
Figure 6: Structure of Streptomycin

After the Second World War, efforts continued to find other novel antibiotics structures, which led to the discovery of the peptide antibiotics (*e.g.*, bacitracin), Chloramphenicol, tetracycline antibiotics (*e.g.*, Chlortetracycline), the macrolide antibiotics (eg. Erythromycin), cyclic peptide antibiotics and cephalosporins.

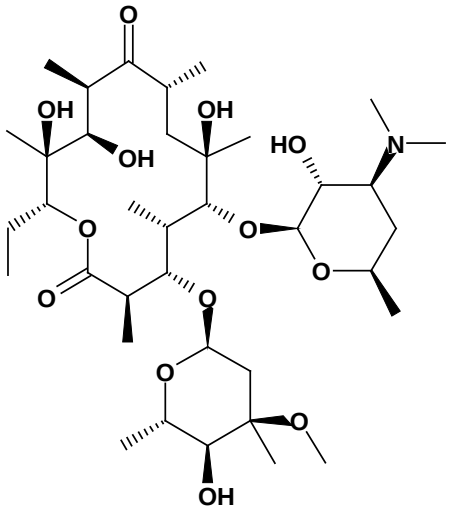
Along with the naturally occurring antibiotics, efforts towards the discovery of synthetic agents resulted in Isoniazid (a pyridine hydrazide structure) in 1952. This drug was found to be effective against human tuberculosis. In 1962, nalidixic acid (the first quinolone antibacterial agent) was discovered and a second generation of this class of drugs was introduced in 1989 with the discovery of Ciprofloxacin.



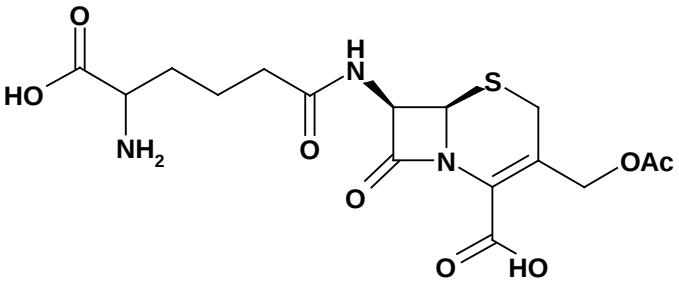
Chloramphenicol 6



Chlortetracycline 7

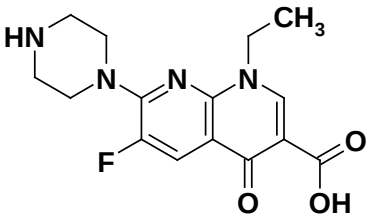


Erythromycin 8

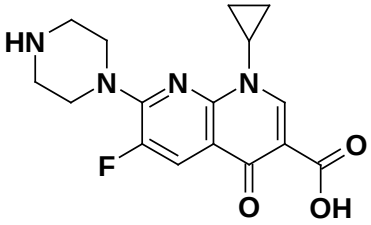


Cephalosporin C 9

Figure 7: Structures of Chloramphenicol, Chlortetracycline, Erythromycin and Cephalosporin C.



Nalidixic acid 10



Ciprofloxacin 11

Figure 8: Structures of Nalidixic acid and Cephalosporin

1.3 SOURCES OF NEW ANTIBACTERIAL AGENTS

The introduction of antibiotics to treat bacterial infections is one of the most successful developments in medicine. However, this breakthrough is now endangered by increasing levels of antibiotic resistance. Antibiotic resistant strains exhibit a greater ability to survive doses of antibiotics that would be lethal to typical strains. This higher resistance reduces the effectiveness of an antibiotic, leading doctors to use alternative antibiotics, which can eventually lead to the evolution of strains resistant to multiple antibiotics. So, this emphasizes the pressing need for discovering new antibacterial agents. The following are the main sources of new antibacterial agents:

- Natural products
- Synthetic products
- Genomics

1.3.1 Natural Products

The discovery of Penicillin gave rise to the concept of seeking naturally occurring drugs.[15] This was true for anti-infectives as well as other types of drugs. In the anti-infective area, treatment is dominated by natural products and natural product analogs such as penicillins, cephalosporins, and Vancomycin.[16] These natural products can come from a number of different sources: bacteria, fungi, plants, and marine environments. Natural products with antibiotic activity that come from bacteria or fungi are generally products of secondary metabolic pathways. These pathways are not required for primary functions of metabolism. When antibiotic-producing organisms face competition in their environment, they turn on genes that encode the antibiotic molecules and use them to initiate chemical war fare on their neighbors. They then have a selective advantage for growth, including the nutrients from their dying neighbors.[17] Organisms which cause human infections do not survive well in soil because they are destroyed by soil-inhabiting organisms. As a result, extensive soil-screening programs have resulted in the discovery of drugs such as Streptomycin, Chloramphenicol, Chlortetracycline, Erythromycin, Neomycin, Bacitracin, and Polymixin.[15] Many of these drugs were isolated from actinomycete species.

The marine environment is another rich source of naturally occurring antibiotics. One of suchconcerns of these compounds is their structural complexity and because of this, it is often difficult to synthesize these molecules on a large-scale.

Plants produce substances that help protect them from microorganisms and herbivores. They also produce chemicals for offensive chemical warfare targeting cell proliferation of pathogens. These chemicals may have a general or specific activity against key target sites in bacteria, fungi and viruses.[18] It is estimated that more than 250,000 species of higher plants exist and only a fraction have been investigated to characterize their chemical constituents for biological effects.[19]

1.3.2 Synthetic products

In contrast to the natural antibiotics, which were primarily isolated from the fermentation of extracts of soil bacteria, the synthetic antibiotics generally originate from rational design and random screening.[20,21] The discovery of the sulfonamide (sulfa drugs) antibiotics, for example Prontosil **3** (Figure **4**), by Domagk in 1932 marked the inception of the target-oriented endeavor of synthetic antibiotics.[22]

There has been a paradigm shift in the development of technologies which enable the synthesis of target compounds in quick time. In the past two decades, a new sub-speciality of chemistry known as combinatorial chemistry has emerged with a great potential to revolutionize the discovery of new medicinal agents and diagnostic reagents. This specialised area of chemistry has increased the production of potential new drugs by using a set of innovative procedures to generate large libraries of compounds for identifying pharmacologically active compounds.[23]

1.3.3 Genomics

The first complete bacterial genome was published for *Haemophilus influenzae* in 1995. Since then, many other bacterial genomes have been completed and are available for use in antibacterial research.[24] As a result of this increased knowledge, the drug discovery paradigm for researchers has shifted away from finding compounds against whole cells to identifying compounds that are active against selected protein targets.[25] Knowing the sequence of the microbial genome has allowed scientists to observe similarities and differences in the genetic makeup of various bacterial species. Many genes that have been sequenced are highly conserved. It is postulated that these genes and the proteins for which they encode are most probably essential for life.[26] Essential genes make up about 25% of the bacterial genome[25] and are responsible for DNA replication, transcription and protein synthesis, lipid biosynthesis, and cell wall assembly.[26] Since these genes are responsible for basic life functions, substances that could block their activity have the potential to be developed into highly effective and broad spectrum antibacterial agents.

1.4 ANTIBACTERIAL AGENTS: MODE OF ACTION

There are five commonly observed mechanisms by which antibacterial agents act (Figure **9**).

- **Inhibition of cell metabolism:** Antibacterial agents which inhibit cell metabolism are called antimetabolites. These compounds inhibit the metabolism of a microorganism, but not the metabolism of the host. They do this by inhibiting an enzyme-catalyzed reaction which is present in the bacterial cell, but not in animal cells. The best known examples of antibacterial agents acting in this way are the sulfonamides.
- **Inhibition of bacterial cell wall synthesis:** Inhibition of cell wall synthesis leads to bacterial cell lysis (bursting) and death. Agents operating in this way include penicillins and cephalosporins. Since animal cells do not have a cell wall, they are unaffected by such agents.
- **Interactions with the plasma membrane:** Some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell. Polymyxins and tyrothricin operate in this way.

- **Disruption of protein synthesis:** Disruption of protein synthesis means that essential enzymes required for the cell's survival can no longer be synthesized. Agents which disrupt protein synthesis include the rifamycins, aminoglycosides, tetracyclines, and Chloramphenicol. **Inhibition of nucleic acid transcription and replication:** Inhibition of nucleic acid function prevents cell division and/or the synthesis of essential enzymes. Agents acting in this way include Nalidixic acid and Proflavin.

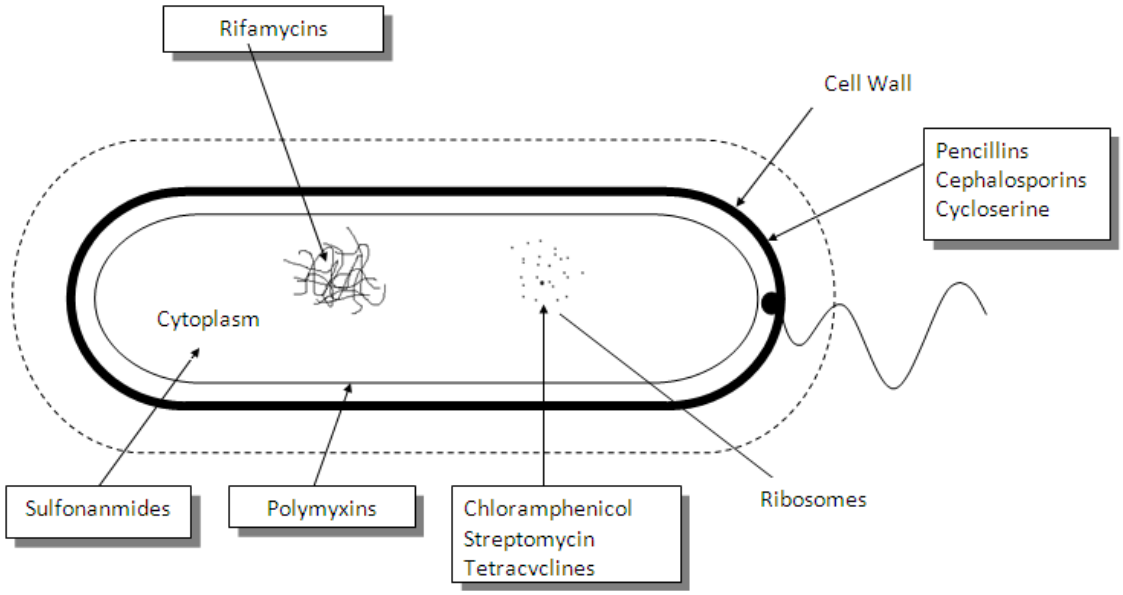
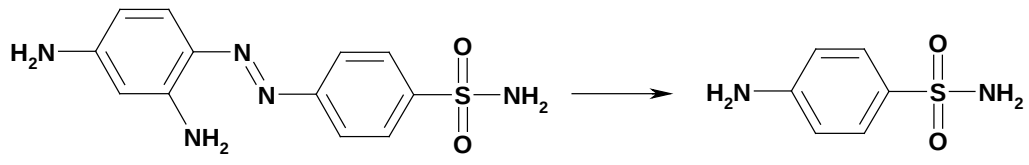


Figure 9: Sites of antibacterial action.

1.4.1 Antimetabolites: Targeting cell metabolism

The best example of antibacterial agents acting as antimetabolites is the sulfonamides (also known as sulfa drugs). Sulfonamides were discovered in 1935 when a red dye called Prontosil **3** was shown to have antibacterial properties *in vivo*. It was surprising that no antibacterial effect was observed *in vitro*. This remained a mystery until it was discovered that Prontosil was not in fact the antibacterial agent.

It was subsequently found that Prontosil was metabolized by bacteria present in the small intestine of the test animal, and broken down to give a product called Sulfanilamide (**12**, Figure **10**). It was this compound which was the true antibacterial agent. Thus, Prontosil was the first example of a prodrug. Sulfanilamide was synthesized in the laboratory and became the first synthetic antibacterial agent active against a wide range of infections. Further developments led to a range of sulfonamides which proved effective against Gram-positive organisms, especially *pneumococci* and *meningococci*. Despite their undoubted benefits, sulfa drugs have proved ineffective against infections such as *Salmonella*—the organism responsible for typhoid. Safety related issues were also observed with the metabolism of these drugs; since toxic products were frequently obtained. This led to the sulfonamides being superseded by Penicillin.



Prontosil 3 **sulfanilamide 12**

Figure 10: Metabolism of Prontosil.

Structure-Activity Relationship (SAR)

The synthesis of a large number of sulfonamide derivatives **13** led to the following understanding:

- The *para*-amino group is essential for activity and must be unsubstituted (i.e. R^1 = H), except for R^1 = acyl (i.e. amides). The amides themselves are inactive but can be metabolized in the body to generate the active compound. Thus the amides are used as prodrugs.
- The aromatic ring and the sulfonamide functionality are both required for activity.
- The aromatic ring must be *para* substituted.
- The sulfonamide nitrogen must be secondary.
- R^2 is the only possible site that can be varied.

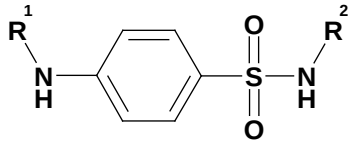


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Figure 11: General structure of sulfonamide analogs

Applications

The sulfa drugs presently have the following applications in medicine:

- Treatment of urinary tract infections
- Eye lotions
- Treatment of infections of mucous membranes
- Treatment of gut infections

Mechanism of action

The sulfonamides act as competitive enzyme inhibitors and block the biosynthesis of folic acid in bacterial cells. The consequences of this are disastrous for the cell. Under normal conditions, folic acid is the precursor for tetrahydrofolate—a compound which is crucial to cell biochemistry since it acts as the carrier for one-carbon units, necessary for many biosynthetic pathways. If tetrahydrofolate is no longer synthesized, then any biosynthetic pathway requiring one-carbon fragments is disrupted. The biosynthesis of nucleic acids is particularly disrupted and this leads to the cessation of cell growth and division. Sulfonamides do not actively kill bacterial cells. They prevent the cells from dividing and spreading. This gives the body's own defense systems enough time to gather their resources and wipe out the invader. Sulfonamides act as inhibitors by mimicking *p*-aminobenzoic acid (PABA) (Figure **12**), one of the normal constituents of folic acid.

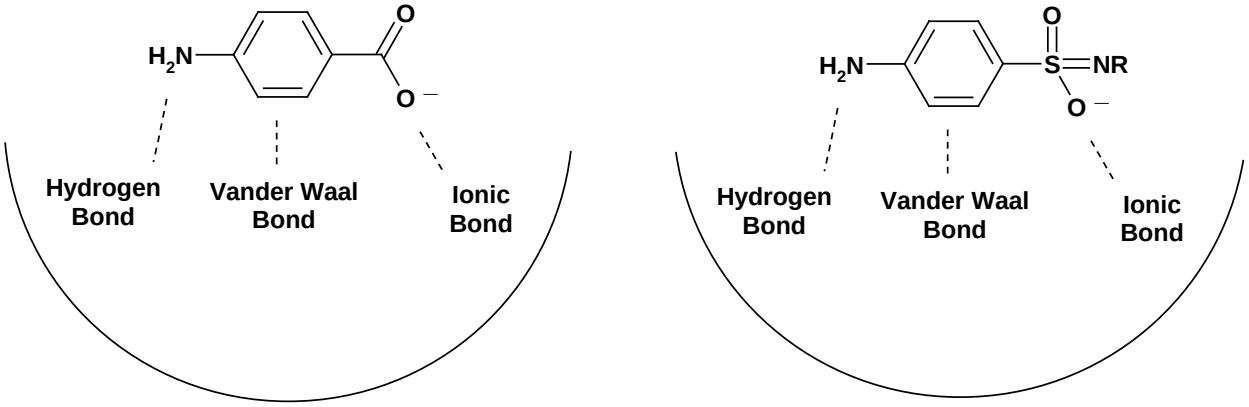

Figure 12: Sulfonamides mimic *p*-aminobenzoic acid (PABA)

Upon binding, sulfonamides prevent PABA from binding. As a result, folic acid is no longer synthesized. Since folic acid is essential to cell growth, the cell will stop dividing. The success of sulfonamides is due to two metabolic differences between mammalian and bacterial cells. Bacteria have a susceptible enzyme, which sulphonamides attack and this enzyme is not present in mammalian cells. Additionally, bacteria lack the transport protein, which would allow them to acquire folic acid from outside the cell, for example, from diet, as in the case of humans.

1.4.2 Cell wall synthesis inhibitors

Penicillins and Cephalosporins are the two major classes of drugs, which act in this fashion.

Penicillins

History of penicillins

In 1877 Pasteur and Joubert discovered that certain moulds could produce toxic substances which killed bacteria. Unfortunately, these substances were also toxic to humans and had no clinical value. However, they demonstrated that moulds could display antibacterial action. In 1928, Fleming noted that a bacterial culture which had been left several weeks open to the air had become infected by a fungal colony. Of more interest was the fact that there was an area surrounding the fungal colony where the bacterial colonies were dying. He correctly concluded that the fungal colony was producing an antibacterial agent which was spreading into the surrounding area.

Fleming spent several years investigating the novel antibacterial substance and showed it to have significant antibacterial properties and to be remarkably non-toxic to humans. Unfortunately, the substance was also unstable and Fleming was unable to isolate and purify the compound. He therefore came to the conclusion that Penicillin was too unstable to be used clinically. The problem of isolating Penicillin was eventually solved in 1938 by Florey and Chain by using a process known as freeze-drying which allowed isolation of the antibiotic under much milder conditions. By 1941, Florey and Chain were able to carry out the first clinical trials on crude extracts of Penicillin and achieved spectacular success. Further developments aimed at producing the new agent in large quantities were developed in the United States by 1944.

Although the use of Penicillin was now widespread, the structure of the compound was still not confirmed and was proving to be a source of serious debate due to the unusual structures being proposed. The issue was finally settled in 1945 when Dorothy Hodgkin established the structure by X-ray analysis. The synthesis of such a highly strained molecule also presented a huge synthetic challenge, which was met successfully by Sheehan, who completed the total synthesis of Penicillin by 1957. The total synthesis was too long to be of commercial use, but the following year Beecham pharmaceutical isolated a biosynthetic intermediate of Penicillin called 6-aminopenicillanic acid (6-APA) which provided a readily accessible biosynthetic intermediate of Penicillin. This revolutionized the field of penicillins by providing the starting material for a huge range of semisynthetic penicillins. Penicillins were used widely and often carelessly, so that the evolution of penicillin resistant bacteria became more and more of a problem. The fight against these penicillin-resistant bacteria was promoted greatly when, in 1976, Beechams discovered a natural product called clavulanic acid which has proved highly effective in protecting penicillins from the bacterial enzymes which attack Penicillin.

Structure of Penicillin

Scientists remained unconvinced regarding the exact the structure of penicillin until an X-ray analysis was carried out. Penicillin is in fact a family of antibiotics which contain a common bicyclic system consisting of a four membered β-lactam ring fused to a five-membered thiazolidine ring which differ by virtue of having different acyl chains. The skeleton of the molecule suggests that it is derived from the amino acids cysteine and valine (Figure **13**). The overall shape of the molecule is like a half-open book, as shown in Figure **14**.

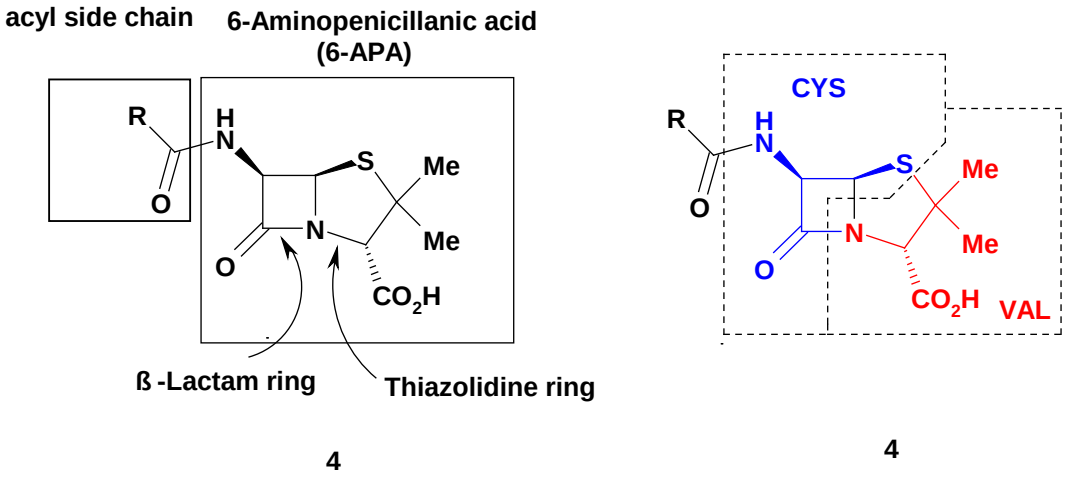

Figure 13: The core structure of penicillin (appears to be derived from cysteine and valine)

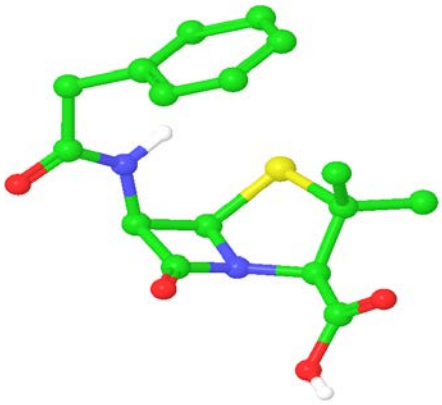

Figure 14: The shape of penicillin G (energy minimized structure generated using Schrodinger suite)

Profile of penicillin G

Penicillin G is active against Gram-positive bacilli (*e.g.*, *staphylococci*, meningitis and gonorrhoea) and a few Gram-negative cocci. However, it does not possess a broad spectrum of activity. It is ineffective orally since it breaks down in the acidic conditions of the stomach. It is sensitive to all known β-lactamases (these are enzymes produced by Penicillin-resistant bacteria which catalyze the degradation of penicillins).

The penicillins are amongst the safest drugs known to medicine. However, some allergic reactions are suffered by some individuals. The problems associated with the use of penicillin G include acid sensitivity, sensitivity to penicillinase, and a narrow spectrum of activity. The purpose of making semisynthetic Penicillin analogues is therefore to find compounds which do not suffer from these disadvantages.

Structure-activity relationship of penicillins

A large number of penicillin analogues have been synthesized and studied. The results of these studies led to the following conclusions:
• Bicyclic ring system: The bicyclic system (with *cis* stereochemistry) is important for activity because it confers strain on the β-lactam ring: the greater the strain, the greater the activity. However, it is also associated with instability of the molecule.
• The free carboxylic acid is essential.
• The acylamino side-chain is essential.
• The sulfur atom is customary but not essential.

Cephalosporins

Discovery and structure of cephalosporin C

The second major group of β-lactam antibiotics to be discovered were the cephalosporins. The first cephalosporin was Cephalosporin C—isolated in 1948 from a fungus obtained from the drain waters on the island of Sardinia. Antibacterial properties were recognized only in 1961 after the establishment of its exact structure. The structure of cephalosporin C (**14**, Figure **15**) has similarities to that of penicillin: it has a bicyclic system containing a four-membered β-lactam ring. Cephalosporins have the β-lactam ring fused with a six-membered dihydrothiazine ring. This larger ring relieves the strain in the bicyclic system to some extent, but it is still a reactive system. The Cephalosporin skeleton reveals that cephalosporins can be derived from the same biosynthetic precursors as penicillin, i.e. cysteine and valine.

Properties of cephalosporin C

Cephalosporin possesses a good ratio of activity against Gram-negative and Gram-positive bacteria; however, the potency is low compared to Penicillin G, but it is relatively more stable to acid hydrolysis. The oral absorption of cephalosporin C is low.

Cephalosporin was found to be very safe and to have low risk of allergenic reactions. It has few clinical uses, is not particularly potent and at first sight seems rather uninteresting. However, Cephalosporin C has been used in the treatment of urinary tract

infections because it concentrates in the urine and survives the body's hydrolytic enzymes. Significant efforts have been directed towards improving the potency of this compound and have led to an understanding of its SAR.

Structure-activity relationship of Cephalosporin C and its analogs

The Structure-activity relationship of cephalosporin C tally closely with those obtained for the penicillins as there are only a limited number of places where modifications can be made (Figure **15**). Structure-activity relationship (SAR) conclusions are summarized as follows:

- The β-lactam ring is essential.
- A free carboxyl group is needed at the 4th position.
- The bicyclic system is essential.
- *Cis* stereochemistry is crucial for activity.

The following positions may be modified:

- The 7-acylamino side-chain
- The 3-acetoxymethyl side-chain
- The substituent at carbon 7

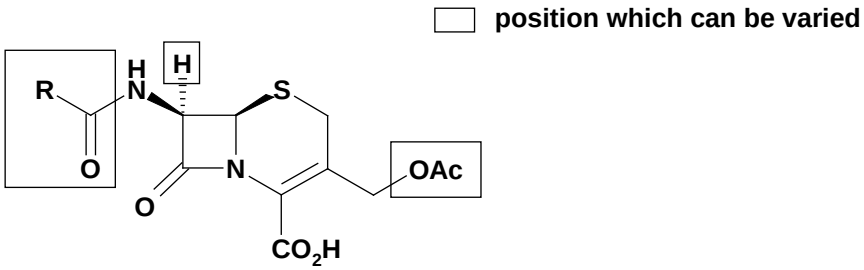

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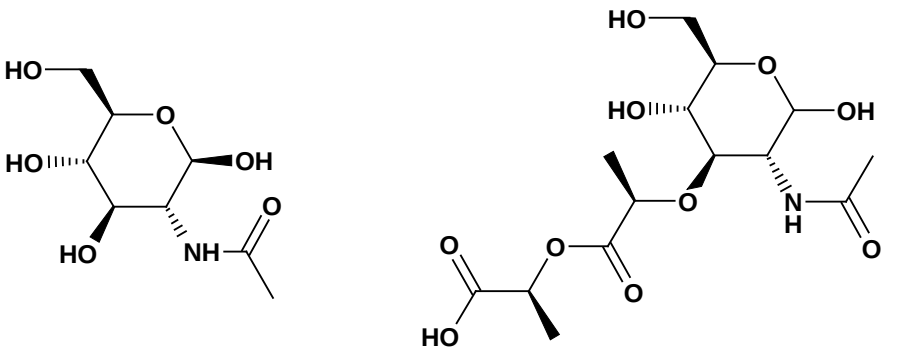
Figure 15: Positions amenable for structural modifications in Cephalosporin C.

Mode of action of penicillins and cephalosporins

Since bacteria have to survive a range of diverse environmental conditions such as a range of pH, temperature, and osmotic pressure, they require a robust cell wall. The cell wall is not present in animal cells; therefore, it is the perfect target for antibacterial agents such as penicillins and cephalosporins. The bacterial cell wall is a peptidoglycan structure; major components being peptide and sugar units. The structure of the wall consists of a parallel series of sugar backbones containing two types of sugar (*N*-acetylmuramic acid (NAM) and *N*-acetyl glucosamine (NAG)) (**14** and **15** in Figure **16**).



N-acetylglucosamine 14 **N-acetylmuramic acid 15**

Figure 16: Sugars contained in the cell wall structure of bacteria.

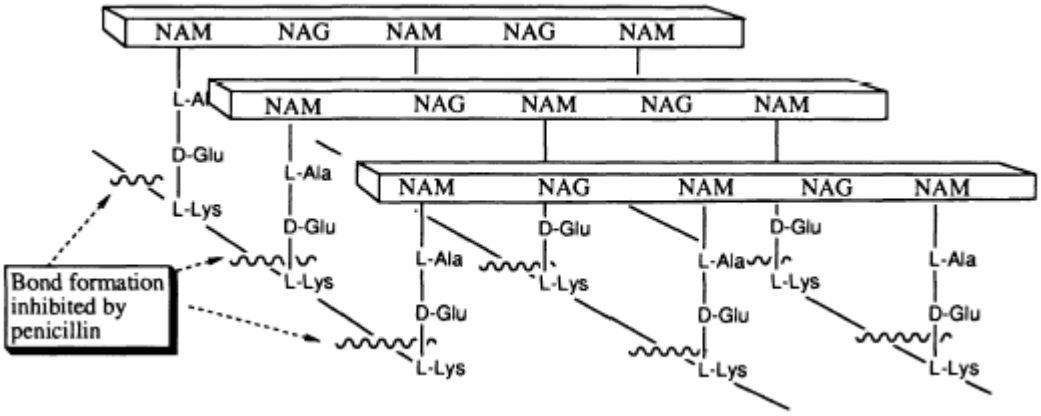


Figure 17: Peptidoglycan structure.

It is the final cross-linking reaction which is inhibited by penicillins and cephalosporins, such that the cell wall framework is not meshed together (Figure **17**). As a result, the wall becomes 'leaky'. Since the salt concentrations inside the cell are greater than those outside the cell, water enters the cell, the cell swells, eventually lyses, and the bacterium dies.

1.4.3 Antibacterial agents targeting plasma membrane

Valinomycin acts as an ion conducting antibiotic and permits the uncontrolled movement of ions across the cell membrane (Figure **18**). Unfortunately, this drug does not show selective toxicity for bacterial over mammalian cells and is therefore of limited use as a therapeutic agent.

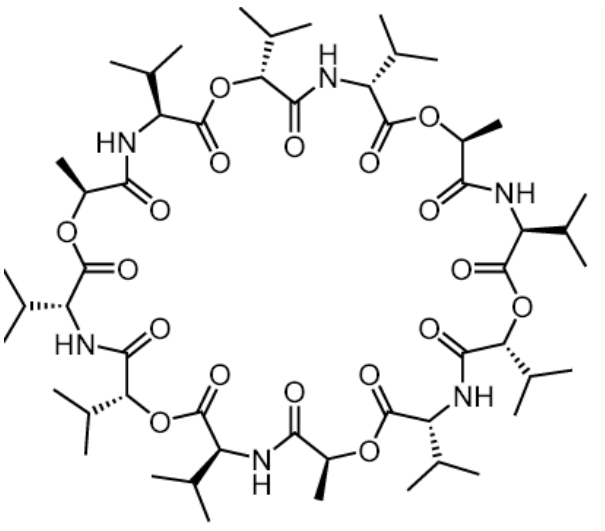


Figure 18: Structure of Valinomycin

Valinomycin is a cyclic structure consisting of three molecules of L-valine, three molecules of D-valine, three molecules of L-lactic acid, and three molecules of 2-hydroxyisovaleric acid. These twelve components are linked in an ordered fashion such that there is an alternating sequence of ester and amide linking bonds around the cyclic structure. This is achieved by the presence of a lactic or hydroxyisovaleric acid unit between each of the six valine units. Valinomycin acts as an ion carrier which forms a doughnut-type structure wherein the polar carbonyl oxygens of the ester and amide groups face inward, while the hydrophobic side-chains of the valine and hydroxyisovaleric acid units point outward. This is clearly a favored configuration because the hydrophobic side-chains can interact *via* van der Waals forces with the fatty lipid interior of the cell membrane, while the polar hydrophilic groups are clustered together in the centre of the doughnut to produce a hydrophilic

environment. This hydrophilic centre is large enough to accommodate a cation and it is found that a potassium ion fits neatly and is complexed by the amide carboxyl groups. Valinomycin can therefore transport a potassium ion from the inner surface of the membrane through the cell wall and deposit it outside, thus disrupting the ionic equilibrium of the cell. Normally, cells have a high concentration of potassium and a low concentration of sodium. The fatty cell membrane prevents passage of ions between the cell and its environment, and ions can only pass through the cell membrane aided by specialized and controlled ion transport systems. Valinomycin introduces an uncontrolled ion transport system which proves fatal.

1.4.4 Protein synthesis inhibitors

Protein synthesis inhibitors are an extremely diverse and ubiquitous class of biochemical molecules. Their functions range from bacterial translation inhibition to the blocking of synthesis of specific amino acid residues necessary for the production of a protein. Most of the antibiotics in this class block the translation step in protein synthesis. These substances are effective antibiotics since they take advantage of the tremendous complexity involved in the synthesis of proteins. Examples of such agents are the rifamycins which act against RNA and the aminoglycosides, tetracyclines, and Chloramphenicol which all act against the ribosome. Selective toxicity is due either to different diffusion rates through the cell barriers of different cell types or to a difference between the target enzymes of different cells.

Rifamycins

Rifampicin (**18**, Figure **19**) is a semisynthetic rifamycin obtained from Rifamycin B (**17**), an antibiotic isolated from *Streptomyces mediterranei*. It inhibits Gram-positive bacteria and works by binding non-covalently to RNA polymerase and thereby inhibiting RNA synthesis.

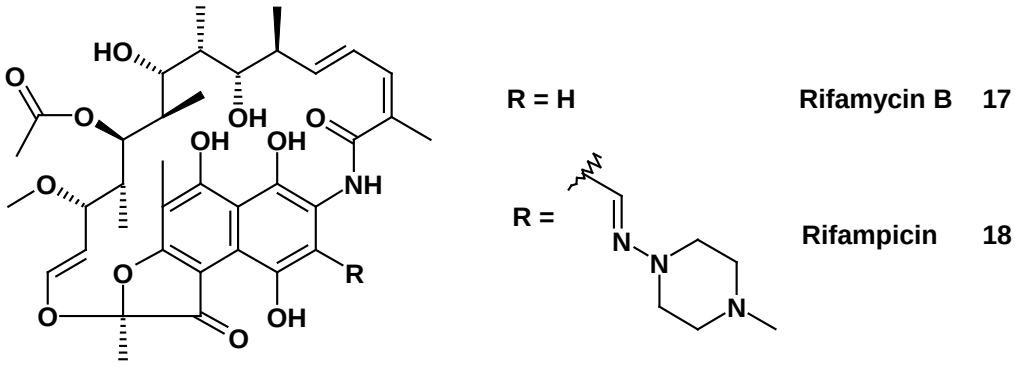


Figure 19: Structures of Rifamycin B and Rifampicin

The DNA-dependent RNA polymerases in eukaryotic cells are unaffected by the action of Rifampicin, since the drug binds to a peptide chain not present in the mammalian RNA polymerase. It is therefore highly selective. The drug is mainly used in the treatment of tuberculosis and *staphylococci* infections that resist Penicillin. It is a very useful antibiotic, showing a high degree of selectivity against bacterial cells over mammalian cells. Unfortunately, wide usage of this drug is limited due to high cost. The flat naphthalene ring and several of the hydroxyl groups are essential for activity.

Aminoglycosides

Streptomycin (from *Streptomyces griseus*) is an example of this class of antibiotics (**5**, Figure **6**). It was the next most important antibiotic to be discovered after Penicillin and proved to be the first antibiotic effective against the lethal disease tuberculous meningitis. The drug works by inhibiting protein synthesis. It binds to the 30S ribosomal subunit and prevents the growth of the protein chain as well as preventing the recognition of the triplet code on mRNA.
The aminoglycoside antibiotics used to be the only compounds effective against the particularly resistant *Pseudomonas aeruginosa* and so far there are very few treatment options available against this bacterial strain.

Tetracyclines

The tetracyclines as a whole have a broad spectrum of activity and are the most widely prescribed form of antibiotic after the penicillins. They are also capable of attacking the malaria parasite.
One of the best known tetracyclines is Chlortetracyclin (**7**, Figure **7**) which was discovered in 1948. It is a broad-spectrum antibiotic; active against both Gram-positive and Gram-negative bacteria. Chlortetracyclin inhibits protein synthesis by binding

to the 30S subunit of the ribosome and prevents aminoacyl-*t*RNA binding to the A site on the ribosome. This prevents the codon-anticodon interaction from taking place. Protein release is also inhibited. Unfortunately, the drug does have side-effects due to the fact that it kills intestinal flora. According to their mode of action, tetracyclines should prevent protein synthesis in mammalian cells as well as in bacterial cells but, because bacterial cells accumulate the drug far more efficiently than mammalian cells, bacteria are more susceptible to the drug.

Chloramphenicol

Chloramphenicol (**6**, Figure **7**) was originally isolated from *Streptomyces Venezuela*, but is now prepared synthetically. It has two chiral centres, but only the 1*R*, 2*R* -diastereomer is active.
SAR studies demonstrate that there must be an electron withdrawing substituent on the aromatic ring (*e.g.*, NO2). The dichloroacetamide group is important, but can be replaced by other electronegative groups. Chloramphenicol is the drug of choice against typhoid and is also used in severe bacterial infections which are insensitive to other antibacterial agents. It has also found widespread use against eye infections. However, the drug is only used in restricted scenarios since it is quite toxic, especially to bone marrow. The NO2 group is suspected to be responsible for its toxicity.
Chloramphenicol binds to the 50S subunit of the ribosome and appears to act by inhibiting the movement of ribosomes along mRNA, probably by inhibiting the peptidyl transferase reaction by which the peptide chain is extended.

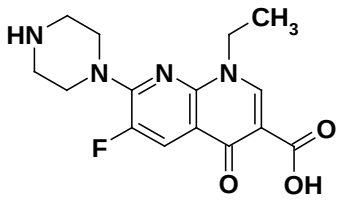
Macrolides

The best known example of this class of compounds is Erythromycin—a metabolite produced by the microorganism *Streptomyces erythreus.* The structure of Erythromycin (**8**, Figure **7**) consists of a macrocylic lactone ring with a sugar and an aminosugar attached. The sugar residues are important for activity.
Erythromycin acts by binding to the 50S subunit. It works in the same way as Chloramphenicol by inhibiting translocation, where the elongated peptide chain attached to tRNA is shifted back from the aminoacyl site to the peptidyl site. Erythromycin was used against penicillin-resistant *staphylococci*, but newer penicillins are now being used for these infections. It is, however, the drug of choice against Legionnaires disease.

1.4.5 Antibacterial agents targeting nucleic acid transcription and replication

Quinolones and fluoroquinolones

The quinolone and fluoroquinolone antibacterial agents (Figures **8**, **20** and **21**) are relatively late arrivals on the antibacterial field, but are proving to be very useful therapeutic agents. The first clinically useful quinolone was Naldixic acid, discovered by Lesher and co-workers in 1962, who generated it from chloroquine.[27] The compound was active against some Gram-negative bacteria and had limited usefulness because of its high protein binding (approximately 90%) and short half-life (about 1.5 h). Bacteria also could quite rapidly develop a resistance to this agent.

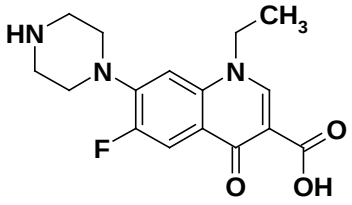


Enoxacin 19

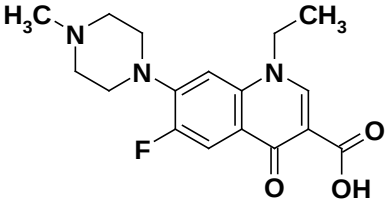
Figure 20: Structure of Enoxacin

In 1968, Kaminsky and Melfezer discovered an oxolinic acid, which was approved by the United States Food and Drug Administration (USFDA).[28] Extensive efforts have been undertaken to develop and derive an array of significantly active drugs of this class. Lead optimization by bioisosteric replacements, homologation of side-chain or branching of side-chain, investigation of stereochemistry and other strategic designs have given rise to agents with broad spectrum activity and minimum side-effects.
Flumequine was the first fluoroquinolone to be reported was patented in 1973 and many analogs have been appeared in clinical use since then (Figures **20** and **21**), including Norfloxacin (1978), Pefloxacin (1979), Enoxacin (1980), Fleroxacin (1981), Ciprofloxacin (1981) and Ofloxacin (1982).[27] Broad spectrum of activity was the major advantage of these compounds over

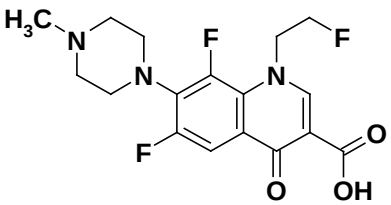
previous non-fluorinated derivatives. A big revolution was made in the 1980s when an analog of naldixic acid, Enoxacin was identified with a significantly increased spectrum of activity against Gram-negative and Gram-positive bacteria. The most successful and widely used fluoroquinolone, ciprofloxacin was marketed in 1986 and since then the value of fluoroquinolones for the treatment of a wide range of infections have become widely recognized.[27,29] This class of compounds has improved pharmacokinetic properties as well as extensive and potent activities against various parasites, bacteria and mycobacteria, including resistant strains, as compared to previously existing bactericidal drugs.[29,30]



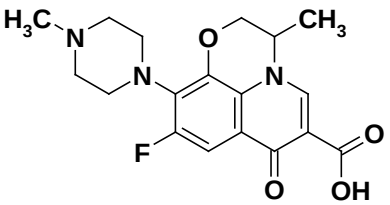
Norfloxacin **20**



Perfloxacin **21**



Fleroxacin **22**



Ofloxacin **23**

Figure 21: Structures of Norfloxacin, Perfloxacin, Fleroxacin and Ofloxacin

Mechanism of action

Fluoroquinolones inhibit the replication and transcription of bacterial DNA, which eventually culminates in cell death.[31,32] They inhibit the activity of DNA gyrase, an essential adenosine triphosphate-hydrolyzing topoisomerase II enzyme and/or prevent the detachment of gyrase from DNA. The topoisomerases exert their bactericidal activity by interacting with DNA.[33] During the processes of replication and transcription, enzymes called helicases unwind and uncoil the DNA double helix leading to excess super coiling of the remaining DNA double helix. A tension is created in this remaining double helix which must be relieved in order to continue transcription. The topoisomerase II enzyme allows the relaxation of supercoiled DNA by breaking both strands of DNA chain, crossing them over, and then resealing them. Bacterial gyrase is sufficiently different from mammalian topoisomerase so that quinolones and fluoroquinolones show 1000-fold selectivity towards bacteria over the corresponding enzyme in humans.

1.5 DRUG RESISTANCE

With a wide range of antibacterial agents available in the market, it may seem surprising that medicinal chemists are still actively seeking new and improved antibacterial agents. The reason for this is mainly due to the ability of bacteria to acquire resistance. Drug resistance arise from a variety of causes. For example, the bacterial cell may change the structure of its cell membrane and prevent the drug from entering the cell. Alternatively, an enzyme may be produced which destroys the drug. Another possibility is that the cell counteracts the action of the drug. For example, if the drug is targeting a specific enzyme, then the bacterium may synthesize an excess of the enzyme. A few of the common reasons of resistance are elaborated below:

1.5.1 Drug resistance by mutation

Bacteria multiply at such a rapid rate that there is always a chance that a mutation will render a bacterial cell resistant to a particular agent. This feature has been known for a long time and is the reason why patients should fully complete antibacterial treatment even though their symptoms may have disappeared well before the end of the course. If this rule were to be obeyed, then the vast majority of the invading bacterial cells would be wiped out, leaving the body's own defense system to mop up any isolated survivors or resistant cells. If, however, the treatment is stopped too soon, then the body's defenses struggle to cope with the survivors. Any isolated resistant bacteria are then given the chance to multiply, resulting in a new infection which will, of course, be completely resistant to the original drug.

1.5.2 Drug resistance by genetic transfer

A second way in which bacterial cells can acquire drug resistance is by gaining that resistance from another bacterial cell. This occurs because it is possible for genetic information to be passed on directly from one bacterial cell to another. There are two main methods by which this can take place—transduction and conjugation. In transduction, small segments of genetic information known as plasmids are transferred by means of bacterial viruses (bacteriophages) from a resistant cell to a non-resistant cell. If the plasmid brought to the infected cell contains the gene required for drug resistance, then the recipient cell will be able to use that information and gain resistance. For example, the genetic information required to synthesize β-lactamases can be passed in in this way, rendering bacteria resistant to penicillins. The problem is particularly prevalent in hospitals where currently over 90 per cent of staphylococcal infections are resistant to antibiotics such as Penicillin, Erythromycin, and Tetracycline.
In conjugation, bacterial cells pass genetic material directly to each other. This is a method used mainly by Gram-negative rod-shaped bacteria in the colon, and involves two cells building a connecting bridge of sex pili, through which the genetic information may pass.

1.5.3 Other factors affecting drug resistance

The more useful a drug is, the more it will be used and the greater the possibilities of resistant bacterial strains emerging. The original penicillins were used widely in human medicine, but were also commonly used in veterinary medicine. Antibacterial agents have also been used in animal feeding and this has also resulted in drug-resistant bacterial strains. The present situation is such that many of the original bacterial strains which were treated so smoothly with penicillin V or penicillin G are now resistant to these agents. In contrast, these penicillins are still highly effective antibacterial agents in poorer, developing nations in Africa, where the use of the drug has been far less widespread. The ease with which different bacteria acquire resistance varies. For example, *Staphylococcus aureus* is notorious for its ability to acquire drug resistance due to the ease with which it can undergo transduction. On the other hand, the microorganism responsible for syphilis seems incapable of acquiring resistance and is still susceptible to the original drugs used against it.

1.6 ANTIBACTERIAL DRUG DEVELOPMENT: CHALLENGES AND OPPORTUNITIES

In the recent times, the approach used to discover antibacterial agent has changed. Traditionally, the theme for successful anti-infective therapy was to discover an agent with excellent pharmacological properties that kills or inhibits disease-causing microorganisms without harming the host. This was pertinent to a broad-spectrum drug that kills a wide array of pathogens, but this criterion has limited the number of suitable drug candidates and the strategies used to discover them. The aims of new approach for successful anti-infective chemotherapeutics are to preserve the efficacy of each agent as long as possible by delaying the emergence of drug resistance and to spare the normal microbiota as much as possible.[34]

There exist formidable challenges before infectious disease clinicians and the antibiotic drug discovery community. There has been an unfortunate retreat of the pharmaceutical industry from this area of research as a result of economic, regulatory, and scientific challenges. Some pharmaceutical companies have decided to discontinue the development of this group of therapeutic agents. In the 1960s, the FDA approved an average 2.9 new antibacterial drugs per year. This decreased to 2.2 drugs per year in the 1990s and further dropped to 1.6 drugs per year between 2000 and 2004. There are many reasons for this decrease. The profit for antibacterial agents is a fraction of the profit obtained from most other types of drugs. The lower profit margin may be attributed to the short term use of the drug when compared to other types of medicine.

Yet, there are reasons to be hopeful. The majority of well-validated targets for antibiotics have been characterized in terms of both structure and function. Furthermore, crystal structures of complexes of these targets with antibiotics are now available and provide drug-target interaction knowledge that was unheard of even a decade ago.

Given the long gap in the introduction of new structural classes of antibiotics—38 years between Streptogramins in 1962 and Linezolid in 2000 and the early development of resistance to a given antibiotic once it is in widespread clinical use, there is a pressing and recurrent need for new molecules with antibiotic properties. Natural and synthetic molecules are both likely to remain important sources for new antibiotics but each offers a distinct challenge.

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