Chapter 1

Introduction

One of the great success stories of medicinal chemistry is the fight against bacterial infection.

1.1 The history of antibacterial agents

While our scientific knowledge of antibacterial agents has only recently been developed, the practical application of antibacterial has existed for centuries. The first known use was by the Chinese about 2,500 years ago. During this time, they discovered that applying the moldy curd of soybeans to infections had certain therapeutic benefits. It was so effective that it became a standard treatment. Evidence suggests that other cultures used antibiotic-type substances as therapeutic agents. The Sudanese-Nubian civilization used a type of tetracycline antibiotic as early as 350 A.D. In Europe during the middle ages, crude plant extracts and cheese curds were also used to fight infection. Although these cultures used antibiotics, the general principles of antibiotic action were not understood until the nineteenth century.

Bacteria were first observed by the Dutch scientist Anton van Leeuwenhoek in 1674, using a single-lens microscope of his own design.[1,2] However, it was not until the nineteenth century that their link with disease was appreciated. Along with his contemporary, Robert Koch, Pasteur was an early advocate of the germ theory of disease. Robert Koch was a pioneer in medical microbiology and worked on cholera, anthrax and tuberculosis. In his research into tuberculosis, Koch finally proved the germ theory, for which he was awarded a Noble Prize in 1905. In Koch's postulates, he set out criteria to test if an organism is the cause of a disease; these postulates are still used today.[3] Though it was known in the nineteenth century that bacteria are the cause of many diseases, no effective antibacterial treatment were available.[4] In 1910, Paul Ehrlich has successfully

developed the first example of a purely synthetic antibiotic, the arsenic-containing compound salvarsan **1**[5] (Fig. 1.1) and proved effective against trypanosomiasis. Later in 1934, proflavine **2** (Fig. 1.2) was introduced and is effective against bacterial infections in deep surface wounds, and was used extensively during Second World War.

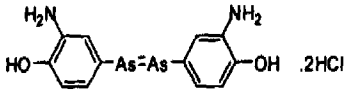


Fig. 1.1 Salvarsan 1

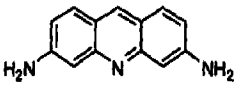


Fig. 1.2 Proflavine 2

To fight against bacterial infections in the bloodstream, in 1935, prontosil **3** (Fig. 1.3) was discovered and was effective against streptococci infections *in vivo*.

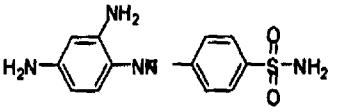


Fig. 1.3 Prontosil3

Despite penicillin's success during 1940's, it was not effective against all the types of infection and the need for new antibacterial agent still remained. In 1944, the antibiotic streptomycin was discovered from a systematic search of soil organisms. After the Second World War, the effort continued to find other novel antibiotic structures. This led to the discovery of peptide antibiotics (1945), chloramphenicol (1947), the tetracycline antibiotics (1948), the macrolide antibiotics (1952), and cephalosporin C (1955). Many antibacterial agents are now available and the vast majority of bacterial diseases have been brought under control (e.g. syphilis, tuberculosis, typhoid, bubonic plague, leprosy, diphtheria, tetanus, gonorrhea).

1.2 The bacterial cell

The success of antibacterial agents owes much to the fact that they can act selectively against bacterial cell rather than animal cells. This is largely due to the fact that bacterial cells and animal cells differ both in their structure and in the biosynthetic pathways, which proceed inside them. The most prominent bacterial cells are 0.5-1.0 μ wide and 1.0-5.0 μ long. The thickness of the cell wall may vary between 80 and 200 Å. The plasma membrane that surrounds the cytoplasm is common to all cells. It consists of phospholipid bilayers, lipids and proteins joined by hydrophobic forces into a fluid dynamic structure. The plasma membrane regulates the movement of ions and molecules into and out of the cell and also houses enzymes and proteins that are specifically required by the cell.

The bacterial cell wall is a unique structure, which surrounds the cell membrane. Although not present in every bacterial species, the cell wall is very important as a cellular component. Structurally, the wall is necessary for maintaining the cell's characteristic shape, countering the effects of osmotic pressure, providing attachment sites for bacteriophages and providing a rigid platform for surface appendages. As in the other organisms, the bacterial cell wall provides structural integrity to the cell. The bacterial cell wall differs from that of all other organisms by the presence of peptidoglycan (poly-N-acetylglucosamine and N-acetylmuramic acid), which is immediately outside the cytoplasmic membrane. Peptidoglycan is responsible for the rigidity of the bacterial cell wall and for the determination of cell shape. There are two main types of bacterial cell walls, Gram-positive and Gram-negative, which differentiated by their Gram staining characteristics.

The Gram-positive cell is characterized by the presence of a very thick peptidoglycan layer. Imbedded in Gram-positive cell are polyalcohols called teichoic acids, some of

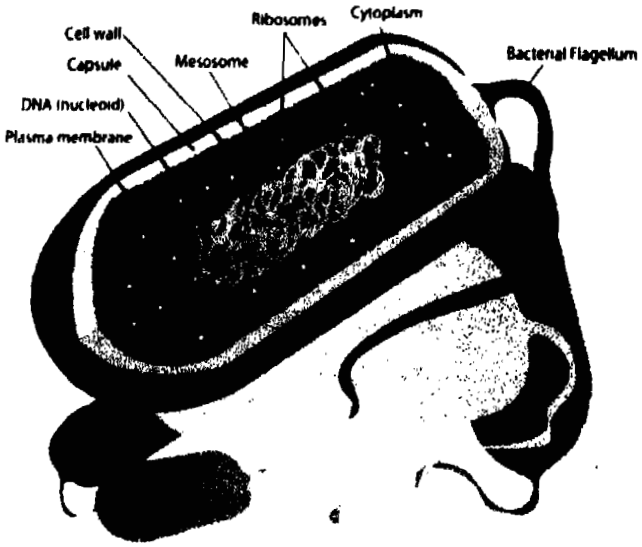
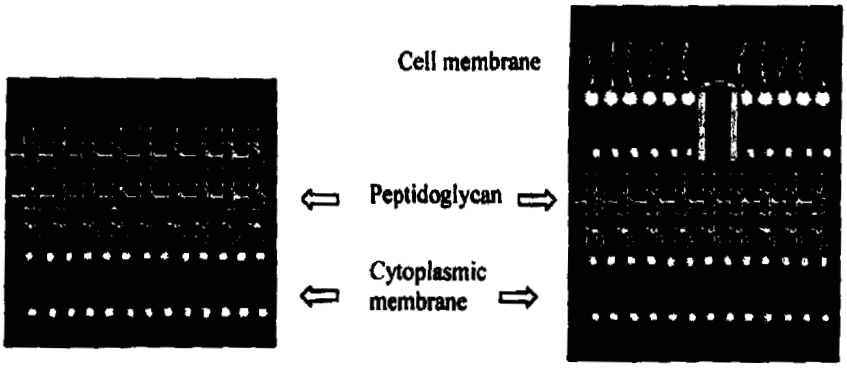


Fig. 1.4 The bacterial cell



Gram-positive cell membrane Gram-negative cellmembrane

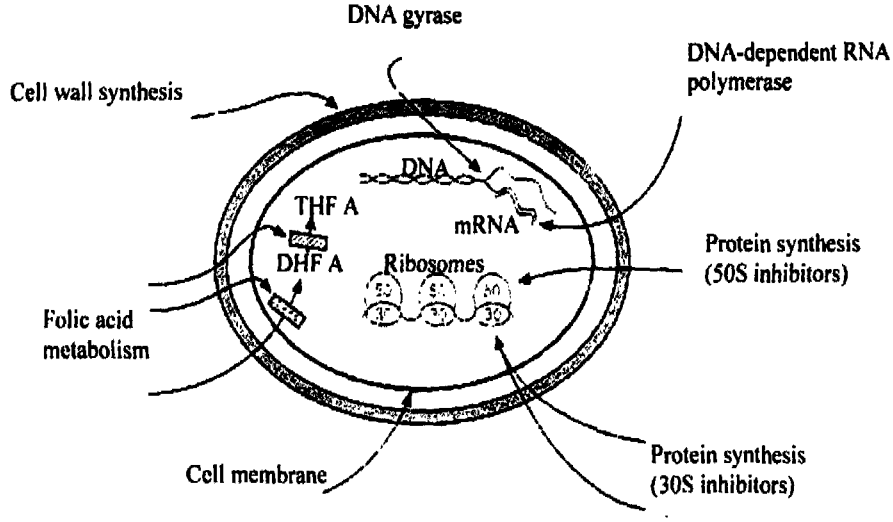
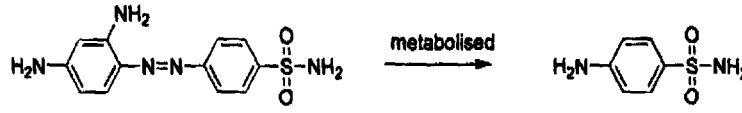


Fig. 1.5 target sites for antibacterial agents

1.3.1 Antibacterial agents that inhibit cell metabolism

The sulfa drugs inhibit the cell metabolism of a microorganism and thereby exhibiting the antibacterial activity. Actually, these sulfa drugs are prodrugs, inactive counterparts of active drug. After intake of these drugs, they are metabolized by the bacteria to give sulfanilamide, which is the true antibacterial agent. Prontosil **3** (Fig. 1.6) is the first drug of this class of compounds.[6,7]



Prontosil 3 sulfanilamide

Fig. 1.6 Metabolism of prontosil

The sulfonamides[8,9] blocks the biosynthesis of the vitamin folic acid in bacterial cells by inhibiting the enzyme responsible for the linking together the component parts of folic

acid. They act as inhibitors by mimicking *p*-aminobenzoic acid (PABA), one of the normal constituents of folic acid. Hence, folic acid is not synthesized. The cell will stop dividing since folic acid is essential to growth (Fig. 1.7).

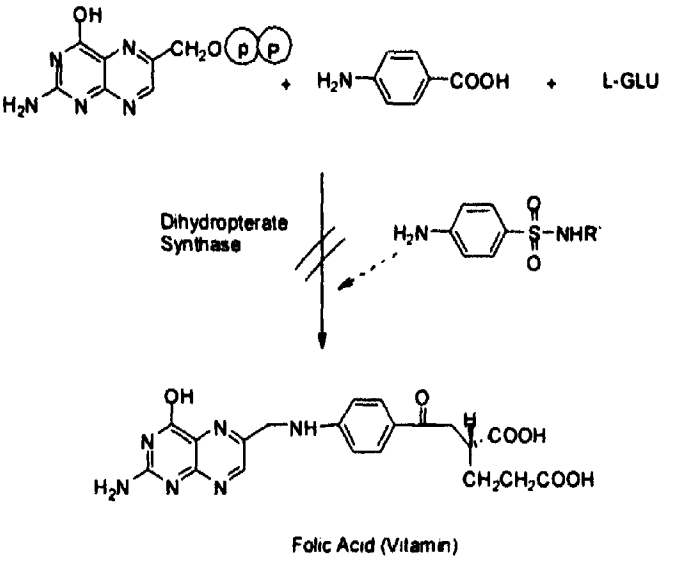


Fig. 1.7 Mechanism of action of sulfonamides.

Apart from the sulfonamides, trimethoprim and sulfones are used as antimetabolites in medical use (Fig. 1.8).

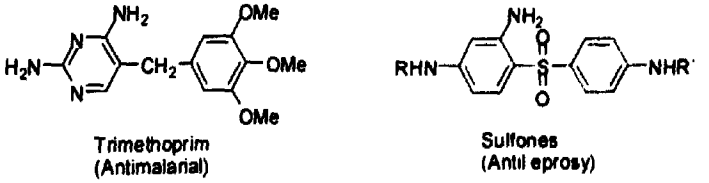


Fig. 1.8 Antimetabolites in medical use.

1.3.2 Inhibitors of cell-wall synthesis

Since bacteria have a cell wall made up of repeating units of peptidoglycan and human cells lacks this feature, it would seem that the bacterial cell wall presents an ideal target

for chemotherapy. The critical attack site of anti-cell-wall agents is the peptidoglycan layer. This layer is essential for the survival of bacteria in hypotonic environments; loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death.

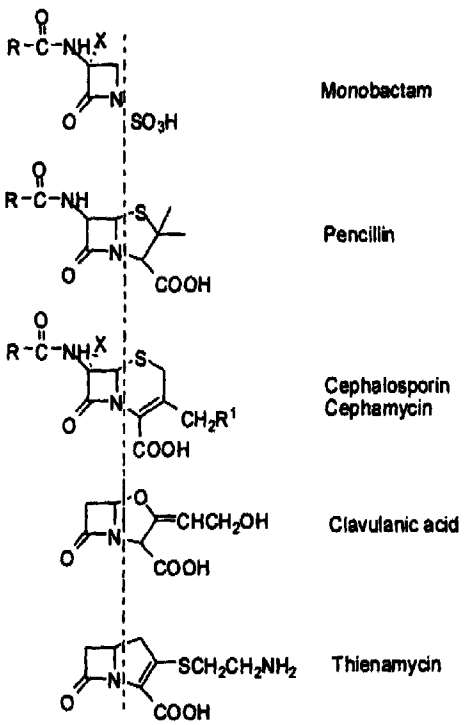


Fig.1.9 Basic structures of ß-lactam antibiotics. Penicillins and cephalosporins/cephamycins are widely used to inhibit both Gram-positive and Gram-negative bacilli.[12-14] Monobactams inhibit only aerobic Gram-negative bacilli, clavulanic acid acts as a ß-lactamase inhibitor, and thienamycin inhibits a wide range of aerobic and anaerobic species. R and R' represent various carbon groups. X can be either hydrogen or a methoxy group.

Peptidoglycan synthesis occurs in three stages. The first stage takes place in the cytoplasm, where the low-molecular-weight precursors UDP-GlcAc and UDP-MurNAc-L-Ala-D-Glu-meso-Dap-D-Ala-D-Ala are synthesized. Membrane-bound enzymes

catalyze the second stage of cell wall synthesis. The non-nucleotide portions of the precursor molecules previously made are transferred. The third stage of cell wall synthesis involves polymerization of the subunits and the attachment of nascent peptidoglycan to the cell wall. The antibacterial agents act at any one these stages, there by inhibiting the bacterial cell wall synthesis.[10,11]

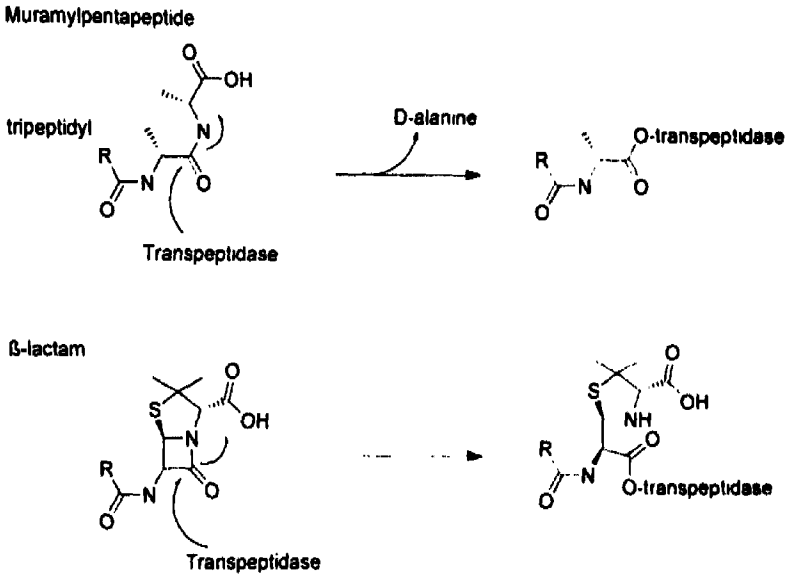


Fig. 1.10 Mechanism of action of ß-lactam antibiotics.

ß-lactams inhibit D-alanyl-D-alanine transpeptidase activity, which is vital in construction of peptiglycan, by acylation forming stable esters with the opened lactam ring attached to the hydroxy group of the enzyme's active site (Fig. 1.10).

Vancomycin

Vancomycin interrupts cell wall synthesis by forming a complex with the C-terminal D-alanine residues of peptidoglycan precursors. Complex formation at the outer surface of the cytoplasmic membrane prevents the transfer of the precursors from a lipid carrier to

the growing peptidoglycan wall by transglycosidases. Biochemical reactions in the cell wall catalyzed by transpeptidases and *D,D*-carboxypeptidase's are also inhibited by vancomycin and other glycopeptide antimicrobials. Because of its large size and complex structure (Fig. 1.11), vancomycin does not penetrate the outer membrane of gram-negative organisms. With resistance to beta-lactams increasing in frequency among staphylococci and enterococci, glycopeptides such as vancomycin remain important therapeutic agents against such bacteria.

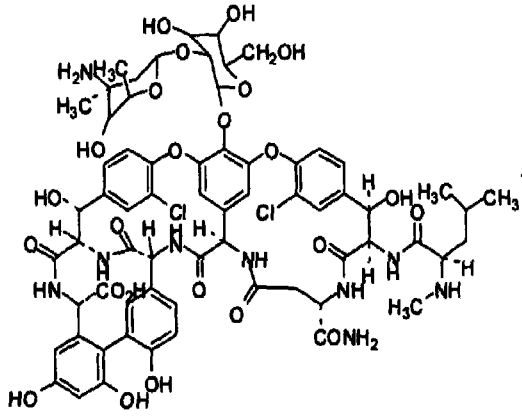


Fig. 1.11 Structure of vancomycin

1.3.3 Antibiotics that affect the function of cytoplasmic membranes

Bacterial Cytoplasmic Membranes

Biologic membranes are composed basically of lipid, protein, and lipoprotein. The cytoplasmic membrane acts as a diffusion barrier for water, ions, nutrients, and transport systems. Most workers now believe that membranes are a lipid matrix with globular proteins randomly distributed to penetrate through the lipid bilayer. A number of antimicrobial agents can cause disorganization of the membrane. These agents can be divided into cationic, anionic, and neutral agents. The best-known compounds are

polymyxin B and colistemethate (polymyxin E). These high-molecular-weight octapeptides inhibit Gram-negative bacteria that have negatively charged lipids at the surface. Since the activity of the polymyxins is antagonized by Mg^{2+} and Ca^{2+}, they probably competitively displace Mg^{2+} or Ca^{2+} from the negatively charged phosphate groups on membrane lipids. Basically, polymyxins disorganize membrane permeability so that nucleic acids and cations leak out and the cell dies. The polymyxins are of virtually no use as systemic agents since they bind to various ligands in body tissues and are potent toxins for the kidney and nervous system. Gramicidins are also membrane-active antibiotics that appear to act by producing aqueous pores in the membranes.

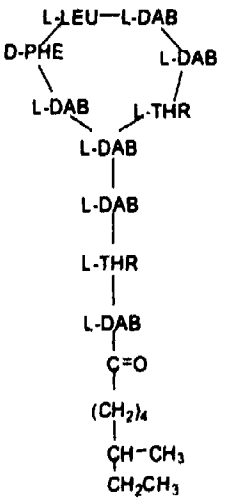
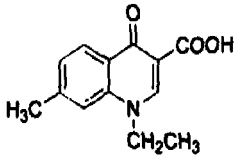


Fig. 1.12 Polymyxin B, polypeptide antibiotic.

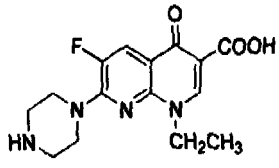
The polypeptide antibiotic polymyxin B (Fig. 1.12) operates within the cell membrane. It binds selectively to the different plasma membranes and causes the leakage of small molecules such as nucleosides from the cell and thereby exhibiting antibacterial activity.[15]

1.3.4 Inhibition of nucleic acid transcription and replication

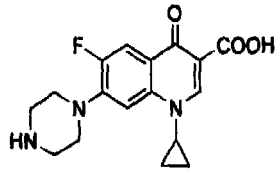
The major group of antibacterial agents that act by blocking DNA synthesis/activity is the quinolone group.[16] The quinolones all act by blocking the A subunit of DNA gyrase and inducing the formation of relaxation complex analogue. DNA gyrase introduces negative super helical turns into duplex DNA, using the energy of ATP. This is the crucial enzyme that maintains the negative super helical tension of the bacterial chromosomes.



Nalidixic acid



Enoxacin



Ciprofloxacin

Fig. 1.13 Quinolones.[17, 18, 19]

Metronidazole represents an antibiotic active against DNA in a different way. This antibiotic, upon being partially reduced, causes the fragmentation of DNA in an, as yet, undefined way. This antibiotic is only effective against anaerobic bacteria and some parasites.

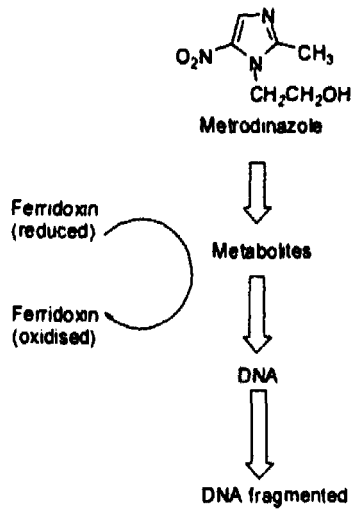


Fig. 1.14 Structure of metronidazole and its mechanism of action: Metronidazole enters an aerobic bacterium where, via the electron transport protein ferredoxin, it is reduced. The drug then binds to DNA, and DNA breakage occurs.

1.3.5 Inhibition of protein synthesis

Protein synthesis is the end result of two major processes, transcription and translation. An antibiotic that inhibits either of these will inhibit protein synthesis.

1.3.5.1 Transcription

During transcription, the genetic information in DNA is transferred to a complementary sequence of RNA nucleotides by the DNA-dependent RNA polymerase. This enzyme is composed of 5 subunits. Antibiotics that either alter the structure of the template DNA or inhibit the RNA polymerase will interfere with the synthesis of RNA, and consequently with protein synthesis.

Rifamycin (Fig. 1.15) inhibits the protein synthesis by selective inhibiting the DNA-dependent RNA polymerase.[20] It does this by binding to the β subunit in a non-covalent fashion.

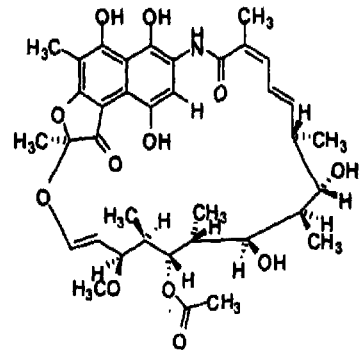


Fig. 1.15 Rifamycin B

1.3.5.2 Translation

In bacterial cells, the translation of mRNA into protein can be divided into three major phases: initiation, elongation, and termination of the peptide chain. Protein synthesis starts with association of mRNA, a 30S ribosomal subunit, and formyl-methionyl-transfer RNA (fMet-tRNA) to form a 30S initiation complex. The formation of this complex also requires guanosine triphosphate (GTP) and the participation of three protein initiation factors. The codon AUG is the initiation signal in mRNA and is recognized by the anticodon of fMet-tRNA. A 50S ribosomal subunit is subsequently added to form 70S initiation complex, and the bound GTP is hydrolyzed. In the elongation phase of protein synthesis, amino acids are added one at a time to a growing polypeptide in a sequence dictated by mRNA. It is this phase that is most susceptible to inhibition by a number of antibiotics. For many of these the ribosome is the target site. There are two binding sites on the ribosome, the P (peptidyl or donor site) and the A (aminoacyl) site. At the end of

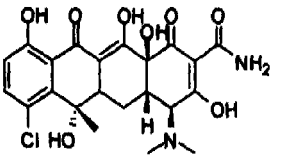
the initiation stage, the fMet-tRNA molecule is empty. In the first step of the elongation cycle, an aminoacyl-tRNA is inserted into the vacant A site on the ribosome. The particular species inserted depends on the mRNA codon that is positioned in the A site. Protein elongation factors and GTP are required for polypeptide chain elongation.

In the next step of the elongation phase, the formylmethionyl residue of the fMet-tRNA located at the peptidyl donor site is release from its linkage to tRNA, and is joined with a peptide bond to the amino group of the aminoacyl-tRNA in acceptor site to form a dipeptidy-tRNA. The enzyme catalyzing this peptide formation is peptidyl transferase, which is part of the 50S ribosomal subunit. Following the formation of peptide bond, an uncharged tRNA occupies the P site, whereas a dipeptidyl tRNA occupies the A site. The final phase of elongation cycle is translocation. The above process is repeated and the polypeptide chain grows from the amino terminal toward the carboxyl terminal amino acid and remains linked to tRNA and bound to the mRNA-ribosome complex during elongation of the chain. When completed it is released during chain termination. The polypeptide is released, and the messenger-ribosome-tRNA complex dissociates.

Several medically important antibiotics act on any one of the above stages and inhibit the protein synthesis and there exhibiting antibacterial activity.

Tetracycline

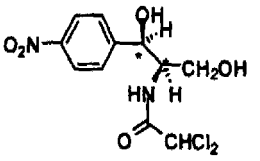
The tetracycline class of compound have a broad spectrum of activity and are the most widely prescribed antibiotics after pencillins.[21-24] Chlortetracycline[25] (Fig. 1.16) inhibits protein synthesis by binding to the 30S subunit of ribosomes and prevents the aminoacyl-tRNA binding to the A site on the ribosome. Thus protein release is inhibited.



Chlortetracyclin

Fig. 1.16

Chloramphenicol[26] (Fig. 1.17) is the drug of choice against typhoid and is also used in several bacterial infections, which are insensitive to other antibacterial agents. Chloramphenicol binds to the 50S subunit of ribosomes and appears to by inhibiting the movement of ribosomes along mRNA, probably by inhibiting the peptidyl transferase reaction by which the peptide chain is extended.



Chloramphenicol

Fig. 1.17

Macrolides

Erythromycin is the best example for the macrolide antibiotics.[27] Erythromycin[28] (Fig. 1.18) acts by binding to the 50S subunit by unknown mechanism. 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.

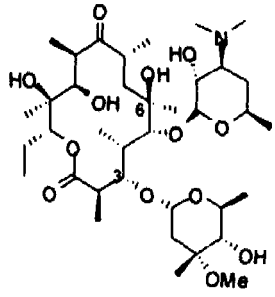


Fig. 1.18 Erythromycin A

Oxazolidinones

Oxazolidinones are a new class of synthetic antibacterial with activity against Gram-positive bacteria and anaerobic bacteria. They selectively bind to the 50S ribosomal subunit and inhibit bacterial translation at the initiation phase of the protein synthesis.

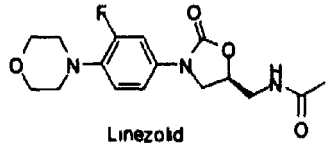


Fig. 1.19

Linezolid (Fig. 1.19),[29] the first drug from this class of compounds, was launched by Pharmacia in 2000.

Though there is availability of wide range of antibacterial agents in medicine, the emergence of bacterial resistance to the antibiotics poses a serious concern for medical

professionals during the past decade. In particular, multidrug resistant Gram-positive bacteria including methillicin resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermitis* (MRSE) and vancomycin resistant entercocci (VRE) are of major concern. To address these problems, medicinal chemists are still actively seeking new and improved antibacterial agents.

1.4 References

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