**19 Antibacterial agents**

The fight against bacterial infections over the last 70 years has been one of the great success stories of medicinal chemistry, yet it remains to be seen whether it will last. Bacteria, such as *Staphylococcus aureus*, have the worry- ing ability to gain resistance to known drugs and so the search for new drugs is never-ending. Although deaths from bacterial infection have dropped in the developed world, bacterial infection is still a major cause of death in the developing world. For example, the World Health Organization estimated that tuberculosis was responsible for about 2 million deaths in 2002 and that 1 in 3 of the world’s population was infected. The same organization estimated that in the year 2000, 1.9 million children died worldwide of respiratory infections with 70% of these deaths occurring in Africa and Asia. They also estimated that, each year, 1.4 million children died from gut infec- tions and the diarrhoea resulting from these infections. In the developed world, deaths from food poisoning due to virulent strains of *Escherichia coli* have attracted widespread publicity, while tuberculosis has returned as a result of the AIDS epidemic.



The topic of antibacterial agents is a large one and terms are used in this chapter which are unique to this particular field. Rather than clutter the text with explana- tions and definitions, Appendix 5 contains explanations of such terms as aerobic and anaerobic organisms; anti- bacterial and antibiotic substances; **cocci**; **bacilli**; strep- tococci; and staphylococci. Appendix 5 also explains briefly the difference between bacteria, algae, protozoa, and fungi. The emphasis in this chapter is on agents that act against bacteria, but some of those described also act against protozoal infections and this may be mentioned in the text.

# 19.1 History of antibacterial agents

There is evidence of antibacterial herbs or potions being used for many centuries. For example, the Chinese used mouldy soybean curd to treat carbuncles, boils, and other

infections. Greek physicians used wine, myrrh, and inor- ganic salts. In the Middle Ages, certain types of honey were used to prevent infections following arrow wounds. Of course in those days, there was no way of knowing that bacteria were the cause of these infections.

Bacteria are single-cell microorganisms first identified in the 1670s by van Leeuwenhoek, following his inven- tion of the microscope. It was not until the nineteenth century, however, that their link with disease was appre- ciated. This followed the elegant experiments carried out by the French scientist Pasteur, who demonstrated that specific bacterial strains were crucial to fermentation and that these, and other, microorganisms were more wide- spread than was previously thought. The possibility that these microorganisms might be responsible for disease began to take hold.

An early advocate of a ‘germ theory of disease’ was the Edinburgh surgeon Lister. Despite the protests of several colleagues who took offence at the suggestion that they might be infecting their own patients, Lister introduced **carbolic acid** as an antiseptic and sterilizing agent for operating theatres and wards. The improvement in surgi- cal survival rates was significant.

During the latter half of the nineteenth century, sci- entists such as Koch were able to identify the microor- ganisms responsible for diseases such as tuberculosis, cholera, and typhoid. Methods of **vaccination** were stud- ied and research was carried out to try and find effec- tive antibacterial agents or antibiotics. The scientist who can lay claim to be the father of chemotherapy—the use of chemicals against infection—was Paul Ehrlich. Ehrlich spent much of his career studying histology, then immunochemistry, and won a Nobel prize for his contri- butions to immunology. In 1904, however, he switched direction and entered a field which he defined as chemo- therapy. Ehrlich’s **principle of chemotherapy** was that a chemical could directly interfere with the proliferation of microorganisms at concentrations tolerated by the host. This concept was popularly known as the **magic bul- let**, where the chemical was seen as a bullet which could

**414 Chapter 19** Antibacterial agents

search out and destroy the invading microorganism without adversely affecting the host. The process is one of **selective toxicity**, where the chemical shows greater tox- icity to the target microorganism than to the host cells. Such selectivity can be represented by a **chemotherapeu- tic index**, which compares the minimum effective dose of a drug with the maximum dose that can be tolerated by the host. This measure of selectivity was eventually replaced by the currently used **therapeutic index**.

By 1910, Ehrlich had successfully developed the first example of a purely synthetic antimicrobial drug. This was the arsenic-containing compound **salvarsan** (Fig. 19.1). Although it was not effective against a wide range of bacterial infections, it did prove effective against the protozoal disease of sleeping sickness (trypanosomiasis) and the spirochete disease of syphilis. The drug was used until 1945 when it was replaced by penicillin (see also Box 19.20).

Over the next 20 years, progress was made against a variety of protozoal diseases, but little progress was made in finding antibacterial agents until the introduction in 1934 of **proflavine** (Fig. 19.1)—a drug which was used during World War II against bacterial infections in deep surface wounds. Unfortunately, it was too toxic to be used against systemic bacterial infections (i.e. those carried in the bloodstream) and there was still an urgent need for agents which would fight these infections.

This need was answered in 1935 when it was discov- ered that a red dye called **prontosil** was effective against streptococcal infections *in vivo*. As discussed later, pron- tosil was recognized eventually as a prodrug for a new class of antibacterial agents—the **sulpha drugs** or **sul- phonamides**. The discovery of these drugs was a real breakthrough, as they represented the first drugs to be effective against systemic bacterial infections. In fact, they were the only effective drugs until penicillin became available in the early 1940s.

Although **penicillin** was discovered in 1928, it was not until 1940 that effective means of isolating it were devel- oped by Florey and Chain. Society was then rewarded with a drug which revolutionized the fight against bac- terial infection and proved even more effective than the sulphonamides. Despite penicillin’s success, it was not effective against all types of infection and the need for new antibacterial agents still remained. Penicillin is an

example of a toxic fungal metabolite that kills bacteria and allows the fungus to compete for nutrients. The reali- zation that fungi might be a source for novel antibiotics spurred scientists into a huge investigation of microbial cultures from all round the globe.

In 1944, the antibiotic **streptomycin** was discovered from a systematic search of soil organisms. It extended the range of chemotherapy to the tubercle bacillus and a variety of Gram-negative bacteria. This com- pound was the first example of a series of antibiotics known as the **aminoglycoside** antibiotics. After World War II, the search continued leading to the discov- ery of **chloramphenicol** (1947), the peptide antibiot- ics (e.g. **bacitracin**, 1945), the **tetracycline** antibiotics (e.g. **chlortetracycline**, 1948), the macrolide antibiotics (e.g. **erythromycin**, 1952), the cyclic peptide antibiot- ics (e.g. **valinomycin**), and the first example of a second major group of β-lactam antibiotics, **cephalosporin C** (1955).

As far as synthetic agents were concerned, **isoniazid** was found to be effective against human tuberculosis in 1952, and in 1962 **nalidixic acid** (the first of the **quino- lone** antibacterial agents) was discovered. A second- generation of this class of drugs was introduced in 1987 with **ciprofloxacin**.

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, gas gangrene, teta- nus, and gonorrhoea). This represents a great achieve- ment for medicinal chemistry and it is perhaps sobering to consider the hazards society faced in the days before penicillin. Septicaemia was a risk faced by mothers dur- ing childbirth and could lead to death. Ear infections were common, especially in children, and could lead to deafness. Pneumonia was a frequent cause of death in hospital wards. Tuberculosis was a major problem, requiring special isolation hospitals built away from populated centres. A simple cut or a wound could lead to severe infection requiring the amputation of a limb, while the threat of peritonitis lowered the success rates of surgical operations. This was in the 1930s—still within living memory for many. Perhaps those of us born since World War II take the success of antibacterial agents too much for granted.

H2N

2HCl

NH2

HO As As OH

H2N

N NH2

Salvarsan Proflavine

**FIGURE 19.1** Salvarsan and proflavine. (The structure of salvarsan shown here is a simplification; it is, in fact, a cyclic trimer with no As = As bonds.)

19.2 **The bacterial cell**

The success of antibacterial agents owes much to the fact that they can act selectively against bacterial cells rather than animal cells. This is largely because bacterial and animal cells differ both in their structure and in their bio- synthetic pathways. Let us consider some of the differ- ences between the bacterial cell (defined as **prokaryotic**) (Fig. 19.2) and the animal cell (defined as **eukaryotic**).

Differences between bacterial and animal cells:

* the bacterial cell does not have a defined nucleus, whereas the animal cell does;
* animal cells contain a variety of structures called organelles (mitochondria, endoplasmic reticulum, etc.), whereas the bacterial cell is relatively simple;
* the biochemistry of a bacterial cell differs significantly from that of an animal cell. For example, bacteria may have to synthesize essential vitamins which animal cells can acquire intact from food. The bacterial cells must have the enzymes to catalyse these reactions. Animal cells do not, because the reactions are not required;
* the bacterial cell has a cell membrane and a cell wall, whereas the animal cell has only a cell membrane. The cell wall is crucial to the bacterial cell’s survival. Bacteria have to survive a wide range of environments and osmotic pressures, whereas animal cells do not. If a bacterial cell lacking a cell wall was placed in an aqueous environment containing a low concentration of salts, water would freely enter the cell as a result of osmotic pressure. This would cause the cell to swell and eventually burst. The scientific term for this is **lysis**. The cell wall does not stop water flowing into the cell directly, but it does prevent the cell from swelling

###### Mechanisms of antibacterial action **415**

and so indirectly prevents water entering the cell. Bacteria can be characterized by a staining technique which allows them to be defined as **Gram-positive** or **Gram-negative** (Appendix 5). Bacteria with a thick cell wall (20–40 nm) are stained purple and defined as Gram-positive. Bacteria with a thin cell wall (2–7 nm) are stained pink and are defined as Gram-negative. Although Gram-negative bacteria have a thin cell wall, they have an additional outer membrane not present in Gram-positive bacteria. This outer membrane is made up of lipopolysaccharides—similar in character to the cell membrane. These differences in cell walls and membranes have important consequences for the different vulnerabilities of Gram-positive and Gram- negative bacteria to antibacterial drugs.

19.3 **Mechanisms of antibacterial action**

There are five main mechanisms by which antibacterial agents act (Fig. 19.2).

* *Inhibition of cell metabolism*: antibacterial agents which inhibit cell metabolism are called **antimetabo- lites**. These compounds inhibit the metabolism of a microorganism, but not the metabolism of the host. They can do this by inhibiting an enzyme-catalysed reaction which is present in the bacterial cell, but not in animal cells. The best-known examples of antibac- terial agents acting in this way are the sulphonamides. It is also possible for antibacterial agents to show selectivity against enzymes which are present in both the bacterial and mammalian cell, as long as there are significant differences in structure between the two.

Sulphonamides

Rifamycins

Quinolones Aminoacridines

Nuclear material DNA/RNA

Penicillins Cephalosporins Cycloserine

Polymyxins

Enzymes

Chloramphenicol Streptomycin Tetracyclines

.........

...... ...

... ..... .

Cytoplasm

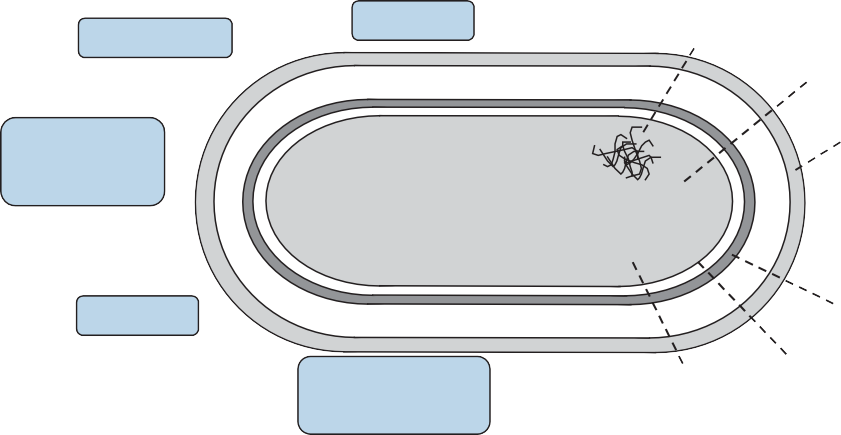
Outer membrane

(Gram-negative bacteria only)

Cell wall Periplasmic space

Ribosomes Plasma membrane

**FIGURE 19.2** The bacterial cell and drug targets.



**416 Chapter 19** Antibacterial agents

* *Inhibition of bacterial cell wall synthesis* leads to bacte- rial cell lysis and death. Agents operating in this way include penicillins, cephalosporins, and glycopeptides such as vancomycin. As animal cells do not have a cell wall, they are unaffected by such agents.
* *Interactions with the plasma membrane*: some antibac- terial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell. Polymyxins and tyrothri- cin operate in this way.
* *Disruption of protein synthesis* means that essential proteins and enzymes required for the cell’s survival can no longer be made. Agents which disrupt protein synthesis include the rifamycins, aminoglycosides, tetracyclines, and chloramphenicol.
* *Inhibition of nucleic acid transcription and replication* prevents cell division and/or the synthesis of essential proteins. Agents acting in this way include nalidixic acid and proflavine.

We now consider these mechanisms in more detail.

was synthesized in the laboratory and became the first synthetic antibacterial agent found to be active against a wide range of infections. Further developments led to a range of sulphonamides which proved effective against Gram-positive organisms, especially pneumococci and meningococci*.*

Despite their undoubted benefits, sulpha drugs have proved ineffective against infections such as *Salmonella*— the organism responsible for typhoid. Other problems have resulted from the way these drugs are metabolized, as toxic products are frequently obtained. This led to the sulphonamides being superseded by penicillin.

19.4.1.2 Structure–activity relationships

The synthesis of a large number of sulphonamide ana- logues (Fig. 19.4) led to the following conclusions:

* the *para*-amino group is essential for activity and must be unsubstituted (i.e. R1 = H). The only excep- tion is when R1 = acyl (i.e. amides). The amides them- selves are inactive but can be metabolized in the body to regenerate the active compound (Fig. 19.5). Thus, amides can be used as sulphonamide prodrugs;
* the aromatic ring and the sulphonamide functional group are both required;
* both the sulphonamide and amino group must be

19.4 **Antibacterial agents which act against cell metabolism (antimetabolites)**

directly attached to the aromatic ring;

* + the aromatic ring must be *para*-substituted only. Extra

19.4.1 **Sulphonamides**

19.4.1.1 The history of sulphonamides

The best example of antibacterial agents acting as anti- metabolites are the sulphonamides (sometimes called the sulpha drugs). The sulphonamide story began in 1935 when it was discovered that a red dye called **pron- tosil** (Fig. 19.3) had antibacterial properties *in vivo* (i.e. when given to laboratory animals). Strangely enough, no antibacterial effect was observed *in vitro*. In other words, prontosil could not kill bacteria grown in the test tube.

substitution eliminates activity for steric reasons;

* the sulphonamide nitrogen must be primary or secondary;
* R2 is the only possible site that can be varied in sulphonamides.

### Sulphanilamide analogues

In sulphanilamide analogues (Fig. 19.4), R2 is often varied by incorporating a large range of heterocyclic or aromatic

This remained a mystery until it was discovered that O



prontosil was metabolized by bacteria present in the small

R1HN S O

intestine of the test animal to give a product called **sul- phanilamide** (Fig. 19.3). It was this compound which was

NHR2

the true antibacterial agent. Thus, prontosil was an early example of a **prodrug** (section 14.6). Sulphanilamide

**FIGURE 19.4** Sulphonamide analogues used in structure–activity relationship studies.

H2N

NH2

O

2



O

N S

N NH

Prontosil

Metabolism

H2N



O

S O NH2

Sulphanilamide

**FIGURE 19.3** Metabolism of prontosil.

###### Antibacterial agents which act against cell metabolism (antimetabolites) **417**



O

S O NHR2

–CH3CO2H



O

S O NHR2

HN

Me

O

H2N

**FIGURE 19.5** Metabolism of an *N*-acyl group to regenerate an active sulphonamide.

structures which affects the extent to which the drug binds to plasma protein. This, in turn, controls the blood levels and lifetime of the drug. Thus, a drug that binds strongly to plasma protein will be released slowly into the blood circulation and will be longer lasting. Varying R2 can also affect the solubility of sulphonamides. To con- clude, variations of R2 affect the pharmacokinetics of the drug, rather than its mechanism of action (Box 19.1).

### Applications of sulphonamides

Before the appearance of penicillin, the sulpha drugs were the drugs of choice in the treatment of infectious diseases. Indeed, they played a significant part in world history by saving Winston Churchill’s life during World War II. After visiting North Africa for the Casablanca conference in 1943, Churchill became gravely ill with

##### **BOX 19.1** Sulphonamide analogues with reduced toxicity



The primary amino group of sulphonamides is acetylated in the body and the resulting amides have reduced solubility which can lead to toxic effects. For example, the metabo- lite formed from **sulphathiazole** (an early sulphonamide) is poorly soluble and can prove fatal if it blocks the kidney tubules (Fig. 1). It is interesting to note that certain popula- tions are more susceptible to this than others. For example, the Japanese and Chinese metabolize sulphathiazole more quickly than the average American and are more susceptible to its toxic effects.

It was discovered that the solubility problem could be overcome by replacing the thiazole ring in sulphathiazole with a pyrimidine ring to give **sulphadiazine** (Fig. 2). The

H2N

O

S O HN

N

*N*-Acetylation

Me C

HN

O

S

HN

O N

S

O

Insoluble

S

reason for the improved solubility lies in the acidity of the sulphonamide NH proton. In sulphathiazole, this proton is not very acidic (high p*K*a). Therefore, sulphathiazole and its metabolite are mostly un-ionized at blood pH. Replacing the thiazole ring with a more electron-withdrawing pyrimidine ring increases the acidity of the NH proton by stabilizing the resulting anion. Therefore, sulphadiazine and its metabo- lite are signiﬁcantly ionized at blood pH. As a consequence, they are more soluble and less toxic. Sulphadiazine was also found to be more active than sulphathiazole and soon replaced it in therapy. Silver sulphadiazine cream is still used topically to prevent infection of burns, although it is really the silver ions which provide the antibacterial effect.

S

HN

O N

p*K*a 6.48

H2N

N

86% Ionized

O

S O N N

N

**FIGURE 1** Metabolism of sulphathiazole.

O

H2N

**FIGURE 2** Sulphadiazine.

**418 Chapter 19** Antibacterial agents

H2N



O

S O N

HN N

MeO OMe

**FIGURE 19.6** Sulphadoxine.

an infection and was bedridden for several weeks. Fortunately, he responded to the novel sulphonamide drugs of the day.

Penicillins largely superseded sulphonamides and, for a long time, sulphonamides took a back seat. There has been a revival of interest, however, with the discovery of a new ‘breed’ of longer-lasting sulphonamides. One example of this new generation is **sulphadoxine** (Fig. 19.6), which is so stable in the body that it need only be taken once a week. The combination of sulphadoxine and **pyrimethamine** is called **Fansidar** and has been used for the treatment of malaria.

The sulpha drugs presently have the following applica- tions in medicine:

* treatment of urinary tract infections;
* eye lotions;
* treatment of infections of mucous membranes;
* treatment of gut infections (Box 19.2).

It is also worth noting that sulphonamides have occa- sionally found uses in other areas of medicine (section 12.4.4.2).

### Mechanism of action

The sulphonamides act as competitive enzyme inhibitors of **dihydropteroate synthetase** and block the biosyn- thesis of **tetrahydrofolate** in bacterial cells (Fig. 19.7). Tetrahydrofolate is important in both human and bacte- rial cells, because it is an enzyme cofactor that provides one carbon units for the synthesis of the pyrimidine nucleic acid bases required for DNA synthesis (section 21.3.1). If pyrimidine and DNA synthesis is blocked, then the cell can no longer grow and divide.

Note that sulphonamides do not actively kill bacterial cells. They do, however, prevent the cells growing and multiplying. This gives the body’s own defence systems enough time to gather their resources and wipe out the invader. Antibacterial agents which inhibit cell growth are classed as **bacteriostatic**, whereas agents such as penicillin which actively kill bacterial cells are classed as

**BOX 19.2** Treatment of intestinal infections



HN

O

S O HN

N

– O2C

Enzyme

+

H2N

O

S O N HN

–

O2C

O

S

Succinyl sulphathiazole

–

CO

2

S

Succinate Sulphathiazole

**FIGURE 1** Succinyl sulphathiazole is a prodrug of sulphathiazole.

O

HN C

O

S O

NHR2

**FIGURE 2** Substitution on the aniline nitrogen with benzoyl groups.

Sulphonamides have been particularly useful against intestinal infections, and can be targeted against these by the use of prodrugs. For example, **succinyl sulphathia- zole** is a prodrug of sulphathiazole (Fig. 1). The succi- nyl moiety contains an acidic group which means that the prodrug is ionized in the intestine. As a result, it is not absorbed into the bloodstream and is retained in

the intestine. Slow enzymatic hydrolysis of the succinyl group then releases the active sulphathiazole where it is needed.

Benzoyl substitution (Fig. 2) on the aniline nitrogen has also given useful prodrugs that are poorly absorbed through the gut wall because they are too hydrophobic (section 11.3). They can be used in the same way.

###### Antibacterial agents which act against cell metabolism (antimetabolites) **419**

H

N

HN

H2N N

O

N

O

H2N CO2H

*para-*Aminobenzoic acid Dihydropteroate synthetase



P P



Reversible

H

H2N N

N

HN

H N

N

O

P = Phosphate

inhibition

Dihydropteroate

CO2H

Sulphonamides

H

H2N N N

HN

H N

H2N CO2H N

CO2H

H O

H

N CO2H

L-Glutamic acid

Dihydrofolate

O H CO2H

H

H2N N N

HN

N H

Dihydrofolate reductase

NADPH



Trimethoprim

H N

H

O

Tetrahydrofolate (coenzyme F)

N CO2H

O H CO2H

**FIGURE 19.7** Mechanism of action of sulphonamides.

**bactericidal**. Because sulphonamides rely on a healthy immune system to complete the job they have started, they are not recommended for patients with a weakened immune system. This includes people with AIDS, as well as patients who are undergoing cancer chemotherapy or have had an organ transplant and are taking immuno- suppressant drugs.

Sulphonamides act as inhibitors by mimicking *p***-aminobenzoic acid** (PABA)—one of the normal sub- strates for dihydropteroate synthetase. The sulphonamide molecule is similar enough in structure to PABA that the enzyme is fooled into accepting it into its active site (Fig. 19.8). Once it is bound, the sulphonamide prevents PABA from binding. As a result, dihydropteroate is no longer

synthesized. One might ask why the enzyme does not join the sulphonamide to the other component of dihydrop- teroate to give a dihydropteroate analogue containing the sulphonamide skeleton. This can in fact occur, but it does the cell no good at all because the analogue is not accepted by the next enzyme in the biosynthetic pathway. Sulphonamides are competitive enzyme inhibitors so inhibition is reversible. This is demonstrated by certain organisms, such as staphylococci, pneumococci, and gonococci, which can acquire resistance by synthesizing more PABA. The more PABA there is in the cell, the more effectively it can compete with the sulphonamide inhibi- tor to reach the enzyme’s active site. In such cases, the dose levels of sulphonamide have to be increased to bring

###### 



O

H2N

C

H-Bond

van der Waals O

interactions

Ionic bond

Active site



H2N

O

S NR

H-Bond

van der Waals O

interactions

Ionic bond

Active site

**FIGURE 19.8** Sulphonamide prevents PABA from binding by mimicking PABA.

**420 Chapter 19** Antibacterial agents

back the same level of inhibition. Resistance to sulphona- mides can also arise by mutations that modify the target enzyme such that it has less affinity for sulphonamides, or by decreased permeability of the cell membrane to the sulphonamide.

Tetrahydrofolate is clearly necessary for the survival of

H2N

NH2

N N

OMe

bacterial cells, but it is also vital for the survival of human cells, so why are the sulpha drugs not toxic to humans? The answer lies in the fact that human cells synthesize tetrahydrofolate in a different manner and do not contain

MeO OMe Trimethoprim (antimalarial)

NH2

the enzyme dihydropteroate synthetase. In human cells, tetrahydrofolate is synthesized from **folic acid**, which is obtained from the diet as a vitamin and is brought across cell membranes by a transport protein.

We could now ask ‘If human cells can acquire folic acid from the diet, why can’t bacterial cells infecting the

N

H2N

N

O

S O

NHR1

human body do the same, then convert it to tetrahydro-

Sulphones (anti-leprosy)

folate?’ In fact, bacterial cells are unable to acquire folic O

acid because they lack the necessary transport protein required to carry it across the cell membrane.

S

To sum up, the success of sulphonamides is due to two

metabolic differences between mammalian and bacterial

H2N

O Me

HN

N O

cells:

* bacteria have a susceptible enzyme which is not pre- sent in mammalian cells;
* bacteria lack the transport protein that would allow them to acquire folic acid from outside the cell.
  + 1. **Examples of other antimetabolites**

Other antimetabolites in medical use include **trimetho- prim** and a group of compounds known as **sulphones** (Fig. 19.9).

### Trimethoprim

Trimethoprim is an orally active diaminopyrimidine structure, which has proved to be a highly selective anti- bacterial and antimalarial agent. It acts against **dihydro- folate reductase**—the enzyme which carries out the con- version of dihydrofolate to tetrahydrofolate—leading to the inhibition of DNA synthesis and cell growth.

Dihydrofolate reductase is present in mammalian cells, as well as bacterial cells, but mutations over millions of years have resulted in a significant difference in structure between the two enzymes such that trimethoprim recog- nizes and inhibits the bacterial enzyme more strongly. In fact, trimethoprim is 100,000 times more active against the bacterial enzyme.

Trimethoprim is often given in conjunction with the sulphonamide **sulphamethoxazole** (Fig. 19.9) in a preparation called **cotrimoxazole**. The sulphonamide inhibits the incorporation of PABA into dihydropteroate,

Sulphamethoxazole

**FIGURE 19.9** Examples of antimetabolites in medical use.

while trimethoprim inhibits dihydrofolate reductase. Therefore, two enzymes in the one biosynthetic route are inhibited (Fig. 19.7). This is a very effective method of inhibiting a biosynthetic route and has the advantage that the doses of both drugs can be kept down to a safe level. To get the same level of inhibition using a single drug, the dose level would have to be much higher, leading to possible side effects. This approach has been described as **sequential blocking**.

Resistance to trimethoprim has been observed in strains of *E. coli* which produce a new form of the target enzyme that has less affinity for the drug.

### Sulphones

The sulphones (Fig. 19.9) are the most important drugs used in the treatment of leprosy. It is believed that they inhibit the same bacterial enzyme inhibited by the sul- phonamides (i.e. dihydropteroate synthetase).

**KEY POINTS**

* The principle of chemotherapy or the magic bullet involves the design of chemicals which show selective toxicity against bacterial cells rather than mammalian cells.
* Early antibacterial agents were salvarsan, prontosil, and the sulphonamides. Following the discovery of penicillin, several classes of antibiotics were isolated from fungal strains.

###### Antibacterial agents which inhibit cell wall synthesis **421**

* The bacterial cell differs in various respects from mamma- lian cells, allowing the identiﬁcation of drug targets which are unique to bacterial cells, or which differ signiﬁcantly from equivalent targets in mammalian cells.
* Antibacterial agents act on ﬁve main targets—cell metabo- lism, the cell wall, the plasma membrane, protein synthesis, and nucleic acid function.
* Sulphonamides require a primary aromatic amine group and a secondary sulphonamide group for good activity.
* Adding an aromatic or heteroaromatic group to the sulphona- mide nitrogen provides a variety of sulphonamides with dif- ferent pharmacokinetic properties.
* *N*-Acetylation of sulphonamides is a common metabolic reaction.
* Sulphonamides are used to treat infections of the urinary tract, gastrointestinal tract, and mucous membranes. They are also used in eye lotions.
* Sulphonamides are similar in structure to *para*-aminobenzoic acid—a component of dihydropteroate. As a result, they can bind to the bacterial enzyme responsible for dihydropteroate synthesis and act as an inhibitor.
* Mammals synthesize tetrahydrofolate from folic acid acquired from the diet. They lack the enzyme targeted by sulphona- mides. Bacteria lack the transport mechanisms required to transport folic acid into their cells.
* Trimethoprim inhibits dihydrofolate reductase—an enzyme which converts folic acid to tetrahydrofolate. It has been used in combination with sulphamethoxazole in a strategy known as sequential blocking.
* Sulphones are used in the treatment of leprosy.

19.5 **Antibacterial agents which inhibit cell wall synthesis**

## Penicillins

### History of penicillins

In 1877, Pasteur and Joubert discovered that certain moulds produced toxic substances which killed bacte- ria. Unfortunately, these substances were also toxic to humans and were of no clinical value. They did demon- strate, however, that moulds could be a potential source of antibacterial agents.

In 1928, Fleming noted that a bacterial culture that 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 anti- bacterial agent which was spreading into the surround- ing area. Recognizing the significance of this, he set out to culture and identify the fungus, and showed it to be a relatively rare species of *Penicillium*. It has since been suggested that the *Penicillium* spore responsible for the fungal colony originated from another laboratory in the building, and that the spore was carried by air currents to be blown through the window of Fleming’s laboratory. This in itself appears to be a remarkable stroke of good fortune. However, a series of other chance events were involved in the story—not least the weather! A period of early cold weather had encouraged the fungus to grow while the bacterial colonies had remained static. A period of warm weather then followed which encouraged the bacteria to grow. These weather conditions were the ideal experimental conditions required for:

* the fungus to produce penicillin during the cold spell;
* the antibacterial properties of penicillin to be revealed during the hot spell.

If the weather had been consistently cold, the bacteria would not have grown significantly and the death of cell colonies close to the fungus would not have been seen. Alternatively, if the weather had been consistently warm, the bacteria would have outgrown the fungus and little penicillin would have been produced. As a final twist to the story, the crucial agar plate had been stacked in a bowl of disinfectant ready for washing up, but was actu- ally placed above the surface of the disinfectant. It says much for Fleming’s observational powers that he both- ered to take any notice of a discarded culture plate and that he spotted the crucial area of inhibition.

Fleming spent several years investigating the novel antibacterial extract and showed it to have significant antibacterial properties while being remarkably non- toxic to mammals. Unfortunately, Fleming was unable to isolate and purify the active principle, and he 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 processes such as freeze-drying and chromatography, which allowed isolation of the antibiotic under much milder conditions than had previously been available. By 1941, Florey and Chain were able to carry out the first clinical trials on crude extracts of penicillin and achieved spec- tacular success. Further developments aimed at produc- ing the new agent in large quantities were developed in the USA, and, by 1944, there was enough penicillin to treat casualties arising from the D-Day landings.

Although the use of penicillin was now widespread, the structure of the compound was still not settled and the unusual structures being proposed proved a source

**422 Chapter 19** Antibacterial agents

R= CH2

H H H

R N S

6-Aminopenicillanic acid Me (6-APA)

Benzylpenicillin (penicillin G)

Acyl side chain 6

O N Me O

R= OCH2

CO2H

Phenoxymethylpenicillin (penicillin V)

-Lactam ring

Thiazolidine ring

**FIGURE 19.10** The structure of penicillin.

H2N

R OH

H**Cys**

CO2H H N

SH

H H H

Me **Val** R N S Me

Me O

O 2

H

CO2H

Biosynthesis

N Me

O H

CO2H

**FIGURE 19.11** The biosynthetic precursors of penicillin.

of furious debate. The issue was finally settled in 1945 when Dorothy Hodgkins established the structure by X-ray crystallographic analysis. The structure was quite surprising at the time, as penicillin was clearly a highly strained molecule, which explained why Fleming had been unsuccessful in purifying it.

The full synthesis of such a highly strained molecule presented a huge challenge—one that was met success- fully by Sheehan in 1957. Unfortunately, the full synthe- sis was too involved to be of commercial use, but, in the following year, Beechams isolated a biosynthetic inter- mediate of penicillin called **6-aminopenicillanic acid (6-APA)**. This revolutionized the field of penicillins by providing the starting material for a huge range of **semi- synthetic** penicillins.

Since then, penicillins have been used widely and often carelessly. As a result, penicillin-resistant bacteria have evolved and have become an increasing problem. The fight against penicillin-resistant bacteria was helped in 1976 when Beechams discovered a natural product called **clavulanic acid**, which proved highly effective in protecting penicillins from the bacterial enzymes which attack them (section 19.5.4.1).

### Structure of benzylpenicillin and phenoxymethylpenicillin

The acyl side chain (R) varies, depending on the com- ponents of the fermentation medium. For example, corn steep liquor (the fermentation medium first used for mass production of penicillin) contains high levels of phenylacetic acid (PhCH2CO2H) and gives **benzylpeni- cillin (penicillin G**; R = benzyl). A fermentation medium containing phenoxyacetic acid (PhOCH2CO2H) gives **phenoxymethylpenicillin** (**penicillin V**; R = PhOCH2) (Fig. 19.10).

 Test your understanding and practise your molecu- lar modelling with Exercise 19.1.

### Properties of benzylpenicillin

Benzylpenicillin (penicillin G) is active against a range of bacterial infections (Box 19.3) and lacks seri- ous side effects for most patients. However, there are various drawbacks. It cannot be taken orally because it is broken down by stomach acids, it has a narrow spectrum of activity, and there are many bacterial infections against which it has no effect—particularly those where the microorganism produces an enzyme called β**-lactamase**. This is an enzyme which hydro- lyses the β-lactam ring of benzylpenicillin and makes

O

Penicillin (Fig. 19.10) contains a highly unstable looking Me



C

NH

H S

H

N

H CO2H

bicyclic system consisting of a four-membered β-lactam R

ring fused to a five-membered thiazolidine ring. The Me

skeleton of the molecule suggests that it is derived from O

the amino acids cysteine and valine, and this has been

established (Fig. 19.11). The overall shape of the molecule

is like a half-open book, as shown in Fig. 19.12. **FIGURE 19.12** The three-dimensional shape of penicillin.

###### Antibacterial agents which inhibit cell wall synthesis **423**

**BOX 19.3** Clinical properties of benzylpenicillin and phenoxymethylpenicillin

**Benzylpenicillin** is active against non-β-lactamase-producing Gram-negative bacteria and those producing β-lactamase Gram-positive bacilli (e.g. *Meningitis*, *Gonorrhoea,* and early enzymes. It is ineffective when taken orally and should be strains of staphylococci) and several Gram-negative cocci administered by intravenous or intramuscular injection. (e.g. *Neisseria*). It is effective for many streptococcal, pneu- **Phenoxymethylpenicillin** is recommended for the treat- mococcal, gonococcal, and meningococcal infections. It is ment of various problems such as tonsillitis, rheumatic also used to treat anthrax, diphtheria, gas-gangrene, lep- fever, otitis media, and oral infections.

tospirosis, and Lyme disease in children. It can be effec- Allergic reactions are suffered by some individuals when tive against tetanus, although **metronidazole** is preferred. As they take penicillins, varying from a rash to immediate ana- penicillin is bactericidal, it is most active against rapidly phylactic shock. **Anaphylactic reactions** occur in 0.2% of dividing bacteria. There are many bacterial species against patients with a fatality rate of 0.001%. Less serious allergic which benzyl penicillin shows no activity, in particular reactions are more common (1–4%).

it inactive. Therefore, there is scope for producing ana- logues with improved properties. Before looking at penicillin analogues, we shall look at penicillin’s mech- anism of action.

### Mechanism of action for penicillin

#### Structure of the cell wall

In order to understand penicillin’s mechanism of action, we have to first look at the structure of the bacterial cell wall and the mechanism by which it is formed. Bacteria have cell walls in order to survive a large range

The wall is a peptidoglycan structure (Fig. 19.13). In other words, it is made up of peptide and sugar units. The structure of the wall consists of a paral- lel series of sugar backbones containing two types of sugar [*N***-acetylmuramic acid** (**NAM**) and *N***- acetylglucosamine** (**NAG**)] (Fig. 19.14). Peptide chains are bound to the NAM sugars and it is inter- esting to note the presence of d-amino acids in these chains. In human biochemistry there are only l-amino acids, whereas bacteria have **racemase** enzymes that can convert l-amino acids into d-amino acids. In the

CH2OH

of environmental conditions, such as varying pH, tem- perature, and osmotic pressure. Without a cell wall, water would continually enter the cell as a result of osmotic pressure, causing the cell to swell and burst (lysis). The cell wall is very porous and does not block the entry of

H O

OR H

HO

H HN

OH

H

CH3

O

water, but it does prevent the cell swelling. Animal cells do not have a cell wall, making it the perfect target for antibacterial agents such as penicillins.



L-Ala

NAM

NAG

NAM

NAG

NAM

NAG

**FIGURE 19.14** Sugars contained in the cell wall structure of bacteria. R = H, *N*-acetylglucosamine (NAG);

R = CHMeCO2H, *N*-acetylmuramic acid (NAM).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| NAM | | NAG | NAM | NAG | NAM | | NAG |
|  |  | | | | |  | |

D-Ala

NAM

NAG

NA

M NA

G NA

M



D-Glu

L-Ala

L-Lys

D-Glu

D-Glu

D-Ala

L-Ala

L-Lys

L-Lys

D-Glu

L-Lys

D-Glu

L-Ala

L-Lys

D-Glu

D-Ala

L-Lys

L-Ala

D-Glu

D-Ala

D-Ala

L-Lys

Pentaglycine link inhibited by penicillin

NAM

NAG

NAM

NAG

NAM

NAG

D-Ala

D-Ala

**FIGURE 19.13** Peptidoglycan structure of bacterial cell walls.

**424 Chapter 19** Antibacterial agents

final stage of cell wall biosynthesis, the peptide chains are linked together by the displacement of d-alanine from one chain by glycine in another.

About 30 enzymes are involved in the overall biosyn- thesis of the cell wall, but it is the final cross-linking reac- tion which is inhibited by penicillin. This leads to a cell wall framework that is no longer interlinked (Fig. 19.15). As a result, the wall becomes fragile and can no longer prevent the cell from swelling and bursting. The enzyme responsible for the cross-linking reaction is known as the **transpeptidase enzyme**. There are several types of the enzyme which vary in character from one bacterial species to another, but they are all inhibited to various degrees by penicillins.

There are significant differences in the thickness of the cell wall between Gram-positive and Gram-negative bac- teria. The cell wall in Gram-positive bacteria consists of 50–100 peptidoglycan layers, whereas in Gram-negative bacteria it consists of only two layers.

#### The transpeptidase enzyme and its inhibition

The transpeptidase enzyme is bound to the outer surface of the cell membrane and is similar to a class of enzymes called the **serine proteases**, so called because they con- tain a serine residue in the active site and catalyse the hydrolysis of peptide bonds. In the normal mechanism (see Fig. 19.16a), serine acts as a nucleophile to split the peptide bond between the two unusual d-alanine units on a peptide chain. The terminal alanine departs the active site, leaving the peptide chain bound to the active site. The pentaglycyl moiety of another peptide chain now enters the active site and the terminal glycine forms a peptide bond to the alanine group, displacing it from serine and linking the two chains together.

It has been proposed that penicillin has a conforma- tion which is similar to the transition-state conforma- tion taken up by the d-Ala-d-Ala moiety during the cross-linking reaction, and that the enzyme mistakes



NAM

L-Ala D-Glu L-Lys D-Ala

D-Ala

NAG

Sugar backbone

NAM NAG Sugar backbone

L-Ala

D-Glu

Gly Gly Gly Gly Gly

Enzyme-OH

L-Lys

D-Ala D-Ala

Gly Gly Gly Gly Gly

Penicillin

Transpeptidase Inhibition

D-Alanine

NAM

L-Ala D-Glu L-Lys D-Ala

D-Ala

NAG

Sugar backbone

NAM NAG Sugar backbone

L-Ala

D-Glu

Gly Gly Gly Gly Gly

L-Lys

D-Ala

Gly Gly Gly Gly Gly

O Enzyme

NAM

L-Ala D-Glu L-Lys

D-Ala

NAG

Sugar backbone

NAM NAG Sugar backbone

L-Ala

D-Glu

Gly Gly Gly Gly Gly

L-Lys

D-Ala

Gly Gly Gly Gly Gly

Cross-linking

**FIGURE 19.15** Cross-linking of bacterial cell walls inhibited by penicillin.

###### Antibacterial agents which inhibit cell wall synthesis **425**

1. Transpeptidase cross-linking



Peptide D-Ala

chain

H

D-Ala

O Me

N

N CO2

Me

H

OH

NH3

Ser Lys

Transpeptidase enzyme



Peptide chain

Peptide chain

NH

O

NH 2 Gly

O

Me

O H NH3

Ser Lys



Peptide Peptide

chain chain

Me

H

N

N

O

H

O

OH

Ser

NH3

Lys

1. Penicillin inhibition

###### 



O

R C NH H

S Me

O

N

H CO2

Me

OH

NH3

Ser Lys

Transpeptidase enzyme



Peptide chain

Blocked

O

R C

Gly

NH H

S Me

O

HN

CO2

Me

O

Ser

NH3

Lys



Blocked

H2O

O

R C NH H

S Me

O

HN

O

CO2

Me

Ser

NH3

Lys

**FIGURE 19.16** Mechanisms of transpeptidase cross-linking and penicillin inhibition.

penicillin for d-Ala-d-Ala and binds it to the active site. Once bound, penicillin is subjected to nucleophilic attack by serine (Fig. 19.16).

The enzyme can attack the β-lactam ring of penicillin and cleave it in the same way as it did with the peptide bond. However, penicillin is cyclic so the molecule is not split in two and nothing leaves the active site. Subsequent hydrolysis of the ester group linking the penicillin to the active site does not take place either, as the penicillin structure blocks access to the pentaglycine chain or water. If penicillin *is* acting as a mimic for a d-Ala-d-Ala moiety, this provides another explanation for its lack of toxicity. Since there are no d-amino acids or d-Ala-d-Ala segments in any human protein, it is unlikely that any of the body’s serine protease enzymes would recognize either the segment or penicillin itself. As a result, peni- cillin is selective for the bacterial transpeptidase enzyme

and is ignored by the body’s own serine proteases.

This theory has one or two anomalies, though. For example, **6-methylpenicillin** (Fig. 19.17) was thought to be

a closer analogue to d-Ala-d-Ala. On that basis, it should fit the active site better and have higher activity. However, when this structure was synthesized, it was found to be inactive. It is now proposed that 6-methoxypenicillin is a closer analogue to acyl-d-Ala-d-Ala than 6-methylpenicil- lin. Indeed, antibacterial penicillin structures containing a 6-methoxy substituent have been developed, for exam- ple **temocillin** (Fig. 19.27). Molecular modelling studies involving overlays of penicillin analogues (section 17.9) have demonstrated that the methyl group of a 6-methoxy substituent is more closely aligned to the methyl group of acyl-d-Ala-d-Ala, than a 6-methyl group would be (see Molecular modelling exercise 19.2).

### Resistance to penicillin

Bacterial strains vary in their susceptibility to penicillin. Some species, such as streptococci, are quite vulnerable, whereas a bacterium like *Pseudomonas aeruginosa* is par- ticularly resistant (see Box 19.4). Other species, such as

H H H

R N S Me

6

O N Me O

H R H

R N S Me

6

O N Me O

H Me

R N

H

O NH Me O H

Penicillin

CO2H

CO2H

R'=Me; 6-Methylpenicillin R'=OMe; 6-Methoxypenicillin

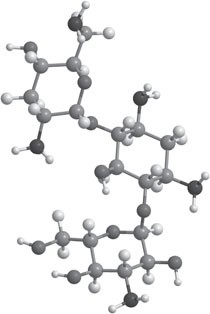
CO2H

Acyl-D-Ala-D-Ala

**FIGURE 19.17** Comparison of penicillin, 6-substituted penicillins, and acyl-d-Ala-d-Ala.

**426 Chapter 19** Antibacterial agents

**BOX 19.4** *Pseudomonas aeruginosa*



*Pseudomonas aeruginosa* is an example of an **opportunis- tic pathogen**. Such organisms are not normally harmful to healthy individuals. Indeed, many people carry the organ- ism without being aware of it, because their immune system keeps it under control. Once that immune system is weak- ened, though, the organism can start multiplying and lead to serious illness. Hospital-bound patients are particularly at risk, especially those suffering from shock or AIDS, or those undergoing cancer chemotherapy. Burn victims are particularly prone to *P. aeruginosa* skin infections and this can lead to septicaemia, which can prove fatal. The organ- ism is also responsible for serious lung infections among patients undergoing mechanical ventilation.

The cells of *P. aeruginosa* are rod-shaped and can appear blue or green in colour, which is why it was given the name aeruginosa. It prefers to grow in moist environments and

H N

H2N

HO

has been isolated from soil, water, plants, animals, and humans. It can even grow in distilled water and contact lens solutions. In hospitals, there are several possible sources of infection, including respiratory equipment, sinks, uncooked vegetables, and ﬂowers brought by visitors.

*Pseudomonas aeruginosa* is a difﬁcult organism to treat because it has an intrinsic resistance to a wide variety of anti- bacterial agents, including many penicillins, cephalosporins, tetracyclines, quinolones, and chloramphenicol. There are two reasons for this. The outer membrane of the cell has a low permeability to drugs and even if a drug does enter the cell, there is an efﬂux system which can pump it back out again. Nevertheless, there are drugs which have proved effective against the organism—in particular aminoglycosides such as tobramycin or gentamicin, and penicillins such as ticarcillin. These are often given in combination with each other.

O

NH2

O

HO

O

H

O

H2N

Tobramycin.

*S. aureus* are initially vulnerable, but acquire resistance when they are exposed to penicillin over a period of time. There are several reasons for this varied susceptibility.

2 O

O

NH2

O

O

HO

HO

O

O

O

OH

O

O

#### Physical barriers

If penicillin is to inhibit the transpeptidase enzyme, it has to reach the outer surface of the bacterial cell membrane where the enzyme is located. Thus, penicillin has to pass through the cell walls of both Gram-positive and Gram- negative bacteria. The cell wall is much thicker in Gram- positive bacteria than in Gram-negative bacteria, so one might think that penicillin would be more effective against Gram-negative bacteria. However, this is not the case. Although the cell wall is a strong, rigid structure, it is also highly porous, which means that small molecules

like penicillin can move through it without difficulty. One can imagine the cell wall being like several layers of chicken wire and the penicillin molecules as small peb- bles able to pass through the gaps.

If the cell wall does not prevent penicillin reaching the cell membrane, what does? As far as Gram-positive bacteria are concerned there *is* no barrier and that is why penicillin G has good activity against these organ- isms. However, Gram-negative bacteria have an outer lipopolysaccharide membrane surrounding the cell wall which is impervious to water and polar molecules, such as penicillin (Fig. 19.18). That can explain why Gram- negative bacteria are generally resistant, but not why some Gram-negative bacteria are susceptible and some are not. Should they not all be resistant?

###### Antibacterial agents which inhibit cell wall synthesis **427**



Outer membrane

Hydrophobic barrier

Porin

Periplasmic space



Lactamase L enzymes

L

L

L

Thin cell wall

L

Cell membrane

Cell

**FIGURE 19.18** Outer surface of a Gram-negative bacterial cell.

The answer lies in protein structures called **porins**, which are located in the outer membrane. These act as pores through which water and essential nutrients can pass to reach the cell. Small drugs such as penicillin can also pass this way, but whether they do or not depends on the structure of the porin, as well as the characteris- tics of the penicillin (i.e. its size, structure, and charge). In general, drugs have less chance of passing through the porins if they are large, have a negative charge, and are hydrophobic. In contrast, a small hydrophilic drug that can exist as a zwitterion can pass through. Therefore, porins play a crucial role in controlling the amount of penicillin capable of reaching the periplasmic space between the outer membrane and cell membranes. If access is slow, the concentration of penicillin at the transpeptidase enzyme may be insufficient to inhibit it effectively.

#### Presence of β-lactamase enzymes

The presence of β-lactamase enzymes is the most important mechanism by which bacteria gain resist- ance to penicillin. β-lactamases are enzymes which have mutated from transpeptidases and so they are quite simi- lar in nature. For example, they have a serine residue in the active site and can open up the β-lactam ring of peni- cillin to form an ester link to the structure. Unlike the

transpeptidase enzyme, β-lactamases are able to hydro- lyse the ester link and shed the ring-opened penicillin. They do this so effectively that 1000 penicillin molecules are hydrolysed per second (Fig. 19.19).

Some Gram-positive bacterial strains are resistant to penicillin because they can release β-lactamase into the surrounding environment such that penicillin is inter- cepted before it reaches the cell membrane. The enzyme eventually dissipates through the cell wall and is lost, so the bacterium has to keep generating the enzyme to maintain its protection. *Staphylococcus aureus* is a Gram- positive bacterium that used to be susceptible to penicil- lin, but 95% of *S. aureus* strains now release a β-lactamase which hydrolyses penicillin G.

Most, if not all, Gram-negative bacteria produce β-lactamases which makes them more resistant to peni- cillins. Moreover, the β-lactamase released is trapped in the periplasmic space between the cell membrane and the outer membrane because it cannot pass through the latter. As a result, any penicillin managing to penetrate the outer membrane encounters a higher concentration of β-lactamase than it would with Gram-positive bac- teria. This might suggest, again, that all Gram-negative bacteria should be resistant to penicillin. However, there are various types of β-lactamase enzyme produced by both Gram-positive and Gram-negative bacteria, and

H H H

R N S Me

H H H

R N S Me

O N Me O

CO2H

-Lactamase

O HO2C HN

Me CO2H

**FIGURE 19.19** β-lactamase deactivation of penicillin.

**428 Chapter 19** Antibacterial agents

these vary in their substrate selectivity. Some are selec- tive for penicillins (**penicillinases**), some for cephalo- sporins (section 19.5.2) (**cephalosporinases**), and some for both penicillins and cephalosporins. The differing levels of enzyme and their differing affinities for differ- ent β-lactams account for the varying susceptibilities of Gram-negative bacteria to different β-lactams.

**High levels of transpeptidase enzyme produced** In some Gram-negative bacteria, excess quantities of transpeptidase are produced and penicillin is incapable

of inactivating all the enzyme molecules present.

#### Affinity of the transpeptidase enzyme to penicillin

There are several forms of the transpeptidase enzyme present within any bacterial cell and these vary in their affinity for the different β-lactams. Differences in the rela- tive proportions of these enzymes across bacterial species account, in part, for the variable susceptibility of these bacteria to different penicillins. For example, early strains of *S. aureus* contained transpeptidase enzymes which had a high affinity for penicillin and were inhibited effec- tively. Penicillin-resistant strains of *S. aureus* acquired a transpeptidase enzyme called **penicillin binding protein 2a** (**PBP2a**), which has a much lower affinity to penicil- lins. The presence of low-affinity transpeptidases is also a problem with enterococci and pneumococci.

#### Transport back across the outer membrane of Gram-negative bacteria

There are proteins in the outer membrane of some Gram- negative bacteria which are capable of pumping penicil- lin out of the periplasmic space, thus lowering its con- centration and effectiveness. The extent to which this happens varies from species to species and also depends on the structure of the penicillin. This is known as an **efflux** process.

#### Mutations and genetic transfers

Mutations can occur which will affect any or all of the above mechanisms such that they are more effective in resisting the effects of β-lactams. Small portions of DNA carrying the genes required for resistance can also be transferred from one cell to another by means of genetic

vehicles called **plasmids**. These are small pieces of circu- lar bacterial extra-chromosomal DNA. If the transferred DNA contains a gene coding for a β-lactamase enzyme or some other method of improved resistance, then the recipient cell acquires immunity. Genetic material can also be transferred between bacterial cells by viruses and by the uptake of free DNA released by dead bacteria.

### Methods of synthesizing penicillin analogues

Having studied the mechanism of action of penicillin G and the various problems surrounding resistance, we now look at how analogues of penicillin G can be syn- thesized which might have improved stability and activ- ity. A method of preparing analogues is required which is cheap, efficient, and flexible. Sheehan’s full synthesis of penicillin is too long and low yielding (1%) to be practi- cal, which limits the options to fermentation methods or semi-synthetic procedures.

#### Fermentation

Originally, the only way to prepare different penicillins was to vary the fermentation conditions. Adding differ- ent carboxylic acids to the fermentation medium resulted in penicillins with different acyl side chains (e.g. **phen- oxymethylpenicillin**; Fig. 19.10). Unfortunately, there was a limitation to the sort of carboxylic acid which was accepted by the biosynthetic route (i.e. only acids of gen- eral formula RCH2CO2H). This, in turn, restricted the variety of analogues which could be obtained. The other major disadvantage was the tedious and time-consuming nature of the method.

#### Semi-synthetic procedure

In 1959, Beechams isolated a biosynthetic intermediate of penicillin from *Penicillium chrysogenum* grown in a fermentation medium which was deficient in a carbox- ylic acid. The intermediate (**6-aminopenicillanic acid; 6-APA**) proved to be one of Sheehan’s synthetic interme- diates, and so it was possible to use this to synthesize a huge number of analogues by a semi-synthetic method. Thus, fermentation yielded 6-APA, which could then be treated with a range of acid chlorides (Fig. 19.20).

H H

H2N S Me

6

N Me

O

O C

R Cl

H H H

R N S Me

6

O N Me O

6-APA

CO2H

CO2H

**FIGURE 19.20** Penicillin analogues synthesized by acylating 6-APA.

###### Antibacterial agents which inhibit cell wall synthesis **429**

H H H

N S

O

6

N

O

Me Penicillin acylase

Me Hydrolysis

H H

H2N S

OH 6 Me

O

+ N Me O

Penicillin G

CO2H

**FIGURE 19.21** Synthesis of 6-APA from penicillin G.

6-APA

CO2H

6-APA is now produced more efficiently by hydrolys- ing penicillin G or penicillin V with an enzyme (**penicil- lin acylase**) (Fig. 19.21), or by a chemical method that allows the hydrolysis of the side chain in the presence of the highly strained β-lactam ring. The latter procedure is described in more detail in section 19.5.2.2, where it is used to hydrolyse the side chain from cephalosporins. We have emphasized the drive to make penicillin analogues with varying acyl side chains, but what is so special about the acyl side chain? Could changes not be made elsewhere in the molecule? In order to answer these questions we need to look at the structure–activity

relationships (SARs) of penicillins.

### Structure–activity relationships of penicillins

A large number of penicillin analogues have been syn- thesized and studied. The results of these studies led to the following SAR conclusions (Fig. 19.22):

* the strained β-lactam ring is essential;
* the free carboxylic acid is essential. This is usually ionized and penicillins are administered as sodium or potassium salts. The carboxylate ion binds to the charged nitrogen of a lysine residue in the binding

the greater the activity, but the greater the instability of the molecule to other factors;

* the acylamino side chain is essential;
* sulphur is usual but not essential (see section 19.5.3);
* the stereochemistry of the bicyclic ring with respect to the acylamino side chain is important.

The results of this analysis led to the inevitable conclu- sion that very little variation is tolerated by the penicil- lin nucleus and that any variations are restricted to the acylamino side chain.

### Penicillin analogues

In this section we consider the penicillin analogues which proved successful in tackling the problems of acid sensitivity, β-lactamase sensitivity, and limited breadth of activity.

#### Acid sensitivity of penicillins

There are three reasons for the acid sensitivity of penicil- lin G.

* *Ring strain*: the bicyclic system in penicillin consists of a four-membered ring fused to a five-membered ring. As a result, penicillin suffers large angle and torsional

site;

* the bicyclic system is important. This confers further strain on the β-lactam ring—the greater the strain,

*cis* Stereochemistry

strains. Acid-catalysed ring-opening relieves these strains by breaking open the more highly strained β-lactam ring (Fig. 19.23).

* *A highly reactive* β*-lactam carbonyl group*: The car- bonyl group in the β-lactam ring is highly suscepti-

ble to nucleophiles and does not behave like a normal

Amide essential

essential



tertiary amide. The latter is resistant to nucleophilic attack because the carbonyl group is stabilized by the

H H H

R N



S

1 Me

2

N 3

Me

Lactam

CO2

6 5

4

O

neighbouring nitrogen atom, as shown in Fig. 19.24. The nitrogen can feed its lone pair of electrons into the carbonyl group to form a dipolar resonance struc-

ture with bond angles of 120°. This resonance stabili-

O Free carboxylate essential

zation is impossible for the β-lactam ring because of the increase in angle strain that would result in hav-

essential

Bicyclic system essential

**FIGURE 19.22** Structure–activity relationships of penicillins.

ing a double bond within a four-membered β-lactam ring. The preferred bond angles for a double bond are 120° but the bond angles of the β-lactam ring are constrained to 90°. As a result, the lone pair is local- ized on the nitrogen atom and the carbonyl group is

**430 Chapter 19** Antibacterial agents

H H H

R N S Me

O N Me O

H H H

R N S Me



O N Me O

–H

H H H

R

N

S

Me

O CO2H HN

Me

H2O

CO2H

OH CO2H

H

CO2H

**FIGURE 19.23** Ring-opening of the β-lactam ring under acidic conditions.

Bond angle 120o

R

C NR2

O

Tertiary amide

R R

C N



O R

-Lactam

Me

S



Me

Bond angle 90o

S



Me

~~X~~

O N H

CO2H

N Me

O

CO2H

Folded ring structure Flat (impossibly strained)

**FIGURE 19.24** Comparison of *tertiary* amide and β-lactam carbonyl groups.

more electrophilic than one would expect for a ter- tiary amide.

* *Influence of the acyl side chain* (**neighbouring group participation**): Fig. 19.25 demonstrates how the neighbouring acyl group can actively participate in a mechanism to open up the lactam ring. Thus, peni- cillin G has a self-destruct mechanism built into its structure.

#### Acid-resistant penicillins

It can be seen that countering acid sensitivity is a diffi- cult task. Nothing can be done about the first two fac- tors, as the β-lactam ring is vital for antibacterial activity. Therefore, only the third factor can be tackled. The task then becomes one of reducing the amount of neighbour- ing group participation taking place. This was achieved by placing an electron-withdrawing group in the side chain which could draw electrons away from the car- bonyl oxygen and reduce its tendency to act as a nucleo- phile (Fig. 19.26).

Phenoxymethylpenicillin (penicillin V) has an electro- negative oxygen on the acyl side chain with the electron- withdrawing effect required. The molecule has better acid stability than penicillin G and is stable enough to survive the acid in the stomach, so it can be given orally.

Other penicillin analogues with an electron-with- drawing substituent (X) on the α-carbon of the side chain (Fig. 19.26) have also proved resistant to acid hydrolysis and can be given orally (e.g. **ampicillin**; see Fig. 19.29).

To conclude, the problem of acid sensitivity is fairly easily solved by having an electron-withdrawing group on the acyl side chain.

#### β-Lactamase-resistant penicillins

The problem of **-lactamases** (or **penicillinases**) became critical in 1960, when the widespread use of penicil- lin G led to an alarming increase of penicillin-resistant

*S. aureus* infections. At one point, 80% of all *S. aureus* infections in hospitals were due to virulent, penicillin- resistant strains. Alarmingly, these strains were also resistant to all other available antibiotics. Fortunately, a solution to the problem was just around the corner—the design of β-lactamase-resistant penicillins.

The strategy of steric shields (section 14.2.1) was used successfully to block penicillin from accessing the penicillinase or β-lactamase active site by placing a bulky group on the side chain (Fig. 19.27). However, there was a problem. If the steric shield was *too* bulky then it also prevented the penicillin from attacking the transpepti- dase target enzyme. Therefore, a great deal of work had

###### Antibacterial agents which inhibit cell wall synthesis **431**

R



N

H H

O

N

O

H H

N

H

O

O

HN

R S N S

-H R S

N

O

O

H

HO2C

S Me

N

N Me

+ N

Me Me

HS C  CO2H

N H

R CO2H

R O O

Penillic acids Penicillenic acids

**FIGURE 19.25** Influence of the acyl side chain on the acid sensitivity of penicillins.

e.w.g H H H O

N



N

PhO

S

H H H N

O

X

H H H N

N

S

N

S



R

O

Reduces

electron O density

O

Penicillin V

O

X = NH2, Cl, PhOCONH,

Heterocycles

**FIGURE 19.26** Reduction of neighbouring group participation with an electron-withdrawing group (e.w.g.).

to be done to find the ideal shield—one large enough to ward off the lactamase enzyme, but sufficiently small to allow the penicillin to bind to the target enzyme. The fact that the β-lactam ring interacts with both enzymes in the same way highlights the difficulty in achieving that goal.

Fortunately, shields *were* found which could make that discrimination. **Methicillin** (Fig. 19.27) was the first effective semi-synthetic penicillin with resistance to the *S. aureus* β-lactamase enzyme and reached the clinic just in time to treat the growing *S. aureus* problem. The



O N

H H

H

S

Bulky group

Me

R

N

Me

O

CO2H

Lactamase

H H

OMe

H

N

S Me

OMe O N Me O

H H H

N S Me

OEt

O

S

N Me

O

Me

H O H

CO2H

O

N S Me

N Me

O

Methicillin

CO2H

Nafcillin

CO2H

Temocillin

CO2H

**FIGURE 19.27** The use of steric shields to blocking penicillin from reaching the β-lactamase active site.

**432 Chapter 19** Antibacterial agents

steric shields are the two *ortho*-methoxy groups on the aromatic ring.

Methicillin is by no means an ideal drug, however. With no electron-withdrawing group on the side chain, it is acid sensitive and has to be injected. It also shows poor activity against many other bacterial strains. Better β-lactamase-resistant agents have since been developed (see Box 19.5), and methicillin is no longer used clini- cally. **Nafcillin** (Fig. 19.27) is a penicillin that is resistant to β-lactamase enzymes and contains a naphthalene ring which acts as its steric shield. **Temocillin** is another β-lactamase-resistant penicillin and is interesting in that it has a 6-methoxy group present (section 19.5.1.4).

In general, β-lactamase-resistant penicillins are kept as ‘reserve troops’. They are only introduced into the fray if an infection proves resistant to a broad-spectrum peni- cillin as a result of the presence of a β-lactamase enzyme

(e.g. penicillin-resistant *S. aureus* and *Staphylococcus epidermidis*).

Unfortunately, 95% of *S. aureus* strains detected in hospitals have become resistant to methicillin and the other β-lactamase-resistant penicillins as a result of mutations to the transpeptidase enzyme. These bacte- ria are referred to as MRSA. The abbreviation stands for methicillin-resistant *S. aureus*, but the term applies to all the β-lactamase-resistant penicillins, not just methicillin.

#### Broad-spectrum penicillins

There are a variety of factors affecting whether a par- ticular bacterial strain will be susceptible to a penicil- lin. The spectrum of activity shown by any penicil- lin depends on its structure, its ability to cross the cell membrane of Gram-negative bacteria, its susceptibility to β-lactamases, its affinity for the transpeptidase tar- get enzyme, and the rate at which it is pumped back out of cells by Gram-negative organisms. All these factors vary in importance across different bacterial species and so there are no clear-cut tactics which can be used



**BOX 19.5** The isoxazolyl penicillins

The incorporation of an isoxazolyl ring into the penicil- lin side chain led to orally active compounds which were stable to the β-lactamase enzyme of *S. aureus*. The isoxa- zolyl ring acts as the steric shield but it is also electron- withdrawing, giving the structure acid stability.

**Oxacillin**, **cloxacillin**, **ﬂucloxacillin**, and **dicloxacillin** are all useful against *S. aureus* infections. The only differ- ence between them is the type of halogen substitution on the aromatic ring. These substituents affect pharmaco- kinetic properties such as absorption and plasma protein binding.

Test your understanding and practise your molecular modelling with Exercise 19.3.

Oxacillin R1 = R2 = H Cloxacillin R1 = Cl, R2 = H Flucloxacillin R1 = Cl, R2 = F Dicloxacillin R1 = Cl, R2 = Cl

Bulky and electron-withdrawing

Me

O

N

H H

N

H

S

Me

R1

O R2

N

Me

O

CO2H

Incorporation of a five-membered heterocycle into a penicillin side chain.

**BOX 19.6** Clinical aspects of β-lactamase- resistant penicillins

**Methicillin** was useful in the 1960s against penicillin- resistant *S. aureus* infections. However, it is no longer used clinically. **Nafcillin** has more intrinsic activity than methicillin against staphylococci and streptococci, and is administered by injection. **Temocillin** is not active against Gram-positive bacteria, or bacteria with altered penicillin- binding proteins. It should be reserved for the treatment of infections caused by β-lactamase producing strains of Gram-negative bacteria, including those resistant to third generation cephalosporins. It is used for the treatment of septicaemia, urinary tract infections, and lower respira- tory tract infections caused by susceptible Gram-negative bacteria.

**Oxacillin**, **cloxacillin**, **ﬂucloxacillin**, and **dicloxacillin** are all useful agents against *S. aureus* infection. Cloxacillin is better absorbed through the gut wall than oxacillin, whereas ﬂucloxacillin is less bound to plasma protein, resulting in higher levels of the free drug in the blood supply. They all show inferior activity to the original penicillins if they are used against bacteria that lack the β-lactamase enzyme. They are also inactive against Gram- negative bacteria. Flucloxacillin is the drug of choice for the treatment of penicillin-resistant staphylococcal infec- tions in the ear. **Co-ﬂuampicil** is a combination of ﬂucloxa- cillin with ampicillin, which is used against streptococcal or staphylococcal infections.

###### Antibacterial agents which inhibit cell wall synthesis **433**

to improve the spectrum of activity. Consequently, the search for broad-spectrum antibiotics was one of trial and error which involved making a huge variety of ana- logues. These changes were again confined to variations in the side chain and gave the following results:

* hydrophobic groups on the side chain (e.g. penicillin

G) favour activity against Gram-positive bacteria, but result in poor activity against Gram-negative bacteria;

* if the hydrophobic character is increased, there is lit- tle effect on Gram-positive activity, but activity drops even further against Gram-negative bacteria;
* hydrophilic groups on the side chain have little effect on Gram-positive activity (e.g. **penicillin T**) or cause a reduction of activity (e.g. **penicillin N**) (Fig. 19.28); however, they lead to an increase in activity against Gram-negative bacteria;
* enhancement of Gram-negative activity is found to be greatest if the hydrophilic group (e.g. NH2, OH, CO2H) is attached to the carbon that is α to the car- bonyl group on the side chain.

Those penicillins having useful activity against both Gram-positive and Gram-negative bacteria are known as **broad-spectrum antibiotics** (Box 19.8)**.** There are three classes of broad-spectrum antibiotics, all of which have an α−hydrophilic group which aids the passage of these penicillins through the porins of the Gram-negative bac- terial outer membrane.

#### Broad-spectrum penicillins: the aminopenicillins

**Ampicillin** (Fig. 19.29; Beechams, 1964) and **amoxicil- lin** are orally active compounds that have a very simi- lar structure, and are commonly used as a first line of

HO2C

H2N H

H H H

N S Me

O N Me O

defence against infection. Both compounds are acid resistant because of the presence of the electron-with- drawing amino group. There are no steric shields present and so these agents are sensitive to β-lactamase enzymes.

Penicillin N

CO2H

Both structures are poorly absorbed through the gut wall

as both the amino group and the carboxylic group are ionized. This problem can be alleviated by using a prod-

H2N

H H H

N S Me

O

N Me

O

rug where one of the polar groups is masked with a pro- tecting group which can be removed metabolically once the prodrug has been absorbed (Box 19.7).

#### Broad-spectrum penicillins:

Penicillin T

CO2H

#### the carboxypenicillins

**Carbenicillin** (Fig. 19.30) was the first example of this

**FIGURE 19.28** Effect of side chain hydrophilic groups on antibacterial activity.

class of compounds. It shows a broad spectrum of activity due to the hydrophilic carboxylic acid group (ionized at

H H H

H2N H

O

N S Me

N Me

O

HO

Phenol

H2N

H H H H

N S Me

O N Me O

CO2H

Ampicillin (Penbritin) Amoxicillin (Amoxil)

**FIGURE 19.29** Broad-spectrum penicillins—the aminopenicillins.

CO2H

CO2R

H H H

O

N S Me

N Me

O

CO2H

H H H

S

O

N S Me

N Me

O

Ticarcillin

|  |  |  |
| --- | --- | --- |
| R = | H | CO2H  Carbenicillin |
| R = | Ph | Carfecillin |
| R = |  | Indanyl carbenicillin |



**FIGURE 19.30** Carboxypenicillins.

CO2H

**434 Chapter 19** Antibacterial agents

**BOX 19.7** Ampicillin prodrugs

Pivampicillin, talampicillin, and bacampicillin are prodrugs of ampicillin (Fig. 1). In all three cases, the esters used to mask the carboxylic acid group seem rather elaborate and one may ask why a simple methyl ester is not used. The answer is that methyl esters of penicillins are not metabolized in humans. The bulky penicillin skeleton is so close to the ester that it acts as a steric shield and prevents the esterase enzymes that catalyse this reaction from accepting the penicillin ester as a substrate. Fortunately, acyloxymethyl esters *are* susceptible to ester- ases. These ‘extended’ esters contain a second ester group fur- ther away from the penicillin nucleus, which is more exposed to attack. The hydrolysis products are inherently unstable and decompose spontaneously to release formaldehyde and reveal

H H N

H

C

O CMe3

O

Pivampicillin

S

Me

O

N

R =

O

Talampicillin

Me

the free carboxylic acid (Fig. 2). The release of formaldehyde is not ideal, as it is a toxic chemical. However, it is formed nat- urally in the body through enzymatic demethylation of various compounds found in the diet and the levels produced from the prodrugs described cause little problem. Moreover, the drugs are only taken for a short period of time.

Such extended esters can be used to prepare prodrugs of other penicillins, but one has to be careful that one does not go to the other extreme and make the penicillin too lipo- philic. For example, the 1-acyloxyalkyl ester of penicillin G is so lipophilic that it has poor solubility in water. Fortunately, the problem can be avoided easily by making the extended ester more polar (e.g. by attaching valine as in Fig. 3).



O

C

O

O O

H

Penicillin O

O

C

OH

H

+ CH2O

Formaldehyde

N

Me

Me

O

C

O

O

O

H N

H

2

Increases polarity

O

R =

H2N H

CO2R

Me O

R =

C

O O Me Bacampicillin

**FIGURE 1** Prodrugs used to aid absorption of ampicillin through the gut wall.

Esterase

Penicillin O C

O

H

O

CMe3 Penicillin

**FIGURE 2** Mechanism by which acyloxymethyl esters are hydrolysed.

H H H N

S

Me

O

Me

O

L-Valine

**FIGURE 3** Polar extended ester for penicillin G.

###### Antibacterial agents which inhibit cell wall synthesis **435**

pH 7) on the side chain. The stereochemistry of this group is important and only one of the two enantiomers is active. **Carfecillin** and **indanyl carbenicillin** (Fig. 19.30) are prodrugs for carbenicillin and show an improved absorption through the gut wall. Aryl esters are better than alkyl esters as the former are more chemically sus-

ceptible to hydrolysis, because of the electron-withdraw- ing inductive effect of the aryl ring. An extended ester is not required in this case as the aryl ester is further from the β-lactam ring and is not shielded (see Box 10.7). **Ticarcillin** is similar in structure to carbenicillin, but has a thiophene ring in place of the phenyl group.

O



R2N

O

HN

Azlocillin

N

O

MeO2S

N

Mezlocillin

N

Et N N Piperacillin

O O

R2N NH

O

H H H

N S Me

6 5 1

7 2

N 3 Me

O

CO2H

**FIGURE 19.31** Ureidopenicillins.

**BOX 19.8** Clinical aspects of broad-spectrum penicillins

**Ampicillin** and **amoxicillin** have a similar spectrum of activity to penicillin G, but are more active against Gram-negative cocci and enterobacteria. They are non-toxic and can be taken orally, but they are sensitive to β-lactamases and are inactive against *P. aeruginosa*. Some patients get diar- rhoea when they take these penicillins. This is a result of poor absorption from the gut, with ampicillin being more poorly absorbed than amoxicillin. If penicillins are used at high doses for prolonged periods, they abolish the normal gut microﬂora and this allows the colonization of resistant Gram-negative bacilli or fungi, which cause the intesti- nal problems. Ampicillin is currently used to treat sinusi- tis, bronchitis and a variety of other infections, including oral, ear, and urinary tract infections. Amoxicillin has been used in the treatment of bronchitis, pneumonia, typhoid, gonorrhoea, Lyme disease, and urinary tract infections. Its spectrum of activity is increased when administered with **clavulanic acid** (section 19.5.4.1).

**Carbenicillin** was the ﬁrst penicillin to show activity against

*P. aeruginosa*. Compared with ampicillin, it is active against a wider range of Gram-negative bacteria and was used par- ticularly against penicillin-resistant strains. However, it is less active than ampicillin against various other bacterial strains and requires high dose levels. Toxic side effects are observed and the drug shows a marked reduction in activity against Gram-positive bacteria. It is also acid sensitive and has to be injected. Better penicillins, such as the ureido- penicillins, have since been developed and so the use of carbenicillin is now discouraged.

**Carfecillin** and **indanyl carbenicillin** proved useful for the treatment of urinary tract infections, but have generally

been superseded by ﬂuoroquinolone antibacterial agents (section 19.8.1).

**Ticarcillin** is administered by injection and has an identi- cal antibacterial spectrum to carbenicillin. However, it has the advantage that smaller doses can be used. It is also 2–4 times more effective against *P. aeruginosa* and has fewer side effects. The drug is used mainly against infections due to *Pseudomonas* and *Proteus* species, and is currently administered with clavulanic acid to broaden its spectrum of activity (section 19.5.4.1).

**Ureidopenicillins** are generally more active than the carboxypenicillins against streptococci and *Haemophilus* species. They show similar activity against Gram-negative aerobic rods such as *P. aeruginosa,* but are gener- ally more active against other Gram-negative bacteria. Unfortunately, they have to be injected. Examples include **azlocillin**, which is 8–16 times more active than car- benicillin against *P. aeruginosa* and is used primarily for the treatment of infections caused by that organism. It is susceptible to -lactamases. **Mezlocillin** has a similar spectrum of activity to carbenicillin, but is more active because it has a higher afﬁnity for transpeptidases and can cross the outer membrane of Gram-negative bacte- ria more effectively. **Piperacillin** is similar to ampicillin in its activity against Gram-positive species. It also has good activity against anaerobic species of both cocci and bacilli, and can be used against a variety of infections. It is more active than ticarcillin against *P. aeruginosa*. Piperacillin can be administered alongside **tazobactam** to widen its spectrum of activity (section 19.5.4.2).

**436 Chapter 19** Antibacterial agents

#### Broad-spectrum penicillins: the ureidopenicillins

**Ureidopenicillins** (Fig. 19.31) are the newest class of broad-spectrum penicillins and have a urea functional group at the α-position. Generally, they have better properties than the **carboxypenicillins** and have largely replaced them in the clinic.

### Synergism of penicillins with other drugs

There are several examples in medicinal chemistry where the presence of one drug enhances the activity of another. In many cases this can be dangerous, leading to an effec- tive overdose of the enhanced drug. In some cases, though, it can be useful. There are two interesting exam- ples where the activity of penicillin has been enhanced by the presence of another drug.

One of these is the effect of clavulanic acid, described in Section 19.5.4.1. The other is the administration of peni- cillins with a compound called **probenecid** (Fig. 19.32). Probenecid is a moderately lipophilic carboxylic acid that can block facilitated transport of penicillin through the kidney tubules. In other words, probenecid slows down the rate at which penicillin is excreted, by compet- ing with it in the excretion mechanism. Probenecid also competes with penicillin for binding sites on albumin. As a result, penicillin levels in the bloodstream are enhanced and the antibacterial activity increases—a useful tactic if faced with a particularly resistant bacterium.

**KEY POINTS**

* Penicillins have a bicyclic structure consisting of a β-lactam ring fused to a thiazolidine ring. The strained β-lactam ring reacts irreversibly with the transpeptidase enzyme responsi- ble for the ﬁnal cross-linking of the bacterial cell wall.
* Penicillin analogues can be prepared by fermentation or by a semi-synthetic synthesis from 6-aminopenicillanic acid. Variation of the penicillin structure is limited to the acyl side chain.
* Penicillins can be made more resistant to acid conditions by incorporating an electron-withdrawing group into the acyl side chain.
* Steric shields can be added to penicillins to protect them from bacterial β-lactamase enzymes.
* Prodrugs of penicillins are useful in masking polar groups and improving absorption from the gastrointestinal tract. Extended esters are used which undergo enzyme-catalysed hydrolysis to produce a product which degrades spontane- ously to release the penicillin.
* Probenecid can be administered with penicillins to hinder the excretion of penicillins.

## Cephalosporins

### Cephalosporin C

#### Discovery and structure of cephalosporin C

The second major group of β-lactam antibiotics to be dis- covered were the cephalosporins. The first cephalosporin (**cephalosporin C**) was derived from a fungus obtained in the mid 1940s from sewer waters on the island of Sardinia. This was the work of an Italian professor who noted that the waters surrounding the sewage outlet peri- odically cleared of microorganisms. He reasoned that an organism might be producing an antibacterial substance and so he collected samples and managed to isolate a fungus called *Cephalosporium acremonium* (now called *Acremonium chrysogenum*). The crude extract from this organism was shown to have antibacterial properties and, in 1948, workers at Oxford University isolated ceph- alosporin C, but it was not until 1961 that the structure was established by X-ray crystallography.

The structure of cephalosporin C (Fig. 19.33) has simi- larities to that of penicillin in that it has a bicyclic system containing a four-membered β-lactam ring, but this time the β-lactam ring is fused to a six-membered dihydro- thiazine ring. Nevertheless, cephalosporins are derived from the same biosynthetic precursors as penicillin (i.e. cysteine and valine) (Fig. 19.34).

#### Properties of cephalosporin C

Cephalosporin C is not particularly potent compared with penicillins (1/1000 the activity of penicillin G), but the antibacterial activity it *does* have is more evenly directed against Gram-negative and Gram-positive bac- teria. Another in-built advantage of cephalosporin C is

7-Aminocephalosporinic acid (7-ACA) 7-Aminoadipic side chain

7 6

8 5

H H H

S

H N

* Broad spectrum activity is associated with the presence of an α-hydrophilic group on the acyl side chain of penicillin.

2

H

CO2H

N 1

2

O N 4 3

O

O Me

C

O -Lactam



CO H O

HO2C S O

N(CH2CH2CH3)2

**FIGURE 19.32** Probenecid.

2

Dihydrothiazine ring

**FIGURE 19.33** Cephalosporin C.

H N SH

H Cys

###### Antibacterial agents which inhibit cell wall synthesis **437**

2

R OH

CO2H

Me Val

HO Me C

O

H H H

R

N

S

N

O

O

C

O H2N

H

CO2H

Me O Biosynthesis

Me

CO2H O

**FIGURE 19.34** Biosynthetic precursors of cephalosporin C.

its greater resistance to acid hydrolysis and β-lactamase enzymes. It is also less likely to cause allergic reactions. Therefore, cephalosporin C was seen as a useful lead compound for the development of further broad-spec- trum antibiotics, hopefully with increased potency.

#### Structure–activity relationships of cephalosporin C

Many analogues of cephalosporin C have been made which demonstrate the importance of the β-lactam ring within the bicyclic system, an ionized carboxylate group at position 4, and the acylamino side chain at position 7. These results tally closely with those obtained for the penicillins. The strain effect of a 6-membered ring fused to a 4-membered ring is less than for penicillin, but this is partially offset by the effect of the acetyloxy group at position 3. This can act as a good leaving group in the inhibition mechanism (Fig. 19.35).

There is a limited number of places where modifica- tions can be made (Fig. 19.36), but there are more pos- sibilities than with penicillins. These are as follows;

* variations of the 7-acylamino side chain;
* variations of the 3-acetoxymethyl side chain;

**7-ACA (7-aminocephalosporinic acid**) either by fer- mentation or by enzymatic hydrolysis of cephalosporin C, thus preventing the semi-synthetic approach analo- gous to the preparation of penicillins from 6-APA (sec- tion 19.5.1.6).

Therefore, a way had to be found of obtaining 7-ACA from cephalosporin C by chemical hydrolysis. This is no easy task, as a secondary amide has to be hydrolysed in the presence of a highly reactive β-lactam ring. Normal hydrolytic procedures are not suitable and so a special method had to be worked out (Fig. 19.37).

The first step of the procedure requires the forma- tion of an imino chloride by the mechanism shown in Fig. 19.38. This is only possible for the secondary amide group, as ring constraints prevent the β-lactam nitrogen forming a double bond within the β-lactam ring. The imino chloride can then be treated with an alcohol to give an imino ether. This functional group is more susceptible to hydrolysis than the β-lactam ring, and so treatment with aqueous acid successfully gives the desired 7-ACA which can then be acylated to give a range of analogues.

* extra substitution at carbon 7.

### Synthesis of cephalosporin analogues at position 7

Access to analogues with varied side chains at posi- tion 7 initially posed a problem. Unlike penicillins, it proved impossible to obtain cephalosporin analogues by fermentation. Similarly, it was not possible to obtain

H H H

N S

R

7

O N 4 3

O Me C

O

O

CO2H

**FIGURE 19.36** Positions for possible modification of cephalosporin C. The shading indicates positions which can be varied.

H H H

R N S



–CH CO–

H H H

R N S

7

O N

O

CO2H

3 2

O Me

C

O

7

O N

O

O CO2H

OH

Ser Enzyme

Ser Enzyme

**FIGURE 19.35** Mechanism by which cephalosporins inhibit the transpeptidase enzyme.

**438 Chapter 19** Antibacterial agents

H H H

1

R N S

7

O N 3

O 4

OAc

PCl5

R1 N H

Cl

O

ROH

R1 N H OR

O

H2O

–R1CO2H

CO2SiMe3

Imino chloride Protecting

group

Imino ether

H2N H H

N

O

S

CO2H

OAc

O

R2 Cl

Acid chloride

H H H

R2 N

O N

O

S

CO2H

OAc

7-ACA

Range of cephalosporins

**FIGURE 19.37** Synthesis of 7-ACA and cephalosporin analogues.

H H

R1 N

7

O

O

PCl4



Cl

Cl

H



R1 N

7

O

Cl3P

O

Cl

H

R1 N

7

Cl

O

Cl3P O

**FIGURE 19.38** Mechanism for imino chloride formation.

### First-generation cephalosporins

Examples of first-generation cephalosporins include **cephalothin**, **cephaloridine**, **cefalexin**, and **cefazolin** (Figs 19.39–19.42). In general, they have a lower activ- ity than comparable penicillins, but a better range. Most are poorly absorbed through the gut wall and have to be injected. As with penicillins, the appearance of resistant organisms has posed a problem, particularly with Gram- negative organisms. These contain β-lactamases which are more effective than the β-lactamases of Gram- positive organisms. Steric shields are successful in pro- tecting cephalosporins from these β-lactamases, but also prevent them from inhibiting the transpeptidase target enzymes.

One of the most commonly used first-generation ceph- alosporins was **cephalothin** (Fig. 19.39). A disadvantage

with cephalothin is the fact that the acetyloxy group at position 3 is readily hydrolysed by esterase enzymes to give the less active alcohol (Fig. 19.40). The acetyloxy group is important to the mechanism of inhibition and acts as a good leaving group, whereas the alcohol is a much poorer leaving group. Therefore, it would be useful if this metabolism could be blocked to prolong activity. Replacing the ester with a metabolically stable pyridinium group gives **cephaloridine** (Fig. 19.41). The pyridine can still act as a good leaving group for the inhibition mecha- nism, but is not cleaved by esterases. Cephaloridine exists as a zwitterion and is soluble in water, but, like most first- generation cephalosporins, it is poorly absorbed through the gut wall and has to be injected.

**Cefalexin** (Fig. 19.41) has a methyl substituent at position 3 (Box 19.9) which appears to help oral absorp- tion. A methyl group would normally be bad for activ- ity as it is not a good leaving group. However, the pres-

H H H

N

S

7

N

3

O

S

O

CO2H

OAc

ence of a hydrophilic amino group at the α-carbon of the 7-acylamino side chain in cefalexin helps to restore activity and cephalexin is one of the few cephalospor- ins which is orally active. The mechanism of absorption through the gut wall is poorly understood and it is not clear why the 3-methyl group is so advantageous for absorption. **Cefazolin** (Fig. 19.42) is another example of

**FIGURE 19.39** Cephalothin. a first-generation cephalosporin.

###### Antibacterial agents which inhibit cell wall synthesis **439**

H H H

N S

S

O

Metabolism

H H H

N S

S

O

7 7

O

Active

N 3

CO2H

OAc

N

O

Decreased activity

3 OH

CO2H

**FIGURE 19.40** Metabolic hydrolysis of cephalothin.

H H H

H H H

Good for absorption. Usually bad for activity

S

1. S N

S

O

H2N H

O

7 7

N 3 N

O

CO2

N 3

1. Me

CO2H

Cephaloridine Cefalexin

**FIGURE 19.41** Cephaloridine and cefalexin.

H H H

### Second-generation cephalosporins



**BOX 19.9** Synthesis of 3-methylated cephalosporins

The synthesis of 3-methylated cephalosporins involves an acid-catalysed ring expansion, where the ﬁve-membered the use of a penicillin starting material as shown below. thiazolidine ring in penicillin is converted to the six-mem- The synthesis, which was ﬁrst demonstrated by Eli Lilly bered dihydrothiazine ring in cephalosporin.

Pharmaceuticals, involves oxidation of sulphur followed by

6

R

H

C N

OH

H

O

O

S

2

Me H2O2

S

N Me N

Me

Me

Toluene/ PTSA

S

N

H CH2

Me

H OH S

N

O

CO2Me

CO2Me

CO2Me

CH3

CO2Me

S

H

OH

– H

H S

N

H

N

OH

H S

N 3

CH3 CH3 CH3

CO Me

2

H CO Me

2

CO Me

2

Synthesis of 3-methylated cephalosporins from a penicillin.

N N C



N

N O

1. S

7

N 3 S S

1. Me

CO2H N N

#### Cephamycins

**Cephamycins** contain a methoxy substituent at position 7, which has proved advantageous. The parent compound **cephamycin C** (Fig. 19.43) was isolated from a culture of *Streptomyces clavuligerus* and was the first β-lactam to be

**FIGURE 19.42** Cefazolin. isolated from a bacterial source. Modification of the side

**440 Chapter 19** Antibacterial agents

chain gave **cefoxitin** (Fig. 19.43), which showed a broader spectrum of activity than most first-generation cephalo- sporins. This is due to greater resistance to β-lactamase enzymes, which may be due to the steric hindrance pro- vided by the methoxy group. Cefoxitin shows good meta- bolic stability to esterases owing to the presence of the urethane group at position 3, rather than an ester (section 14.2.2).

 Test your understanding and practise your molecu- lar modelling with Exercise 19.4.

#### Oximinocephalosporins

The development of **oximinocephalosporins** has been a major advance in cephalosporin research. These struc- tures contain an iminomethoxy group at the α-position of the acyl side chain, which significantly increases the stability of cephalosporins against the β-lactamases pro- duced by some organisms (e.g. *Haemophilus influenza*). The first useful agent in this class of compounds was **cefuroxime** (Fig. 19.44), which, like cefoxitin, has an increased resistance to β-lactamases and mammalian esterases. Unlike cefoxitin, cefuroxime retains activity against streptococci and, to a lesser extent, staphylococci.

### Third-generation cephalosporins

Replacing the furan ring of the aforesaid oximinocephalo- sporins with an aminothiazole ring enhances the penetra-

tion of cephalosporins through the outer membrane of Gram-negative bacteria, and may also increase affinity for the transpeptidase enzyme. As a result, third-generation cephalosporins containing this ring have a marked increase in activity against these bacteria. A variety of such struc- tures have been prepared, such as **ceftazidime**, **cefotaxime**, **ceftizoxime**, and **ceftriaxone** (Figs 19.44 and 19.45), with different substituents at position 3 to vary the pharmaco- kinetic properties. They play a major role in antimicrobial therapy because of their activity against Gram-negative bacteria, many of which are resistant to other β-lactams. As such infections are uncommon outside hospitals, phy- sicians are discouraged from prescribing these drugs rou- tinely and they are viewed as ‘reserve troops’ to be used for troublesome infections which do not respond to the more commonly prescribed β-lactams.

### Fourth-generation cephalosporins

**Cefepime**and**cefpirome**(Fig.19.45)areoximinocephalo- sporins which have been classed as fourth-generation cephalosporins. They are zwitterionic compounds hav- ing a positively charged substituent at position 3 and a negatively charged carboxylate group at position 4. This property appears to radically enhance the ability of these compounds to penetrate the outer membrane of Gram- negative bacteria. They are also found to have a good affinity for the transpeptidase enzyme and a low affinity for a variety of β-lactamases.

H OMe H

Stabilizes neighbouring carbonyl group

H OMe H S

HO2C N S N

S

O

7 7

H2N H

O N 3

O

CO2H

O NH2 C

O

N 3

O

CO2H

O NH2 C

O

Cephamycin C

Cefoxitin

**FIGURE 19.43** Cephamycin C and cefoxitin.

Me Iminomethoxy group

O

N

Me

Me CO2H

O

N

H H H

N

S

7

N

3

N

O

Furan ring

O

H H H

N S

C 7

O N

O

3 O NH2 C

Aminothiazole

ring C

S

N O

H2N

Cefuroxime

CO2H O

Ceftazidime

CO2

**FIGURE 19.44** Oximinocephalosporins.

###### Antibacterial agents which inhibit cell wall synthesis **441**

H2N

Me O

N

H H H

N

S

7

N

3

O

N

S

C O

R

CH2OCOMe Cefotaxime

H

Ceftizoxime

Me

CH2

N

Cefepime

Me

CH2S N N N

Ceftriaxone

CH2 N

Cefpirome

OH

O

Substituents (R)

CO2H

**FIGURE 19.45** Third- and fourth-generation oximinocephalosporins.

### Fifth-generation cephalosporins

**Ceftaroline fosamil** (Fig. 19.46) is a fifth-generation cephalosporin that has activity against various strains of MRSA and multi-resistant *Streptococcus pneumonia* (MDRSP). It acts as a prodrug for **ceftaroline**, and the 1,3-thiazole ring is thought to be important for its activ- ity against MRSA.

### Resistance to cephalosporins

The activity of a specific cephalosporin against a particu- lar bacterial cell is dependent on the same factors as those for penicillins. i.e. the ability to reach the transpeptidase enzyme, stability to any β-lactamases which might be present, and the affinity of the antibiotic for the target. For example, most cephalosporins (with the exception of cephaloridine) are stable to the β-lactamase produced by

*S. aureus* and can reach the transpeptidase enzyme without difficulty. Therefore, the relative ability of cephalosporins to inhibit *S. aureus* comes down to their affinity for the target transpeptidase enzyme. Agents such as the cephamycins and ceftazidime have poor affinity, whereas other cephalo- sporins have a higher affinity. The MRSA organism con- tains a modified transpeptidase enzyme (**PBP2a**) for which both penicillins and cephalosporins have poor affinity.

**KEY POINTS**

* Cephalosporins contain a strained β-lactam ring fused to a dihydrothiazine ring.
* In general, ﬁrst-generation cephalosporins offer advan- tages over penicillins in that they have greater stability to acid conditions and β-lactamases, and have a good ratio of activity against Gram-positive and Gram-negative bacteria. However, they have poor oral availability and are generally lower in activity.
* Variation of the 7-acylamino side chain alters antimicro- bial activity, whereas variation of the side chain at position 3 predominantly alters the metabolic and pharmacokinetic properties of the compound. Introduction of a methoxy sub- stitution at C-7 is possible.
* Semisynthetic cephalosporins can be prepared from 7-amino- cephalosporanic acid (7-ACA).
* 7-ACA is obtained from the chemical hydrolysis of cephalo- sporins. This requires prior activation of the side chain to make it more reactive than the β-lactam ring.
* Deacetylation of cephalosporins occurs metabolically to pro- duce inactive metabolites. Metabolism can be blocked by replacing the susceptible acetoxy group with metabolically stable groups.

Me

O

N

N

S

N

O

H H

N S



N

HN O –

1,3–thiazole ring

Me



S

+

N

N

S

X CO2

Ceftaroline; X = H

Ceftaroline fosamil; X = P(=O)(OH)2

**FIGURE 19.46** Ceftaroline and ceftaroline fosamil.

**442 Chapter 19** Antibacterial agents

**BOX 19.10** Clinical aspects of cephalosporins

In general, cephalosporins are useful broad-spectrum anti- bacterial agents for the treatment of septicaemia, pneu- monia, meningitis, biliary tract infections, peritonitis, and urinary tract infections. **Cephalosporin C** itself has been used in the treatment of urinary tract infections, as it is found to concentrate in the urine and survive the body’s hydrolytic enzymes.

First-generation cephalosporins

First-generation cephalosporins have good activity against Gram-positive cocci and they can be used to treat some community-derived Gram-negative infections (i.e. infec- tions not caught in a hospital). They can also be used against *S. aureus* and streptococcal infections when peni- cillins have to be avoided. **Cephalothin** is more active than penicillin G against some Gram-negative bacteria and is less likely to cause allergic reactions. It can also be used against β-lactamase producing *S. aureus* strains.

**Cefalexin** is useful for the treatment of urinary tract infections which do not respond to other drugs or which occur in pregnancy. It is also useful in treating infections of the respiratory tract, ear, skin, and mouth. **Cefazolin** is recommended for use as a prophylactic to prevent infec- tion when surgical procedures are used to implant foreign bodies.

Second-generation cephalosporins

In general, the second-generation cephalosporins have variable activity against Gram-positive cocci, but increased activity against Gram-negative bacteria. **Cefoxitin** is active against bowel ﬂora, including *Bacteroides fragilis*, and was once recommended for peritonitis. **Cefuroxime** has a wide spectrum of activity and is useful against organisms which have become resistant to penicillin. However, it is not active against ‘difﬁcult’ bacteria, such as *P. aeruginosa*. It is used clinically against *Neisseria gonorrhoeae* and respiratory

infections caused by *H. inﬂuenza*, *Moraxella catarrhalis*, and susceptible strains of *S. pneumoniae*. It is also used for surgical prophylaxis, as well as for the treatment of Lyme disease. **Cefotaxime** is used in surgical prophylaxis and for the treatment of gonorrhoea, meningitis, and infections caused by *Haemophilus epiglottis.*

Third-generation cephalosporins

Third-generation cephalosporins have good activity against Gram-negative bacteria, but vary in their activity against Gram-positive cocci. The ability to attack *P. aeruginosa* also varies from structure to structure, and they lack activ- ity against the MRSA organisms and *Enterobacter* spe- cies. **Ceftazidime** is an injectable cephalosporin which has excellent activity against *P. aeruginosa*, as well as other Gram-negative bacteria. Because the drug can cross the blood–brain barrier it can be used to treat meningi- tis. Compared with the other aminothiazole structures, ceftazidime has good activity against streptococci, but loses activity against strains of methicillin-susceptible

*S. aureus*. This is because of a decreased binding afﬁn- ity for the transpeptidase enzyme present in *S. aureus*. **Ceftriaxone** is used for surgical prophylaxis and as a pro- phylactic for meningococcal meningitis.

Fourth and ﬁfth-generation cephalosporins

Fourth-generation cephalosporins have activity against Gram-positive cocci and a broad array of Gram-negative bacteria, including *P. aeruginosa* and many of the entero- bacterial species. **Cefpirome** is administered as an intra- venous injection or infusion, and has been used against a variety of sensitive Gram-positive and Gram-negative bacteria. **Ceftaroline fosamil** has been licensed for the treatment of bacterial pneumonia and acute bacterial skin infections.

* A methyl substituent at position 3 is good for oral absorption but bad for activity unless a hydrophilic group is present at the α-position of the acyl side chain.
* 3-Methylated cephalosporins can be synthesized from penicillins.
* Cephamycins are cephalosporins containing a methoxy group at position 7.
* Oximinocephalosporins have resulted in several generations of cephalosporins with increased potency and a broader spectrum of activity, particularly against Gram-negative bacteria.

## Other β-lactam antibiotics

Although penicillins and cephalosporins are the best known and most researched β-lactams, there are other β-lactam structures which are of great interest in the antibacterial field.

### Carbapenems

**Thienamycin** (Fig. 19.47) was the first example of this class of compounds and was isolated from *Streptomyces*

*cattleya* in 1976. It is potent, with an extraordinarily

###### Antibacterial agents which inhibit cell wall synthesis **443**

Plays a role

Acylamino side chain absent

Opposite stereochemistry

to penicillins O



Carbon H

in -lactamase resistance

H OH

Me

H  R

1

6 5

H OH

Me

H Me

1

N C R2 N

R1

2 S

N

O 3

CO2

6 5 2 S N

O 3

CO2

Carbapenam nucleus Thienamycin R = NH+

Double bond leading to high ring strain and increase in lactam reactivity

Meropenem; R1 = R2 = Me

CO2

Imipenem R =

3

NH–CH=NH

Ertapenem; R1 = H, R2 =

**FIGURE 19.47** Carbapenems.

broad range of activity against Gram-positive and Gram- negative bacteria, including *P. aeruginosa*. It has low toxicity and shows a high resistance to β-lactamases. This resistance has been ascribed to the presence of the hydroxyethyl side chain. Unfortunately, it shows poor metabolic and chemical stability, and is not absorbed from the gastrointestinal tract. The surprising features in thienamycin are the missing sulphur atom and acylamino side chain, both of which were thought to be essen- tial to antibacterial activity. Furthermore, the stereo- chemistry of the side chain at substituent 6 is opposite from the usual stereochemistry in penicillins—another factor contributing to the resistance of this agent to

β-lactamases. **Imipenem** and **meropenem** are clinically useful analogues of thienamycin (Box 19.11). Imipenem is susceptible to metabolism by a dehydropeptidase enzyme, whereas meropenem is more resistant as a result of the different substituent at position 2. **Ertapenem** was approved in 2002 and is similar in structure to mero- penem. It has an extra substituent on the carbapenem ring (R1 = Me) which provides further stability against dehydropeptidases, while the ionized benzoic acid con- tributes to high protein binding and prolongs the half- life of the drug such that once-daily dosing is feasible. In general, the **carbapenems** have the broadest spectrum of activity of all the β-lactam antibiotics.

**BOX 19.11** Clinical aspects of miscellaneous β-lactam antibiotics

**Imipenem** is active against a variety of aerobic, anaerobic, to dehydropeptidases. Both meropenem and imipenem pen- Gram-positive, and Gram-negative infections, and can be etrate the outer membrane of Gram-negative bacteria through effective against some infections which do not respond to porins, but meropenem enters more efﬁciently, resulting in cephalosporins, or infections which have become resistant higher activity against these bacteria. The drug has been to more conventional β-lactams. It can be used against used to treat pneumonia, meningitis, abdominal infections, hospital-acquired septicaemia and for surgical prophy- and urinary tract infections. **Ertapenem** is administered by laxis. The structure is metabolized by a dehydropeptidase intravenous infusion and is used for the treatment of abdomi- enzyme to produce metabolites that are toxic to the kidney, nal infections, acute gynaecological infections, community- but this can be alleviated by administrating the drug along- acquired pneumonia, and diabetic foot infections of the skin side **cilastatin**—a dehydropeptidase inhibitor which protects and soft tissue. It is also used as a prophylactic for colorectal imipenem from metabolism. Administration is by intramus- surgery. **Aztreonam** is used against Gram-negative infections, cular injection or by intravenous infusion. **Meropenem** is including *P. aeruginosa*, *H. inﬂuenzae*, and *Neisseria menin-* also effective against a variety of aerobic, anaerobic, Gram- *gitidis*. It is administered by intravenous injection and can be positive, and Gram-negative infections, and is administered used safely in patients with allergies to penicillin or cephalo- by intravenous injection or infusion. Meropenem is slightly sporins. It has no activity against Gram-positive organisms or less active than imipenem against Gram-positive bacteria, **anaerobic bacteria** because it does not bind to the transpepti- but is more active against Gram-negative bacteria. Unlike dases produced by these organisms. However, it can bind to, imipenem, meropenem is active against *P. aeruginosa* and and inhibit, the transpeptidases produced by Gram-negative can be administered on its own because it is more resistant aerobic organisms.

**444 Chapter 19** Antibacterial agents

### Monobactams

Monocyclic β-lactams such as the **nocardicins** (Fig. 19.48) have been isolated from natural sources. At least seven nocardicins have been isolated by the Japanese company Fujisawa. They show moderate activity *in vitro* against a narrow group of Gram-negative bacte- ria, including *P. aeruginosa*. Surprisingly, they contain a single β-lactam ring, demonstrating that a fused sec- ond ring is not always essential for antibacterial activ- ity. One explanation for this is that nocardicins might have a different mechanism of action from penicillins and cephalosporins—possibly by inhibiting a differ- ent enzyme involved in cell wall synthesis. This would help to explain why nocardicins are inactive against Gram-positive bacteria and generally show a different spectrum of activity from the other β-lactam antibiot- ics. They also show low levels of toxicity. **Aztreonam** (Fig. 19.48) is an example of a monobactam which has reached the clinic and was developed from a naturally occurring monobactam isolated from *Chromobacterium violaceum*.

## β-Lactamase inhibitors

### Clavulanic acid

Clavulanic acid (Fig. 19.49) was isolated from *S. cla- vuligerus* by Beechams in 1976. It has weak and unim- portant antibiotic activity, but it is a powerful and irre-

OH

N

O

versible inhibitor of most β-lactamases, which means that it is used as a sentry drug (section 14.7.1) in com- bination with traditional penicillins, such as amoxicillin (**Augmentin**). This allows the dose levels of amoxicillin to be decreased and also increases the spectrum of activ- ity. However, it should be noted that there are various types of β-lactamases. Although clavulanic acid is effec- tive against most of these, it is not effective against all. Clavulanic acid is also administered intravenously with ticarcillin as **Timentin**.

The structure of clavulanic acid was the first example of a naturally occurring compound where the β-lactam ring was not fused to a sulphur-containing ring; instead, it is fused to an oxazolidine ring. The structure is also unusual in that it does not have an acylamino side chain. Many analogues have now been made and the essen-

tial requirements for β-lactamase inhibition are:

* a strained β-lactam ring;
* the enol ether;
* the *Z* configuration for the double bond of the enol ether (activity is reduced but not eliminated if the double bond is *E*);
* no substitution at C-6;
* (*R*)-stereochemistry at positions 2 and 5;
* the carboxylic acid group.

It is also thought that the 9-hydroxyl group is involved in a hydrogen bonding interaction with the active site of the β-lactamase enzyme. Clavulanic acid is a mecha- nism-based irreversible inhibitor and can be classed as a

Me

Me CO2H

H H N

3 4

O 2 N

O

H

D

CO2H

OH

H2N

O

N

H Me

3 4

2

N

N

S

O

N

O SO–

3

H2N

H

CO2H

Nocardicin A

**FIGURE 19.48** Monobactams.

Sulphur replaced by O

Aztreonam

No acylamino side chain

6

7

H

O

5 4

3

N1 2

9 OH H

H enol ether

O Z OH

N H

O

-Lactam

H CO2H

O H CO2H

Oxazolidine ring

**FIGURE 19.49** Clavulanic acid.

###### Antibacterial agents which inhibit cell wall synthesis **445**

**suicide substrate**. The mechanism of inhibition is shown in section 7.5.

### Penicillanic acid sulphone derivatives

The agents **sulbactam** and **tazobactam** have also been developed as β**-lactamase inhibitors** and are used clini- cally (Fig. 19.50). They, too, act as suicide substrates for β-lactamase enzymes and have similar properties. Sulbactam has a broader spectrum of activity against β-lactamases than clavulanic acid, but is less potent. It is combined with ampicillin for intravenous administra- tion in a preparation called **Unasyn**. Tazobactam is simi- lar to sulbactam and has a similar spectrum of activity against β-lactamases. However, its potency is more like clavulanic acid. It is administered intravenously with piperacillin in a preparation called **Tazocin** or **Zosyn**, which has the broadest spectrum of activity of the vari- ous combinations described so far.

### Olivanic acids

The **olivanic acids** (e.g. **MM 13902**) (Fig. 19.51) were isolated from strains of *Streptomyces olivaceus* and are carbapenem structures like thienamycin. They are very strong inhibitors of β-lactamase—in some cases 1000 times more potent than clavulanic acid. They are also effective against the β-lactamases which break down cephalosporins and are unaffected by clavulanic acid. Unfortunately, olivanic acids lack chemical stability.

## Other drugs which act on bacterial cell wall biosynthesis

β-Lactams are not the only antibacterial agents that inhibit cell wall biosynthesis. The antibacterial agents

from two l-alanine units which are first racemized then linked together.

NAM, with its pentapeptide chain, is then linked to a **C55 carrier lipid** with the aid of a **translocase** enzyme and carried to the outer surface of the cell membrane, where the lipid carrier acts as an anchor to hold the glyco- peptide in place for the subsequent steps. These steps involve the addition of *N*-acetylglucosamine (NAG) and a pentaglycine chain to give the complete ‘building block’. A **transglycosidase** enzyme catalyses the attachment of the disaccharide building block to the growing cell wall and, at the same time, the carrier lipid is released to pick up another molecule of NAM/pentapeptide. Cross- linking between the various chains of the cell wall finally takes place, catalysed by the transpeptidase enzyme as described previously (section 19.5.1.4).

### D-Cycloserine and bacitracin

d-Cycloserine (Fig. 19.53) is a simple molecule produced by *Streptomyces garyphalus*, which has broad-spectrum activity and acts within the cytoplasm to prevent the formation of d-Ala-d-Ala. It does this by mimicking the structure of d-alanine and inhibiting the enzymes **l-alanine racemase** (responsible for racemizing l-Ala to d-Ala) and **d-Ala-d-Ala ligase** (responsible for linking the two d-alanine units together).

Bacitracin is a polypeptide complex produced by *Bacillus subtilis*, which binds to the lipid carrier respon- sible for transporting the NAM/pentapeptide unit across the cell membrane, thus preventing it from carrying out that role.

Me

H O

S

Me

H

6

1

O

O

N

O

3

HN

O

**vancomycin**, **d-cycloserine**, and **bacitracin** also inhibit biosynthesis, though at different stages. In order to syn- thesize the cell wall, *N*-acetylmuramic acid (NAM) is linked to three amino acids, then to the dipeptide d-Ala- d-Ala (Fig. 19.52). The d-Ala-d-Ala dipeptide is derived

HO 2

S

CO2H

**FIGURE 19.51** MM 13902.

H O O



S

1 Me

6 5

7

H O O

S Me

6



H O O

S Me



6

2

N 3 Me

O  O

CO2 Na

O

N 3 Me Me

O Me

O Me

O

N 3

O

CO2

N N N

Sulbactam

Sulbactam pivoxal

**FIGURE 19.50** Penicillanic acid sulphones.

Tazobactam

**446 Chapter 19** Antibacterial agents

Cross-linking

Growing cell wall

Vancomycin



Transglycosidation

Gly

NAG

Cell membrane

Bacitracin

Carrier lipid

Cytoplasm

L -Ala D-Glu L -Lys

NAM

L-Ala

D-Ala

D-Ala-D-Ala

Amino acid

Cycloserine

**FIGURE 19.52** Cell wall biosynthesis.

H

O N O

H NH2

HO

O

Me H

NH2

undergo oxidative coupling with each other to produce three cyclic moieties within the structure. Chlorination, hydroxylation, and the final addition of two sugar units then complete the structure (Fig. 19.55).

The cyclizations described transform a highly flexible

D-Cycloserine

D-Alanine

heptapeptide molecule into a rigid structure that holds

the peptide backbone in a fixed conformation. Moreover,

**FIGURE 19.53** d-Cycloserine as a mimic for d-alanine.

### The glycopeptides: vancomycin and vancomycin analogues

**Vancomycin** (Fig. 19.54) is a narrow-spectrum bac- tericidal glycopeptide produced by a microorganism called *Streptomyces orientalis* found in Borneo and India. Aptly, its name is derived from the verb ‘to vanquish’. Vancomycin was introduced in 1956 for the treatment of infections caused by penicillin-resistant *S. aureus*, but was discontinued when methicillin became available. It has since been reintroduced and is now the main stand-by drug for treating MRSA. Vancomycin and related glyco- peptides are often the last resort in treating patients with drug-resistant infections. As such, they have become extremely important and a great deal of research is cur- rently being carried out in this area.

Vancomycin is derived biosynthetically from a linear heptapeptide containing five aromatic residues. These

there is an extra element of rigidity to the structure, which may not be apparent at first sight. The aromatic rings (A–E) cannot rotate and are fixed in space because of hindered single bond rotation. For example, the aro- matic rings C and E have a chloro substituent which prevents these rings becoming coplanar with ring D. Similarly, rings A and B have phenol substituents which prevent them becoming coplanar.

The fixed conformation of the hexapeptide chain is important to vancomycin’s unique mechanism of action, which involves targeting the cell wall’s building blocks rather than a protein or a nucleic acid. To be specific, there is a pocket in the vancomycin structure into which the tail of the building block’s pentapeptide moiety can fit. The pentapeptide is then held there by the forma- tion of five hydrogen bonds between it and the hexa- peptide chain of vancomycin (Fig. 19.54). Dimerization can now occur where a highly stable vancomycin dimer is bound to two tails. Because vancomycin is a large molecule, it caps the tails and acts as a steric shield,

###### Antibacterial agents which inhibit cell wall synthesis **447**



H3N HO

HO

Me

HO

CH2OH

O

Vancomycin

O O O Cl Me O O



HO

C

O

H

O

H

N

H

N CO2

H

B

A

HO

OH

OH

D E

Cl OH H3C

O

CH3

H H O

N O

N N

Heptapeptide

Vancomycin

Building block

Cell membrane

O H N H H H

CONH2

H NH2Me

backbone

O H Me H O N

Cell wall building block

N O

H-Bonds

H O Me H

L-Lys-D-Ala-D-Ala 'tail'

**FIGURE 19.54** Vancomycin and its binding interactions to the l-Lys-d-Ala-d-Ala moiety.

Chlorination

Glycosidations

Chlorination



H OH H



Hydroxylation

O

Tyr

O

O

Tyr

O



Hydroxylation O

H

HO2C N

N H

O

HO OH

H H

N N

N H

O O

H2NOC Asn

N H

Val

NHMe

OH Oxidative couplings

**FIGURE 19.55** Reactions involved in the biosynthesis of vancomycin.

blocking access to the transglycosidase and transpepti- dase enzymes (Fig. 19.56).

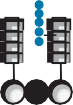
Dimerization occurs head to tail such that the hepta- peptide chains of each vancomycin molecule interact through four hydrogen bonds (Fig. 19.57). The sugar and chloro-groups also play an important role in this dimeri- zation, and activity drops if either of these groups is absent.

Because vancomycin is such a large molecule, it is unable to cross the outer cell membrane of Gram- negative bacteria and, consequently, lacks activity against those organisms. It is also unable to cross the inner cell

membrane of Gram-positive bacteria, but this is not required as the construction of the cell wall takes place outside the cell membrane.

Bacterial resistance to vancomycin has been slow to develop, although some hospital strains of *S. aureus* were identified in 1996 which *do* show resistance (**VRSA**). Of particular concern was the appearance of **vancomycin- resistant enterococci** (**VRE**) in 1989. These are organ- isms that can cause life-threatening gut infections in patients whose immune system is weakened. Resistance in the latter organisms has arisen from a modification

**448 Chapter 19** Antibacterial agents



Vancomycin dimer

Growing cell wall

Building block

**FIGURE 19.56** ‘Capping’ of pentapeptide ‘tails’ by vancomycin.

of the cell wall precursors where the terminal d-alanine group in the pentapeptide chain has been replaced by d-lactic acid, resulting in a terminal ester link rather

than an amide link (Fig. 19.58). This removes one of the NH groups involved in the hydrogen bonding interac- tion with vancomycin. It may not sound like much, but it is sufficient to weaken the binding affinity and make the antibiotic ineffective. The modified building block is still acceptable to the transglycosylase and transpepti- dase enzymes. In the latter case, lactate acts as the leaving group rather than d-alanine.

**Teicoplanin** is a medication that contains five very similar structures which were isolated from a soil micro- organism called *Actinoplanes teichomyceticus* and which differ only in the nature of a long alkyl substituent. One example is taicoplanin A2–5 (Fig. 19.59). The teicoplan- ins belong to the vancomycin family but do not dimarize. The long alkyl chain plays an importart role is anchoring the antibiotic to the outer surface of the cell membrane where it is perfectly placed to interact with the building

H Me

O

N

D-Ala-D-Ala-L-Lys tail O

H N

Cell wall building block



NH Me

O

H R3

H Me



H O

H



R7

5 O2C N

Heptapeptide

2 H N

N

H O R

N N

backbone

R1

O O

O R2

R6

H

N

O

O

N

R4 H

H N



R5

O

H

O R6

R4

O O

N

R2 O

N

R Heptapeptide



N CO2



R7

H

O H N R3 H

H NH2Me

1

backbone

O H Me H O N

Cell wall building block

N O

H O Me H

L-Lys- D-Ala-D-Ala tail

**FIGURE 19.57** Dimerization of vancomycin. The dashed lines represent hydrogen bonds.

O H Me H O N



Cell wall building block

N O

O H Me O O

N O



Cell wall building block

H O Me H H O Me H

L-Lys- D-Ala-D-Ala tail L-Lys-D-Ala-D-Lactate tail

**FIGURE 19.58** Modification of the pentapeptide chain leading to resistance.

###### Antibacterial agents which inhibit cell wall synthesis **449**

Teicoplanin

Building block

Alkyl chain anchor

Cell membrane

Alkyl anchor

O

HO

HO CH2OH

OH

HO NHAc

HO O

O

O

N O

H Cl



D

H

N

O

H

O O



C E

Cl OH



O

N

H

NH3

H

O

H H H



O O

N

H

O N

N

Heptapeptide backbone H H

N CO2

H B

HO OH

A

O

HO O OH

HO

O

OH

HO

OH

**FIGURE 19.59** Teicoplanin A2–5.

blocks for cell wall synthesis (Fig. 19.59). Teicoplanin is used clinically for the treatment of Gram-positive infec- tions and is less toxic than vancomycin.

Another naturally occurring member of the vancomy- cin family is **eremomycin** (Fig. 19.60). A biphenyl hydro- phobic ‘tail’ was added to act as an anchor, resulting in a compound (**LY 333328**), which is 1000 times more active than vancomycin. Further modifications involved removal of a tetrahydropyran ring to leave an alcohol group (R4), modification of the hydrophobic tail (R2) and addition of a side chain with a phosphate group (R3), to give **telavancin**, which was approved in 2009.

Although the complexity of the glycopeptides is an advantage in their targeting and selectivity, it is a prob- lem when it comes to synthesizing analogues. Therefore, work has been carried out to try and prepare simplified analogues of vancomycin which are easier to synthesize, yet retain the desired selectivity. Structures such as those shown in Fig. 19.61 have been prepared which are capa- ble of binding to d-Ala -d-Ala and d-Ala-d-Lac. These now represent lead compounds for the development of future antibacterial agents.

There are another two mechanisms by which **gly- copeptides** may have an antibacterial activity. Firstly, it is possible that glycopeptide dimers disrupt the cell membrane structure. This is supported by the fact that glycopeptide antibacterial agents enhance the activ-

ity of aminoglycosides by increasing their absorption through the cell membrane. Secondly, RNA synthesis is known to be disrupted in the presence of glycopeptides. The possibility of three different mechanisms of action explains why bacteria are slow to acquire resistance to the glycopeptides.

**KEY POINTS**

* β-Lactamase inhibitors are β-lactam structures that have negligible antibacterial activity but inhibit β-lactamases. They can be administered alongside penicillins to protect them from β-lactamases and to broaden their spectrum of activity.
* Carbapenems and monobactams are examples of other

β-lactam structures with clinically useful antibacterial activity.

* Glycopeptides, such as vancomycin, bind to the building blocks for cell wall synthesis, preventing their incorporation into the cell wall. They also block the cross-linking reaction for those units already incorporated in the wall. The glyco- peptides are the drugs of last resort against drug-resistant strains of bacteria.
* Bacitracin binds to and inhibits the carrier lipid respon- sible for carrying the cell wall components across the cell membrane.
* Cycloserine inhibits the synthesis of D-Ala-D-Ala.

**450 Chapter 19** Antibacterial agents

+

R2H2N Me



HO HO

CH2OH

Me O

HO O

O

O Cl

O O

R4O

H

R1

O H H

O

N

N

H – CO2

N

H

HO

OH OH

H O O

OH H3C CH3 O

N N

H

O H N H

H

CONH2

H NH2Me

+

R3



+

H3N Me HO

+

Eremomycin R1 = R2 = R3 = H R4 = Me

LY 333328 R1 = C1, R2 = CI

O

CH2

H3N Me HO

R3=H R4 = Me

O

Telavancin R1 = CI, R2 = (CH2)2NH(CH2)9CH3 R3 = CH2NHCH2PO3H2, R4 = H

**FIGURE 19.60** Eremomycin, LY 333328, and telavancin.

H N

AA1-AA2-AA3

OH

O

H

N

O

H

O O

N H H

CONH2

H3C

N

O

H

H NH2Me

CH3

the uncontrolled movement of ions across the cell mem- brane. These agents are described in section 10.6.

19.6.2 **Polymyxin B**

The polypeptide antibiotic **polymyxin B** (Fig. 19.62) derives from a soil bacterium called *Bacillus polymyxa*. It also operates within the cell membrane and shows a

**FIGURE 19.61** Simplified analogues of the glycopeptides.

19.6 **Antibacterial agents which act on the plasma membrane structure**

## 19.6.1 Valinomycin and gramicidin A

The peptides **valinomycin** and **gramicidin A** both act as ion-conducting antibiotics (ionophores) and allow

selective toxicity for bacterial cells over animal cells. This appears to be related to the ability of the compound to bind selectively to the different plasma membranes. The mechanism of this selectivity is not fully understood. Polymyxin B acts like valinomycin (section 10.6.2), but it causes the leakage of small molecules such as nucleosides from the cell.

## Killer nanotubes

Work is in progress to design cyclic peptides which will self-assemble in the cell membranes of bacteria to form tubules that have been labelled as killer **nanotubes** (section 10.6.1).

###### Antibacterial agents which act on the plasma membrane structure **451**

**BOX 19.12** Clinical aspects of cycloserine, bacitracin, and vancomycin

**D-Cycloserine** is administered orally in combination with the prophylaxis and treatment of endocarditis and other seri- other drugs to treat tuberculosis that is resistant to ﬁrst-line ous infections caused by Gram-positive cocci. Vancomycin is drugs. **Bacitracin** is used alongside **polymyxin B sulphate** for also given orally to treat gut infections caused by *Clostridium* the topical treatment of skin infections. The same prepara- *difﬁcile*. This organism may appear following the use of tion is also used for the topical treatment of eye infections broad-spectrum antibiotics and produces harmful toxins. caused by *P. aeruginosa*. **Neomycin sulphate** and bacitracin Vancomycin has also been used in eye drops. Teicoplanin are used together for the topical treatment of skin infections can be administered once daily and is used for potentially as a cream or dusting powder. serious Gram-positive infections, including endocarditis, **Vancomycin** and **teicoplanin** are bactericidal and are dialysis-associated peritonitis, and serious infections caused active against aerobic and anaerobic Gram-positive bacte- by *S. aureus*. It is also used as a prophylactic in endocarditis ria, including MRSA. Vancomycin is not absorbed orally and and orthopaedic surgery. Telavancin was approved in 2009

is administered by intravenous injection every 12 hours for for the treatment of skin infections which include MRSA.

L-Dab

L-Dab

L-Leu

D-Phe

L-Thr NH

L-Dab

H

O

H L-Dab

L-Thr

L-Dab CO

Me

(CH2)4 CH

Me

**FIGURE 19.62** Polymyxin B (Dab = α,γ-Diaminobutyric acid with peptide link through the α-amino group).

N

CO2H

NH

O

H

HN

N

O

O

NH2

Lipid chain

O

H N

N N

H H H

CO2H

O

H H

1. N Me

N H

O O

Me O

O NH2

1. (CH2)3

O

O

H N

N

H H

O

O

NH2

OH

O

N H

O NH

O

NH

CO2H

Me

CO2H

**FIGURE 19.63** Daptomycin.

## Cyclic lipopeptides

**Daptomycin** (Fig. 19.63) is a member of a new class of antibacterial agents called the cyclic lipopeptides. It is a natural product derived from a bacterial strain *Streptomyces roseosporus*, and works by disrupting mul- tiple functions of the bacterial cell membrane. The lipid portion of the molecule is derived from decanoic acid

and the yield of product obtained is increased if decanoic acid is added to the fermentation medium.

**KEY POINTS**

* Ionophores act on the plasma membrane and result in the uncontrolled movement of ions across the cell membrane, leading to cell death.

**452 Chapter 19** Antibacterial agents

**BOX 19.13** Clinical aspects of drugs acting on the plasma membrane

**Valinomycin** and **gramicidin A** show no selective toxicity for and **neomycin** for the treatment of eye infections. The ear-drop bacterial cells over mammalian cells and are therefore use- preparation **Otosporin** contains **hydrocortisone** and polymyxin B less as systemic therapeutic agents. However, gramicidin is to treat ear infections and inﬂammation.

present as a minor constituent in some topical applications. **Daptomycin** was approved in 2003 for the treatment of **Polymyxin B** is injected intramuscularly and is useful against Gram-positive infections. It is administered by intravenous *Pseudomonas* strains that are resistant to other antibacterial infusion and has a spectrum of activity similar to vancomy- agents. It can be used topically for the treatment of minor skin cin. In order to guard against the development of drug resist- infections and has good activity against Gram-negative bacte- ance, the drug is held in reserve for skin and soft tissue ria. It is less effective against Gram-positive bacteria, as it is infections caused by drug-resistant Gram-positive bacteria, difﬁcult for such a big molecule to pass through the thicker such as MRSA. It can be administered alongside other anti- cell wall. It is used in combination with **bacitracin** for the bacterial agents for mixed infections involving Gram-positive

treatment of eye and skin infections, or with **dexamethasone** bacteria, Gram-negative bacteria, and some anaerobes.

* Polymyxin B operates selectively on the plasma membrane of bacteria and causes the uncontrolled movement of small molecules across the membrane.
* Cyclic peptides are being designed which will self-assemble to form nanotubes in the cell membranes of bacteria.
* Cyclic lipopeptides are a new class of antibiotic.

ticle (see section 6.2.2) made up of a 30S subunit and a 50S subunit. The 30S subunit binds messenger RNA (mRNA) and initiates protein synthesis. The 50S subu- nit combines with the 30S subunit-mRNA complex to form a ribosome, then binds aminoacyl transfer RNA (tRNA) and catalyses the building of the protein chain.

There are two main binding sites for the tRNA mole-

cules. The peptidyl site (P-site) binds the tRNA bearing the peptide chain. The acceptor aminoacyl site (A-site) binds the tRNA bearing the next amino acid, to which the peptide chain will be transferred (see also section 6.2.2). The ribosomes of eukaryotic cells are bigger (80S), consisting of 60S and 40S subunits. They are suf- ficiently different in structure from prokaryotic ribo-

19.7 **Antibacterial agents which impair protein synthesis: translation**

The agents described in this section all inhibit protein synthesis by binding to ribosomes and inhibiting dif- ferent stages of the translation process (Fig. 19.64). Selective toxicity is due to either different diffusion rates through the cell barriers of bacterial versus mam- malian cells or to a difference between the ribosomal target structures. The bacterial ribosome is a 70S par-

somes that it is possible for some drugs to distinguish between them.

## Aminoglycosides

**Streptomycin** (Fig. 19.65) was isolated from the soil microorganism *Streptomyces griseus* in 1944 and is an

Oxazolidinones bind to 50S subunit





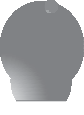
Growing protein



P A

Amino acid tRNA

Chloramphenicol blocks peptide chain transfer



P

A

Macrolides block translocation



50S

Ribosome



A



30S mRNA

Tetracycline blocks tRNA binding

Aminoglycosides block translocation

**FIGURE 19.64** Stages at which antibacterial agents inhibit translation.

###### Antibacterial agents which impair protein synthesis: translation **453**

H2N

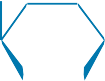
NH

NHH HN

OH H

NH

NH2



H

Streptidine

CH2NH2

H O H

H

H H

H O

Purpurosamine

H HO

H OH

H H2N

H

O H HO O H



H O

CHO

Me H

OH

H

H

Streptose

Garosamine

NHMe H

Me H

O

HO O

CH OH

H HO O

OH

H H

2-Deoxystreptamine

2

H MeHN

H H

*N*-Methyl-

L-glucosamine

H NH2

NH2 H

OH H

Streptomycin (from *Streptomyces griseus*) Gentamicin C1a

**FIGURE 19.65** Aminoglycosides.

**BOX 19.14** Clinical aspects of aminoglycosides

Aminoglycosides are fast acting, but they can also cause ear and kidney problems if the dose levels are not carefully controlled. They are effective in the treatment of infections caused by aerobic Gram-negative bacteria, including *P. aer- uginosa*. Indeed, they used to be the only compounds effec- tive against that organism. Some Gram-negative bacteria are resistant to aminoglycosides due mainly to enzymes which cat- alyse reactions such as *O*-phosphorylations, *O*-adenylations (addition of an adenine group), and *N*-acylations. Resistance can also occur from alterations of the ribosomes such that they bind aminoglycosides less strongly or by less efﬁcient uptake mechanisms. Because the aminoglycosides are polar in nature, they have to be injected. They are also unable to cross the blood–brain barrier efﬁciently and so they can- not be used for the treatment of meningitis unless they are injected directly into the central nervous system (CNS). The activity of aminoglycosides is increased if they are admin- istered with agents which disrupt cell wall synthesis, as this increases uptake into the cell. However, bacteriostatic agents should not be taken with aminoglycosides, because

these inhibit the energy-dependent uptake process by which the aminoglycosides cross the cell membrane.

**Streptomycin** was the ﬁrst effective agent used against tuberculosis. However, resistance soon developed and a multidrug therapy involving streptomycin, **isoniazid**, and ***para*-aminosalicylic acid** was used until the early 1970s. At that point, **rifampicin** became available, allowing different multidrug therapies to be developed. Streptomycin is now rarely used for the treatment of tuberculosis, unless there is a known resistance to isoniazid, in which case it is adminis- tered by intramuscular injection. Streptomycin is still used to treat enterococcal endocarditis and as an adjunct to **doxy- cycline** in brucellosis.

**Gentamycin** is administered by intramuscular or slow intravenous injection for the treatment of a number of infec- tions, including septicaemia; neonatal sepsis; CNS infec- tions (including meningitis); biliary tract infections; acute pyelonephritis or prostatis, endocarditis; and pneumonia in hospital patients. It can be used topically in drops for the treatment of eye and ear infections.

example of an aminoglycoside—a carbohydrate struc- ture which includes basic amine groups. Streptomycin was the next most important antibiotic to be discov- ered after penicillin and a variety of other aminogly- cosides have been subsequently isolated from various organisms, for example **gentamicin C1a** (Fig. 19.65). The aminoglycosides work best in slightly alkaline con- ditions. At pH 7.4, they have a positive charge that is beneficial to activity by aiding absorption through the outer membrane of Gram-negative bacteria. An ionic

interaction takes place with various negatively charged groups on the outer surface of the cell membrane which displaces magnesium and calcium ions. These ions normally act as bridges between lipopolysaccharides, and their displacement results in rearrangement of cell membrane components to produce pores through which an aminoglycoside can pass. The drug then crosses the cell membrane by an energy-dependent pro- cess and is trapped inside the cell where it accumulates to relatively high concentrations.

**454 Chapter 19** Antibacterial agents

Binding to bacterial ribosomes now takes place to inhibit protein synthesis. The binding is specifically to the 30S ribosomal subunit and prevents the movement of the ribosome along mRNA so that the triplet code on mRNA can no longer be read. In some cases, protein synthesis is terminated and the shortened proteins end up in the cell membrane. This can lead to a further increase in cell per- meability, resulting in an even greater uptake of the drug. Aminoglycosides are bactericidal rather than bacteriostatic and it is thought that their activity may be due to their effects both on the ribosomes and the outer cell membrane. Because the ribosomes in human cells are different in structure from those in bacterial cells, they have a much lower binding affinity for the aminoglycosides, which

explains the selectivity of these drugs.

## Tetracyclines

The tetracyclines are bacteriostatic antibiotics which have a broad spectrum of activity and are the most widely pre- scribed form of antibiotic after penicillins. They are also

capable of attacking the malarial parasite. One of the best known tetracyclines is **chlortetracycline** (**aureomycin**) (Fig. 19.66), which was isolated in 1948 from a mud- growing microorganism in Missouri called *Streptomyces aureofaciens*—so-called because of its golden colour. Further tetracyclines, such as **tetracycline** and **doxycy- cline** (Fig. 19.66), have been synthesized or discovered.

The tetracyclines inhibit protein synthesis by bind- ing to the 30S subunit of ribosomes and preventing aminoacyl-tRNA from binding. This stops the further addition of amino acids to the growing protein chain. Protein release is also inhibited.

In the case of Gram-negative bacteria, tetracyclines cross the outer membrane by passive diffusion through the porins. Passage across the inner membrane is dependent on a pH gradient, which suggests that a pro- ton-driven carrier is involved. Selectivity is due to the ability of bacterial cells to concentrate these agents faster than human cells. This is fortunate because tetracyclines are capable of inhibiting protein synthesis in mammalian cells—particularly in mitochondria.

##### **BOX 19.15** Clinical aspects of tetracyclines and chloramphenicol

The tetracyclines are broad-spectrum antibiotics with activ- ity against both Gram-positive and Gram-negative bacteria. Commonly used tetracyclines in the clinic are **tetracycline**, **demeclocycline**, **doxycycline**, **lymecycline**, **minocycline**, and **oxytetracycline**. The use of chlortetracycline has decreased over the years because it kills the intestinal ﬂora that pro- duce vitamin K. However, it is still administered alongside tetracycline and demeclocycline in the preparation **Deteclo**. In general, the tetracyclines can be divided into short- lasting compounds, such as chlortetracycline, an interme- diate group of compounds which includes demeclocycline, and longer-acting compounds which include doxycycline. Minocycline has the broadest spectrum of activity. The tetracyclines were used originally for many types of respira- tory infections, but have been largely replaced by β-lactams because of the problems of resistance. However, they are still the agents of choice for the treatment of Lyme disease, rickettsia, and infections caused by *Chlamydia* species. They are also used to treat acne and a variety of differ- ent infections including respiratory and genital infections. Doxycycline has been found to be useful for the treatment and prophylaxis of malaria, and is cheaper than other anti- malarial agents. One drawback is the possibility of skin hypersensitivity to sunlight. The drug can also be used for the treatment of a variety of diseases including syphilis, sinusitis, oral herpes simplex, and acne. It is a possible

agent for the treatment or prophylaxis of anthrax.

Tetracyclines should be avoided for young children and pregnant mothers as they can bind to developing teeth and bone leading to tooth discolouration. Resistance to tetra- cyclines can arise through several mechanisms. Some organisms have effective efﬂux mechanisms which pump the drug back out of the cell. Resistance can also arise from alterations in the bacterial ribosomes such that they have lower afﬁnity for the agents.

**Chloramphenicol** is a potent broad-spectrum antibiotic and, in some regions of the world, it is the drug of choice for the treatment of typhoid when more expensive drugs cannot be afforded. It can also be used in severe bacterial infec- tions which are insensitive to other antibacterial agents and is widely used for eye infections. It can also be used for ear infections, but the preparation can cause hypersensitivity reactions in about 10% of patients. The drug should only be used in these restricted scenarios as it is quite toxic, especially to bone marrow. The drug is metabolized inad- equately in babies leading to a combination of symptoms described as the **gray baby syndrome**, which can be fatal. In adults, the drug undergoes a phase II conjugation reaction to form a glucuronic acid conjugate (section 11.5.5), which is excreted. This reaction fails to take place efﬁciently in newborn babies and so the drug levels increase to toxic lev- els. Bacteria with resistance to the drug contain an enzyme called **chloramphenicol acetyltransferase**, which catalyses the acylation of the hydroxyl groups.

###### Antibacterial agents which impair protein synthesis: translation **455**

OH O OH O O

OH

OH H

H

R1 X R2 Y

Chlortetracycline (Aureomycin) (R1 = Cl, R2 = Me, X = OH, Y = H) Tetracycline (R1 = H, R2 = Me, X = OH, Y = H)

Doxycycline (Vibramycin) (R1 = H, R2 = Me, X = H, Y = OH) Demeclocycline (R1 = Cl, R2 = H, X = OH, Y = H)

H

NMe2

NH2

OH

O2N

\*

\* CH2OH

H

HN CHCl2

O

Chloramphenicol

**FIGURE 19.66** Tetracyclines and chloramphenicol. The asterisks indicate asymmetric centres.

Widespread resistance to tetracyclines has occurred, caused partly by the use of tetracyclines to cure animal infections and as a food additive to promote the growth of newborn animals.

 Test your understanding and practise your molecu- lar modelling with Exercise 19.5.

## Chloramphenicol

Chloramphenicol (Fig. 19.66) was originally isolated from a microorganism called *Streptomyces venezuela* found in a field near Caracas, Venezuela. It is now pre- pared synthetically and has two asymmetric centres. Only the *R,R*-isomer is active.

Chloramphenicol binds to the 50S subunit of ribo- somes 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. Since it binds to the same region as mac- rolides and **lincosamides**, these drugs cannot be used in

combination. The nitro group and both alcohol groups are involved in binding interactions. The dichloroaceta- mide group is also important, but can be replaced by other electronegative groups. Chloramphenicol is quite toxic and the nitro substituent is thought to be respon- sible for this.

## Macrolides

**Macrolides** are bacteriostatic agents. The best-known example of this class of compounds is **erythromycin**—a metabolite isolated in 1952 from the soil microorganism *Streptomyces erythreus* found in the Philippines, and one of the safest antibiotics in clinical use. The structure (Fig. 19.67) consists of a 14-membered macrocyclic lactone ring with a sugar and an amino sugar attached. The sugar residues are important for activity.

Erythromycin acts by binding to the 50S subunit of bacterial ribosomes to inhibit translocation, but other mechanisms of action also appear likely. Because erythromycin and chloramphenicol bind to the same

O

Me

OH

Me

OH

Me Me

Me X

Me HO NMe2

O

Me

Me

H

N R1 R3

H

O O

O O

Lactone Me

Aminosugar (desosamine)

Me

N

H O H C HO



C Me

O H

O

Me Sugar

OH H

H

SMe

Me OMe (cladinose) OH

Erythromycin; X = OH Clarithromycin; X = OMe

H OR2

**FIGURE 19.67** Macrolides and lincosamides.

Lincomycin R1=R2=H, R3=OH Clindamycin R1=Cl, R2=R3=H

Clindamycin phosphate R1=Cl, R2=PO32–, R3=H

**456 Chapter 19** Antibacterial agents

O



## Lincosamides

Me Me

HO OH

Me Me

OH

Acid conditions HO

Me

Me Me

O O Me

The lincosamide antibiotics (Fig. 19.67) have similar antibacterial properties to the macrolides and act in the same fashion. **Lincomycin** was the first of these agents

Erythromycin Erythromycin

**FIGURE 19.68** Intramolecular ketal formation in erythromycin.

region of the ribosome, they should not be adminis- tered together as they will compete with each other and be less effective.

Erythromycin is unstable to stomach acids, but can be taken orally in a tablet form. The formulation of the tablet involves a coating that is designed to protect the tablet during its passage through the stomach, but which is soluble once it reaches the intestines (entero- soluble). The acid sensitivity of erythromycin is due to the presence of a ketone and two alcohol groups which are set up for the acid-catalysed intramolecular forma- tion of a ketal (Fig. 19.68). One way of preventing this is to protect the hydroxyl groups. For example, **clarithro- mycin** is a methoxy analogue of erythromycin which is more stable to gastric juices and has improved oral absorption. Another method of increasing acid stability is to increase the size of the macrocycle to a 16-mem- bered ring.

**Azithromycin** (Fig. 19.69) contains a 15-membered macrocycle where an *N*-methyl group has been incorpo- rated into the macrocycle. It is one of the world’s best-sell- ing drugs. **Telithromycin** (Fig. 19.69) is a semi-synthetic derivative of erythromycin and reached the European market in 2001. The cladinose sugar in erythromycin has been replaced with a keto-group and a carbamate ring has been fused to the macrocyclic ring. The two hydroxyl groups that cause the intramolecular ketal formation in erythromycin have been masked, one as a methoxy group and the other as part of the carbamate ring.

and was isolated in 1962 from a soil organism called *Streptomyces lincolnensis* found near Lincoln, Nebraska. Chemical modification led to the clinically useful **clinda- mycin** with increased activity.

## Streptogramins

**Pritinamycin** is a mixture of macrolactone structures obtained from *Streptomyces pristinaespiralis*. Two of the components (**quinupristin** and **dalfopristin**) have been isolated. These agents bind to different regions of the bacterial ribosome’s 50S subunit form a complex. It is found that binding of dalfopristin increases the binding affinity for quinupristin, and so the two agents act in synergy with each other. Quinupristin inhibits peptide chain elongation, while dalfopristin interferes with the transfer of the peptide chain from one tRNA to the next.

## Oxazolidinones

The **oxazolidinones** are a new class of synthetic anti- bacterial agents discovered in recent years. They inhibit protein synthesis at a much earlier stage than previous agents, and, consequently, do not suffer the same resist- ance problems. Before protein synthesis can start, a 70S ribosome has to be formed by the combination of a 30S ribosome with a 50S ribosome. The oxazolidinones bind to the 50S ribosome and prevent this from happening. As a result, translation cannot even start. Other agents that inhibit protein synthesis do so during the translation process itself (Fig. 19.64). **Linezolid** (Fig. 19.70) was the first of this class of compounds to reach the market in 2000, and by 2010, it was netting sales of £716 million

O

H

Me

O O

Me

HO O

HO Me

OH

Me OMe

Me

Me

O

H

Me2N

Me

OH

O Me

Keto O group

MeO Me Me

O O

N

N

N

Me H N

Me N

NMe2

Me O

MeO O

Me MeMe OH OH

*N*-Methyl

O Carbamate

ring

Me

group

Azithromycin Telithromycin

**FIGURE 19.69** Azithromycin and telithromycin.

###### Agents that act on nucleic acid transcription and replication **457**

O N

O

N

O

H

F

Linezolid

O

Me

NH NH

N

N

N H

O

O

O

H

N Me

NH

F

Radezolid

**FIGURE 19.70** Oxazolidinones.

**BOX 19.16** Clinical aspects of macrolides, lincosamides, streptogramins, and oxazolidinones

Macrolides

**Erythromycin** has an antibacterial spectrum that is similar to penicillins and can be used as an alternative to penicillins for those patients having penicillin allergies. It has been used against penicillin-resistant staphylococci, but newer penicil- lins are now preferred for these infections owing to increased resistance against erythromycin. It is very useful for the treat- ment of respiratory infections, including whooping cough and Legionnaires’ disease. It can also be used to treat syphilis and diphtheria, as well as oral and skin infections. Topically, it can be used for the treatment of acne. **Clarithromycin** has slightly greater activity than erythromycin, with fewer gastrointesti- nal side effects. Therefore, it is often prescribed instead of erythromycin. Clarithromycin is one of the drugs used in the treatment of ulcers caused by the presence of *Helicobacter pylori* (section 25.4). **Azithromycin** is slightly less active than erythromycin against Gram-positive infections, but is more active against Gram-negative infections, including

*H. inﬂuenza*—against which erythromycin shows poor activ- ity. Azithromycin can also be used for the treatment of Lyme disease. **Telithromycin** has a similar spectrum of activity to other macrolides. It should only be used for speciﬁed infec- tions such as pneumonia, tonsillitis, and sinusitis.

Resistance to macrolides is due to effective efﬂux mecha- nisms which pump the drug back out the cell. The ribo- somal target site may also change in character such that binding is weakened. Enzyme-catalysed modiﬁcations can also occur. Recently there has been research into ﬁnding novel macrolides which can be effective against respiratory infections due to resistant strains of *S. pneumoniae*, as well as the organism *H. inﬂuenza*.

Lincosamides

**Clindamycin** can be taken orally and is active against Gram-positive cocci, including streptococci and penicil- lin-resistant staphylococci. It is active against peripheral infections involving the anaerobic *B. fragilis*, and is recom- mended for the treatment of joint and bone infections caused by staphylococci. It is also used topically for the treatment of acne.

Streptogramins

**Pritinamycin** has been used orally in the treatment of Gram- positive cocci infections, including MRSA. **Quinupristin** and **dalfopristin** are used intravenously in combination (**Synercid**). At present, these agents are reserved for life- threatening Gram-positive infections for which there are no alternative therapies; for example hospital-acquired pneumonia, skin and soft tissue infections, and infections caused by vancomycin-resistant *Enterococcus faecium*.

Oxazolidinones

The oxazolidinones have a broad spectrum of activity and are active against bacterial strains which have acquired resistance to other antibacterial agents acting against protein synthesis. **Linezolid** has good activity against most clinically important Gram-positive bacteria, includ- ing MRSA. It can also be taken orally with 100% uptake from the gastrointestinal tract. Unfortunately, there is a high level of side effects related to its use and, as it is a bacteriostatic agent, there is a greater risk of bacterial resistance developing.

per year. X-ray crystallographic studies have revealed how the structure binds to the ribosome, and that has allowed the development of analogues which bind more strongly. **Radezolid** is one such structure which binds 10,000 times more strongly as a result of extra binding interactions (*extension strategy*, section 13.3.2). It is cur- rently undergoing clinical trials.

19.8 **Agents that act on nucleic acid transcription and replication**

## Quinolones and ﬂuoroquinolones

The quinolone and **fluoroquinolone** antibacterial agents are particularly useful in the treatment of urinary tract

**458 Chapter 19** Antibacterial agents

O

Me N N

CO2H

F 6

Piperazine

8



N 7 N

O

3 CO2H

N

O

F CO2H

1

N

CH2CH3 HN CH2CH3



N

HN

Nalidixic acid

Enoxacin

**FIGURE 19.71** Quinolones and fluoroquinolones.

Ciprofloxacin

infections and infections which prove resistant to the more established antibacterial agents.

**Nalidixic acid** (Fig. 19.71), synthesized in 1962, was the first therapeutically useful agent in this class of com- pounds. Various analogues were synthesized but offered no great advantage. However, a breakthrough was made in the 1980s with the development of **enoxacin** (Fig. 19.71), which showed improved broad-spectrum activity. The development of enoxacin was based on the discovery that a single fluorine atom at position 6 greatly increased both activity and cellular uptake. A basic sub- stituent, such as a piperazinyl ring at position 7, was also

beneficial for a variety of pharmacokinetic reasons due to the ability of the basic substituent to form a zwitterion with the carboxylic acid group at position 3.

The introduction of a cyclopropyl substituent at posi- tion 1 further increased broad-spectrum activity, while replacement of the nitrogen at position 8 with carbon reduced adverse reactions and increased activity against

*S. aureus*. This led to **ciprofloxacin** (Fig. 19.71 and Box 19.17), the most active of the fluoroquinolones against Gram-negative bacteria.

The quinolones and fluoroquinolones inhibit the rep- lication and transcription of bacterial DNA by stabilizing

**BOX 19.17** Synthesis of ciproﬂoxacin



O

O

O

F F F

Cl

Cl

Cl

CH2(CO2Et)2

Mg(OEt)2

CH(CO2Et)2

Cl

a)H+/Δ

b)H /EtOH

CH2CO2Et

+

Cl

Cl

Cl

O

O

O

HC(OEt)3

Ac2O

F

CO2Et

F

CO2Et

F

CO2Et

CHOEt

Cl

Cl

NH2

CH

Cl

Cl

HN

Base

–HCl Cl

N

O

O

F

H+

CO2H

F

CO2H

Cl

N

HN NH

N

N

HN

Synthesis of ciprofloxacin.

The synthesis of ciproﬂoxacin is a seven-stage route and is applicable to a wide range of ﬂuoroquinolones. It involves the construction of the ‘right-hand’ pyridone ring onto the

ﬂuoro-substituted aromatic ring. The cyclopropyl substitu- ent is incorporated just before ring closure and the piperazi- nyl substituent is added at the ﬁnal stage of the synthesis.

###### Agents that act on nucleic acid transcription and replication **459**

O

N

O

Me

F CO2H

H



N

N

Me

F

N

NH H

O

N

OMe

CO2H

H2N

O

F

N N

Cl

CO2H

Levofloxacin

(Ofloxacin is the racemate)

Moxifloxacin Besifloxacin

**FIGURE 19.72** Third- and fourth-generation fluoroquinolones.

the complex formed between DNA and topoisomerases (section 9.2). In Gram-positive bacteria, the stabilized complexes are between DNA and **topoisomerase IV**, with the drugs showing a 1000-fold selectivity for the bacterial enzyme over the corresponding enzyme in human cells. In Gram-negative bacteria, the main target for fluoroquinolones is the complex between DNA and a topoisomerase II enzyme called **DNA gyrase**. It has the same role as topoisomerase IV in reverse and is required when the DNA double helix is being supercoiled after replication and transcription.

A large number of fluoroquinolones have now been syn- thesized. Those agents having good activity all have a simi- lar bicyclic ring system, which includes a pyridone ring and a carboxylic acid at position 3. A problem with first- and second-generation fluoroquinolones is that they gen- erally show only moderate activity against *S. aureus,* fol- lowed by rapidly developing drug resistance. Furthermore,

only marginal activity is shown against anaerobes and

*S. pneumoniae*. Third- and fourth-generation fluoroqui- nolones, such as **ofloxacin**, **levofloxacin**, **moxifloxacin**, and **besifloxacin** (Fig. 19.72) began to be developed in the early 1990s to tackle these issues. Ofloxacin has an asym- metric centre and is sold as a racemic mixture of both enantiomers, one of which is active and one of which is not. Levofloxacin is the active enantiomer of oflaxacin and is twice as active as the racemate.

## Aminoacridines

Aminoacridine agents, such as the yellow-coloured **pro- flavine**, are topical antibacterial agents which were used particularly during World War II to treat deep surface wounds. The best agents are completely ionized at pH 7 and they interact directly with bacterial DNA by inter- calation (section 9.1). Despite the success of these drugs

**BOX 19.18** Clinical aspects of quinolones and ﬂuoroquinolones

**Nalidixic acid** is active against Gram-negative bacteria and back out of the cell. Less common resistance mechanisms is useful in the short-term therapy of uncomplicated urinary include mutations to the topoisomerase enzymes which tract infections. It can be taken orally but, unfortunately, reduce their afﬁnity to the agents, and alteration of porins bacteria can develop a rapid resistance to it. **Enoxacin** has in the outer membrane of Gram-negative organisms to limit a greatly increased spectrum of activity against Gram- access.

negative and Gram-positive bacteria. It also shows improved Third-generation ﬂuoroquinolones show improved activity oral absorption, tissue distribution, and metabolic stability, against *S. pneumoniae*, while maintaining activity against as well as an improvement in the level and spectrum of enterobacteria. **Oﬂoxacin** is administered orally or by intra- activity, particularly against Gram-negative bacteria, such venous infusion to treat septicaemia, gonorrhoea, and infec- as *P. aeruginosa*. **Ciproﬂoxacillin** is used in the treatment of tions of the urinary tract, lower respiratory tract, skin, and a large range of infections involving the urinary, respiratory, soft tissue. **Levoﬂoxacin** has a greater activity against pneu- and gastrointestinal tracts (e.g. travellers’ diarrhoea), as mococci than ciproﬂoxacillin and is a second-line treatment well as infections of skin, bone, and joints. It is also used for community-acquired pneumonia. It is also used for for gonorrhoea and septicaemia, and as part of a cocktail acute sinusitis, chronic bronchitis, urinary tract infections, of drugs for anthrax. It has been claimed that ciproﬂoxacin skin infections, and soft tissue infections. **Moxiﬂoxacin** also may be the most active broad-spectrum antibacterial agent has greater activity against pneumococci than ciproﬂoxacin. on the market. In contrast to nalidixic acid, resistance to the It is used to treat sinusitis and is a second-line treatment ﬂuoroquinolones is slow to appear, but, when it does appear, of community-acquired pneumonia. **Besiﬂoxacin** is a fourth- it is mainly due to efﬂux mechanisms which pump the drug generation ﬂuoroquinolone approved in 2009.

**460 Chapter 19** Antibacterial agents

as topical agents, they are not suitable for the treatment of systemic bacterial infections because they are toxic to host cells.

## Rifamycins

**Rifampicin** (Fig. 19.73) is a semi-synthetic rifamycin made from **rifamycin B**—an antibiotic which was iso- lated from *Streptomyces mediterranei* in 1957. It inhibits Gram-positive bacteria and works by binding non-cova- lently to **DNA-dependent RNA polymerase** and inhib- iting the start of RNA synthesis. The DNA-dependent RNA polymerases in eukaryotic cells are unaffected because the drug binds to a peptide chain not present in

the mammalian RNA polymerase. It is, therefore, highly selective. The flat naphthalene ring and several of the hydroxyl groups are essential for activity and the mol- ecule exists as a zwitterion, giving it good solubility both in lipids and aqueous acid. **Rifaximin** is another semi- synthetic analogue that was approved in 2004 for the treatment of diarrhoea and *E. coli* infection.

## Nitroimidazoles and nitrofurantoin

**Metronidazole** (Fig. 19.73) is a nitroimidazole struc- ture which was introduced in 1959 as an anti-protozoal agent, but began to be used as an antibacterial agent in the 1970s. The nitro group is reduced when the drug

Me Me

HO

H

O

OH

O

Me

Me

Me

OH

OH

NH

O

R

O OH

Me

O

O

Me Me

HO

O

H OH O

Me Me Me O

O O

Me Me

Me OH OH

Me Me Me NH

O N

O N

Me O

Rifaximin

R =

H

Rifamycin B

R =

CH=N–N

NH Rifampicin

OH

O2N N Me N

NO2

O N

N

N

O

NH N N

O N

Me

Me

H

HO

Me Me

Me

CO2H O

Me

O

Metronidazole

Nitrofurantoin Methenamine

O

NHNH2 N

HO H

Me

H H



Fusidic acid

CH2CH3



N NH2

O

N

N OH

HO N

H H

CH2CH3

Isoniazid Pyrazinamide (+)Ethambutol

**FIGURE 19.73** Miscellaneous agents.

enters the bacterial cell, which lowers the concentration of metronidazole within the cell and sets up a concen- tration gradient down which more drug can flow. The reduction mechanism also proves toxic to the cell as free radicals are formed which act on DNA. **Nitrofurantoin** also undergoes reduction within bacterial cells to form radical species that act on DNA.

## Inhibitors of bacterial RNA polymerase

A recent addition to the arsenal of clinically useful antibi- otics is **fidaxomicin** (Fig. 19.74), which is a natural product obtained from a *Dactylosporangium* Gram-positive bacte- rial strain. The agent is a macrocycle and was approved in 2011 as a narrow spectrum bactericidal agent for the treat- ment of *C. difficile* infections in the gastrointestinal tract. It inhibits transcription in *C. difficile* by inhibiting RNA polymerase, and has a minimal effect on other gut flora.

19.9 **Miscellaneous agents**

A variety of miscellaneous agents are shown in Fig. 19.73. **Methenamine** is used to treat urinary tract infections where it degrades in acid conditions to give formaldehyde as the active agent (section 14.6.6). **Fusidic acid** is a ster- oid structure derived from the fungus *Fusidium coccineum* and is used as a topical antibacterial agent. **Isoniazid** is the most widely used drug for the treatment of tubercu- losis. It acts by inhibiting the synthetic pathways leading to mycolic acid, an important constituent of mycobacte-

###### Miscellaneous agents **461**

peroxidase enzyme. Resistant strains of tuberculosis block the action of this enzyme. **Ethambutol** and **pyrazinamide** are synthetic compounds which are both front-line drugs in the treatment of tuberculosis. Ethambutol inhibits **ara- binosyl transferase** enzymes that are involved in the bio- synthesis of the mycobacterial cell wall.

**KEY POINTS**

* Aminoglycosides, tetracyclines, chloramphenicol, strepto- gramins, lincosamides, and macrolides inhibit protein syn- thesis by binding to the bacterial ribosomes involved in the translation process.
* Resistance can arise from a variety of mechanisms, such as drug efﬂux, altered binding afﬁnity of the ribosome, altered membrane permeability, and metabolic reactions.
* Oxazolidinones prevent the formation of the 70S ribosome by binding to the 50S subunit.
* Quinolones and ﬂuoroquinolones inhibit topoisomer- ase enzymes, resulting in inhibition of replication and transcription.
* Aminoacridines are useful topical antibacterial agents which can intercalate with bacterial DNA and hinder replication and transcription.
* Rifamycins inhibit the enzyme RNA polymerase and pre- vent RNA synthesis. In turn, this prevents protein synthesis. Rifampicin is used to treat tuberculosis and staphylococcus infections. Fidaxomicin is a macrocycle which also targets RNA polymerase.
* Nitroimidazoles are used against infections caused by proto- zoa and anaerobic bacteria.

rial cell walls. It is activated in bacterial cells by a catalase-

Cl OH

Me

Cl

Me

HO O

O OH

O OH

Me

Me

Me Me

Me O Me

O

O

O OH

OMe

Me O O H

H Me

Me O HO OH

**FIGURE 19.74** Fidaxomicin.

**462 Chapter 19** Antibacterial agents

**BOX 19.19** Clinical aspects of rifamycins and miscellaneous agents

**Rifampicin** is bactericidal and is used mainly in the treat- ment of tuberculosis and staphylococci infections that resist penicillin. It is used in combination with dapsone in treating leprosy and is also used for the treatment of bru- cellosis, legionnaires’ disease, and serious staphylococ- cal infection. It is a very useful antibiotic, showing a high degree of selectivity against bacterial cells over mammalian cells. Unfortunately, it is also expensive, which discourages its use against a wider range of infections. Rifampicin is a key component of any anti-tuberculosis regimen, but it poses a special problem when treating tuberculosis in AIDS patients, as it enhances the activity of the cytochrome P450 enzyme family (CYP3A). These enzymes metabolize the HIV protease inhibitors used in HIV therapy, thus lowering their effectiveness. Increased cytochrome P450 activity also decreases the effect of oral anticoagulants, oral contracep- tives, and barbiturates.

**Metronidazole** has good activity in treating infections caused by anaerobic bacteria and protozoa, including difﬁcult-to-treat organisms, such as *B. fragilis* and *C. dif- ﬁcile*. It is well distributed round the body and crosses the blood–brain barrier, so it can be used for the treatment of brain abscesses and other central nervous system infections involving anaerobic bacteria. Metronitrazole is used for the treatment of leg ulcers, bacterial vaginosis, pelvic inﬂam-

matory disease, and can also be used as an alternative to penicillins for oral infections, including tooth abscesses. It is administered with amoxicillin (or with tetracycline and bismuth) in the treatment of gastric ulcers involving *H. pylori* (section 25.4). The drug is effective against *Giardia* infections derived from polluted water supplies—a common hazard when visiting the third world. Finally, nitroimida- zoles, such as metronidazole, are commonly combined with cephalosporins or aminoglycosides to treat infections involving both aerobic and anaerobic organisms. Resistance is rare, though not of the question. **Nitrofurantoin** is used to treat uncomplicated urinary tract infections.

**Methenamine** can be used to treat urinary tract infections, but only if the urine is acidic and the infection is in the lower urinary tract. It can be used as a prophylactic, and as a treatment for chronic and recurrent lower urinary tract infections.

**Fusidic acid** is a topical antibacterial agent that is used in eye drops and skin creams. It can penetrate intact and damaged skin, so it is useful for the treatment of boils. It has also been used to eradicate MRSA colonies carried in the nasal passages of hospital patients and health workers. **Isoniazid** is the most widely used drug for the treatment of tuberculosis and is part of a four-drug cocktail which is the

ﬁrst choice treatment for the initial phase of the disease.

19.10 **Drug resistance**

Medicinal chemists are still actively seeking new and improved antibacterial agents to combat the worry- ing ability of bacteria to acquire resistance to current drugs. For example, 60% of *S. pneumoniae* strains are resistant to β-lactams, and 60% of *S. aureus* strains are resistant to **methicillin**. The last resort in treat- ing *S. aureus* infections is **vancomycin**, but resistance is also beginning to appear to that antibiotic. Some strains of *E. faecalis* appearing in urinary and wound infections are resistant to all known antibiotics and are untreatable. If antibiotic resistance continues to grow, medicine could be plunged back to the 1930s. Indeed, many of today’s advanced surgical procedures would become too risky to carry out due to the risks of infec- tion. Old diseases are already making a comeback. For example, a new antibiotic-resistant strain of tuberculo- sis [**multidrug-resistant TB** (**MDRTB**)] appeared in New York and took 4 years and $10 million to bring under control. These strains were resistant to two of the front-line drugs used against tuberculosis (isonia- zid and rifamycin), and had various levels of resistance

against another two (streptomycin and ethambutol). Other examples of bacterial strains acquiring resistance include penicillin-resistant meningococci and pneumo- cocci in South Africa, penicillin-resistant gonococci in Asia and Africa, ampicillin-resistant *H. influenza* in the USA and Europe, and chloramphenicol-resistant meningococci in France and Southeast Asia. Resistance to trimethoprim in some of the developing nations has meant that the drug has become ineffective as a treat- ment for dysentery.

Drug resistance can arise because of a variety of factors described in section 19.5.1.5, but the cell must have the necessary genetic information. This information can be obtained by mutation or by the transfer of genes between cells.

## 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 complete a full course of antibacterial treatment

even though their symptoms may have disappeared well before the end of the course. If this rule is adhered to, the vast majority of invading bacterial cells will be wiped out, leaving the body’s own defences to mop-up any isolated survivors or resistant cells. If the treatment is stopped too soon, however, then the body’s defences struggle to cope with the survivors. Any isolated resistant cell is then given the chance to multiply, resulting in a new infection which will, of course, be completely resistant to the origi- nal drug. This was a major factor in the appearance of MDRTB.

Mutations occur naturally and randomly, and do not require the presence of a drug. Indeed, it is likely that a drug-resistant cell is present in a bacterial population even before the drug is encountered. This was demon- strated with the identification of **streptomycin**-**resistant** cells from old cultures of *E. coli*, which had been freeze- dried to prevent multiplication before the introduction of streptomycin into medicine.

## Drug resistance by genetic transfer

A second way in which bacterial cells can acquire drug resistance is by gaining that resistance from another bac- terial cell. This occurs because it is possible for genetic information to be passed on 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 infor- mation known as **plasmids** are transferred by means of bacterial viruses (**bacteriophages**) which leave the resistant cell and infect a non-resistant cell. If the plas- mid contains the gene required for drug resistance, then the recipient cell will be able to use that infor- mation and gain resistance. For example, the genetic information required to synthesize β**-lactamases** can be passed on in this way, rendering bacteria resistant to penicillins. The problem is particularly prevalent in hospitals where over 90% of staphylococcal infections are currently resistant to antibiotics such as penicillin, erythromycin, and tetracycline. It may seem odd that hospitals should be a source of drug-resistant strains of bacteria. In fact, they are the perfect breeding ground. Drugs commonly used in hospitals are present in the air in trace amounts. It has been shown that breath- ing in these trace amounts kills sensitive bacteria in the nose and allows the nostrils to act as a breeding ground for resistant strains.

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 can pass.

###### Drug resistance **463**

* + 1. **Other factors affecting drug resistance**

The more useful a drug is, the more it will be prescribed, and the greater the possibilities of resistant bacterial strains emerging. The original penicillins were used widely in human medicine, but were also used com- monly in veterinary medicine. Antibacterial agents have also been used in animal feeding to increase animal weight and this, more than anything else, has resulted in drug-resistant bacterial strains. It is sobering to think that many of the original bacterial strains which were treated so dramatically with penicillin V or penicillin G are now resistant to those early penicillins. In contrast, these two drugs are still highly effective antibacterial agents in poorer, developing African nations, where the use (and abuse) of the drugs has been far less widespread. The ease with which different bacteria acquire resist- ance varies. For example, *S. aureus* is notorious for its ability to acquire drug resistance owing to the ease with which it can undergo transduction. However, the micro- organism responsible for syphilis seems incapable of acquiring resistance and is still susceptible to the original

drugs used against it.

## The way ahead

The ability of bacteria to gain resistance to drugs is an ever-present challenge to the medicinal chemist and it is important to continue designing new antibacterial agents. Identifying potential new targets is essential in this never-ending battle. The sequencing of genomes and a study of the proteins present in bacterial cells prom- ises to give more detailed understanding of the molecu- lar details of infectious agents leading to the identifica- tion of new drug targets. For example, *Mycobacterium tuberculosis*—the causative agent of tuberculosis—has a complex cell wall where three types of polymers are attached to peptidoglycan. The detailed mechanisms by which these polymers are synthesized and incorporated into the cell wall are being investigated to identify new targets for antibacterial drugs which will disrupt the cell wall structure.

It is also beginning to be appreciated that the drugs with the least susceptibility to resistance are those with several different modes of action. Therefore, designing drugs which act on a number of different targets, rather than one specific target, is more likely to be successful.

Examples of new targets include kinase enzymes. There has already been success in designing kinase inhibitors as anticancer agents (section 21.6.2) and sev- eral research groups are now looking at agents that might prove to be selective inhibitors of bacterial kinases. Other potential targets are the enzymes known as **aminoacyl**

**464 Chapter 19** Antibacterial agents

**tRNA synthetases**. These enzymes are an ancient group of enzymes responsible for attaching amino acids to tRNA. Because they are ancient, there is a considerable sequence divergence between the bacterial and human enzymes, making selective inhibition possible. **Isoleucyl tRNA synthetase** is one such enzyme which is known to be inhibited by **mupirocin** (Fig. 19.75)—a clinically useful antibiotic isolated from *Pseudomonas fluorescens* with activity against MRSA. Mupirocin is used as a topi- cal agent for skin infections and has also been used to combat the transmission of *S. aureus* within hospitals by treating the nasal passages of patients and hospital staff. Unfortunately, the widespread use of the agent for this purpose has led to strains of *S. aureus* with increasing resistance to the drug. Research is now being carried out to find novel inhibitors for a different aminoacyl tRNA synthetase present in *S. aureus*, namely **tyrosine tRNA synthetase**. The strategy of targeting aminoacyl tRNA synthetases is also proving fruitful in the search for novel antifungal agents. **Tavaborole** inhibits **leucine tRNA synthetase** and is undergoing clinical trials for the treat- ment of fungal nail infections.

Another potential approach in countering resistance is to modify antibiotics such that they gain resistance to the mechanisms of resistance used against them! For example, **kanamycin** is an aminoglycoside which is no longer used because resistant bacteria can phosphoryl- ate one of the hydroxyl groups present (Fig. 19.76).

An active analogue has been synthesized which replaces the susceptible alcohol with a ketone (Fig. 19.77).

This ketone is in equilibrium with the hydrated gem-diol. When phosphorylation occurs on the diol, the phosphate group thus formed acts as a good leaving group and the ketone is regenerated. *In vitro* tests showed that this agent was active against strains of bacteria which are resistant to kanamycin.

Another approach is to design molecules with an inbuilt self-destruct mechanism. One of the problems with antibiotics in medicine or veterinary practice is that much of the active antibiotic is excreted, giving bacteria in the environment the opportunity to gain resistance. This problem could be reduced by incorporating a self- destruct mechanism which kicks in once the antibiotic is excreted. For example, work has been carried out on a cephalosporin containing a protected hydrazine group (Fig. 19.78). The protecting group concerned is *ortho*- nitrobenzylcarbamate which is susceptible to light. Once the antibiotic is excreted and exposed to light, the pro- tecting group is lost, allowing the nucleophilic hydrazine moiety to react with the β-lactam ring and deactivate the molecule. This works *in vitro* but has still be tested *in vivo.*

Recent research into drug combinations has shown that there can be a beneficial effect on antibacterial activ- ity *in vitro* if one administers an antibacterial drug with another drug, even if the other drug has no antibacte- rial activity itself. For example, a small dose of the tetra- cycline agent **minocycline** showed better activity than expected when it was administered along with the anti- diarrhoeal drug **loperamide** (Box 24.3). Further studies

OH

HO O

Me

OH

CO2H B

O

Me Me O

F

O H

OH Mupirocin Tavaborole

**FIGURE 19.75** Inhibitors of aminoacyl tRNA synthetases.

HO

HO

NH2 O

HO

O

HO P

NH2 O

HO H2N O

HO O

HO H2N O

Kanamycin

HO

NH2

O

OH O

Phosphorylation

NH2 OH

HO

Inactive

NH2

O

OH O

NH2 OH

OH OH

**FIGURE 19.76** The phosphorylation reaction causing resistance to kanamycin.

###### Drug resistance **465**

HO

Ketone analogue

NH2

HO

O HO

NH2

O

Hydrated diol

of kanamycin O

HO

OR



HO HO OR

Dephosphorylation

NH2

HO

O O

Phosphorylation

HO P

HO O HO HO OR

**FIGURE 19.77** Analogue of kanamycin which is resistant to phosphorylation.

NO2 *o*-NBC protecting group

O O

HN

H H H

NH

N S

C

Light

HN

PhH2C



NH2

H H H

N

S

C

O

N

O

O

CO2

O

O

PhH2C O

N

CO2

O Me Me

C C

O O

HN HN

NH

S

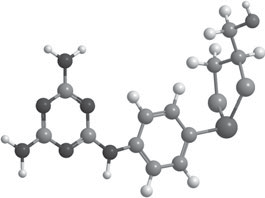
O N

Further degradation

Inactive

CO2

**FIGURE 19.78** Self-destruct mechanism.



**BOX 19.20** Organoarsenicals as antiparasitic drugs

The ﬁrst effective antimicrobial drug to be synthesized was treatment of trypanosomiasis and sleeping sickness. This is the organoarsenical, **salvarsan** (section 19.1). In the late despite the fact that it has to be injected and can kill 1 in 1940s, another organoarsenical called **melarsoprol** was 20 patients treated with it (Fig. 1).

introduced into medicine and is the ﬁrst-choice drug for the

O

NH2

S

S

N N

As

S

OH

S

As

H2N N

N

H

**FIGURE 1** Melarsoprol.

(*Continued* )

**466 Chapter 19** Antibacterial agents

**Box 19.20** Organoarsenicals as antiparasitic drugs (*Continued* )

One of the mechanisms by which melarsoprol might act is leads to a loss of cell motility and eventual cell death. Other through a reaction with the cysteine residues of enzymes mechanisms of action have been proposed.

involved in glycolysis (Fig. 2). The blocking of glycolysis

S

Enzyme

HS-enzyme

HS

R

As S

OH

R

S

As S

Enzyme

HS

OH

**FIGURE 2** Mechanism of action of melarsoprol.

are needed to see if this effect occurs *in vivo*, but it might be another way of tackling drug resistance.

**KEY POINTS**

* Bacterial strains vary in their ability to gain resistance to anti- bacterial drugs. *Staphylococcus aureus* is quick to gain anti- bacterial resistance. The MRSA strain is a *S. aureus* strain that is resistant to most antibacterials, including methicillin.
* Vancomycin is the antibacterial agent of last resort in the treatment of resistant bacterial strains.
* There are many mechanisms by which bacteria can acquire resistance against antibacterial agents, but they all result from a change in the cell’s genetic make-up.
* Drug resistance can result from mutation of a cell’s genetic information or from transfer of genetic information from one cell to another. Genetic information can be transferred from one cell to another by transduction or conjugation.
* Care has to be taken to use antibacterial agents in a respon- sible manner to reduce the chances of resistance developing.
* It is important to identify new targets which can be used for the design of novel antibacterial agents.

# QUESTIONS

1. How would you convert penicillin G to 6-aminopenicillanic acid (6-APA) using chemical reagents? Suggest how you would make ampicillin from 6-APA.

**5.** Discuss whether you think the following penicillin analogue would be a useful antibacterial agent.

NH2

O

1. Penicillin is produced biosynthetically from cysteine and valine. If the biosynthetic pathway could accept different amino acids, what sort of penicillin analogues might be formed if valine was replaced by alanine, phenylalanine, glycine, or lysine? What sort of penicillin analogue might

H H H N

N

O

S

O

CO2H

O CH3

be formed if cysteine was replaced by serine? (See Appendix 1 for amino acid structures.)

1. Referring to Question 2, why do you think penicillin analogues like this are not formed during the fermentation process?
2. The activity of sulphonamides is decreased if they are taken at the same time as procaine. Suggest why this might be the case.

Penicillin analogue

1. Explain what effect replacing the methoxy groups on methicillin with ethoxy groups might have on the properties of the agent.
2. What effect might the bicyclic ring system of cephalosporins have on their chemical and biological properties compared with the bicyclic ring system of penicillins, and why?

###### Further reading **467**

1. The following structure is an analogue of cefoxitin. What O

sort of properties do you think it might have compared to F

cefoxitin itself?

N



N

HN

CO2H

H OMe H

N S



S

O

N

O CF3



F

N

N

H2N

O



O

F

N

HN

O

Cefoxitin analogue

C

CO2H O

Me

F

CO2H

H N

CO2H

N

1. Show the mechanism by which the prodrug bacampicillin

(Box 19.7) is converted to ampicillin. What are the Me

by-products?

HN F

H

F

1. Which of the following structures would you expect to have the best antibacterial activity?
2. Devise a synthesis for the structure chosen in Question 10.

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*Titles for general further reading are listed on p. 763.*