The rational redesign of penicillins to help combat penicillin resistance

This month’s science article explores the link between chemical structures of penicillins and their clinical importance. Timothy J. Snape and Alison M. Astles re-emphasise the link between chemistry and pharmacy practice in order to shed some light on why there is a need for so many penicillin antibiotics.

The number of natural penicillins and synthetic penicillin analogues — based on the β-lactam structure (Figure 1) — is huge, and many have made it to the market to help our fight against bacterial infections. However, is it just a case of another generic or do these drugs deserve their market share based purely on their unique chemical structure and, therefore, their unique antibacterial activity? This article looks into the structural similarities and differences between a number of penicillin antibiotics and argues that subtle changes in chemical structure make their market share a necessity.

The penicillin antibiotics are arguably the most commonly known serendipitous discovery of the past 100 years, which provides an excellent example of Pasteur’s aphorism “chance favours only the prepared mind”. On returning from his holiday in 1928, Alexander Fleming almost destroyed the culture plates that had been contaminated with a rare strain of penicillium mould and which happened to be a highly efficient producer of penicillins. Only Fleming’s tenacity and prepared mind enabled him to understand the importance of what he had discovered — a mould that produced an agent (later termed penicillin) that was effective against potentially pathogenic organisms — since, unlike other culture plates that also swelled and produced enzymes, this mould remained solid and healthy.

For this discovery, he was ultimately awarded a Nobel Prize in medicine in 1945, along with H.W. Florey and E. B. Chain, who had demonstrated the successful application of penicillin to humans in 1943 in response to the huge numbers of soldiers who were dying due to wound-related infections in the 1939–45 war. Despite penicillin’s huge success, it was not effective against all types of bacterial infection, thus new drugs were needed to help treat these diseases that remained elusive. The penicillin class of antibiotic has seen a huge number of structural analogues produced in efforts to overcome their sensitivity to acid, their sensitivity to β-lactamase enzymes produced by bacteria (see Panel) and their limited breadth of activity. Again, we turn to their chemical structures for an answer as to how this might be achieved.

Bacterial cell wall
First, we need to take a brief look at the mechanism of action of these penicillin antibiotics to understand how chemical structure can be correlated to antibacterial activity. Bacteria rely on their cell wall for survival. Without it, water would continually enter the cell through osmotic pressure and the cell would burst. The cell wall does not prevent water passing through, but it does prevent the cell from swelling and, since animal cells do not have a cell wall, the wall itself becomes a perfect target for drugs (eg, penicillins) to enable selective attack against bacterial cells over animal (human) cells.

The bacterial cell wall consists of a cross-linked array of amino acid chains and sugar units, and the final step in the biosynthesis of the cell wall is the cross-linking of the amino acid chains. It is at this point where penicillin antibiotics work by inhibiting this process and ultimately leaving the cell wall in a weakened, non-cross-linked state. As a result, the cell wall is now fragile and is unable to prevent the cell from swelling and bursting. The crucial enzyme that is responsible for the cross-linking process is blocked by the penicillins and it has been shown that penicillins mimic the amino acid chain of the cell wall and, as such, the enzyme mistakes the penicillin for the amino acid chain. Consequently, the enzyme is blocked, the cell cannot generate a cell wall and the cell dies.

Structural features
The success of penicillins is dependent on a number of things, including the ability of the drug to get to its site of action unsalted, the ability of the drug to fight off any attack by the bacteria’s defence mechanism, the ability of the drug to bind to the cell-wall building enzyme and block its natural function, and the
rate at which the drug is pumped back out of the cell. These factors can be controlled to a differing extent by manipulating the drug’s chemical structure. Thus, it stands to reason (within certain parameters) that the larger the number of analogues that are prepared and tested, the greater the chance of finding a structure that satisfies most demands on it. Also, the greater the number of demands there are, the greater the number of drugs that will ultimately reach the market. After all, necessity is the mother of invention.

Inevitably, during the preparation of a large number of penicillin analogues, certain structural features were highlighted that have been found to be essential for antibiotic activity. These are (see Figure 1):

- The strained β-lactam ring
- The carboxylic acid
- The fused 4- and 5-membered ring systems
- The amide side-chain
- The cis-stereochemistry (i.e., the two rings must be fused so that the H-atoms are both on the same side of the molecule)

Since little change to the structure of the penicillins can be tolerated while still maintaining potent antibacterial activity, most analogues tend to have structural variation at the site labelled “R” in Figure 1. We will look at how changes at this point in the structure have led to a number of commonly prescribed analogues that tend to have structural variation at the site labelled “R” in Figure 1. We will look at how changes at this point in the structure have led to a number of commonly prescribed analogues that tend to maintain potent antibacterial activity, most of which are significantly different to enable them to overcome the shortfalls of the others.

Penicillin redesign

The widespread use of the penicillins in the latter half of the 20th century led to an alarming number of penicillin-resistant infections that were also untreatable with all other available antibiotics. Thus, new drugs that could overcome these penicillin-resistant strains were desperately needed. Interestingly, the answer came not from discovering a new class of a more potent antibiotic, but from the rational redesign of the chemical structures of the existing penicillins to “design out of them” their chemical susceptibility to these resistant strains.

As described (see Panel), certain bacteria secrete β-lactamase enzymes. These enzymes accept the penicillin into their active site and subsequently cleave open the strained β-lactam ring. However, if the drug was redesigned so as to be too big to fit into the enzyme’s active site, yet still maintain its ability to prevent the cross-linking of the bacterial cell wall, then it would no longer be susceptible to the β-lactamase enzyme, but would maintain its antibacterial activity.

In the event, such a bulk was added to the structures at position “R” (Figure 1), and meticillin was the first semi-synthetic penicillin to show resistance to the β-lactamase enzyme of *Staphylococcus aureus*. Temocillin is another β-lactamase-resistant penicillin that acts in this way. Incidentally, 95 per cent of *S aureus* strains detected in hospitals have become resistant to meticillin and other β-lactamase resistant penicillins resulting in the so-called meticillin-resistant *S aureus* epidemic. This is the result of continual bacterial evolution, meaning the fight must go on to find new drugs to combat these increasingly resistant bacteria. Meticillin is now discontinued.

β-lactam ring

The β-lactam ring is an interesting structure in chemistry. Without getting too bogged down in physical organic chemistry, the bond angles in the β-lactam ring (i.e., a square) are 90 degrees, whereas the components (orbitals) that make up the chemical bonds in the carbonyl group (C=O) would prefer to be 120 degrees. This difference in assumed and desired bond angles produces strain on the ring at the carbonyl group. As a result of this strain, the ring is predisposed to pop open given the chance, and this is even more likely in the presence of gastric acid and various cellular molecules that would like to attack it.

Unfortunately, the β-lactam ring is essential for antibacterial activity and so not much can be done to alter this apparent weakness in structure. One of the main problems of the β-lactam ring’s susceptibility to attack comes not just from cellular molecules, such as water and the amino acids in proteins, but from another part of the drug itself (i.e., the drug’s own functional groups can participate in the undesired ring-opening of the β-lactam ring, rendering the drug inactive). In knowing this, the answer to the problem reveals itself — why not alter those groups that cause a problem and, in the process, generate a more acid-resistant analogue?

Fortunately, the group in question lies around the part of the molecule labelled “R” (Figure 1) and thus efforts were undertaken to change it in the hope of maintaining antibacterial activity. Drugs (natural or synthetic) that exploit this feature and thus result in acid-resistant antibiotics include phenoxymethylpenicillin, ampicillin and amoxicillin, while flucloxacillin is a treatment that combines the features of acid resistance and β-lactamase resistance.

Interestingly, the only structural difference between amoxicillin and ampicillin is that amoxicillin has a hydroxyl (OH) group on the benzene ring of the side-chain “R”. Such a difference means that ampicillin must be taken before food or on an empty stomach, whereas with amoxicillin, timing in relation to food does not matter. This can be rationalised by the presence of the hydroxyl group in amoxicillin, which increases the drug’s bioavailability compared with ampicillin. As such, little or no effect on the absorption of amoxicillin is observed in the fasting versus non-fasting state, whereas ampicillin absorption is significantly reduced in the non-fasted state.

Making up children’s antibiotics with water is a routine part of a pharmacist’s day, and patients are counselled to keep the bottle in the refrigerator in order to slow down water-promoted opening of the strained β-lactam ring and thus maintain antibiotic activity for the duration of the treatment.

Penicillin sensitivity

As we have seen, the chemical susceptibility of the β-lactam ring is pivotal for it to inhibit vital bacterial enzymes and prevent cell wall cross-linking. Similarly, we have also seen how this chemically fragile motif is problematic with regard to acid sensitivity and β-lactamase resistance. Additionally, the β-lactam ring is also responsible (in part) for the allergic reaction to the penicillins. When the β-lactam ring is split open, it may link with specific cellular proteins, creating an antigen that can stimulate an immune response, resulting in hypersensitivity to the drug. This also accounts for the cross-sensitivity to cephalosporin antibiotics, which share the β-lactam ring structure.

Conclusions

Due to the number of constraints imposed on penicillin antibiotics in order for them to be active and the fact that each of these constraints has differing importance across the different bacterial species, it is not surprising that there are no hard-and-fast rules as to what makes a good broad-spectrum antibiotic. Nevertheless, broad-spectrum penicillins have been found through the synthesis of a large number of structural analogues — largely confined to changes in the “R” group — and current clinical use of such antibiotics is focused on ampicillin, amoxicillin and the antipseudomonal penicillins piperacillin and ticarcillin. With the ongoing resistance to antibiotics, it is inevitable that new and more effective drugs will need to be developed in order to combat resistance in the future, and it is almost guaranteed that chemists and pharmacists will play an important role in the development of these drugs.

BACTERIAL RESISTANCE

Some species of bacteria are resistant to the action of the penicillin antibiotics because they secrete a defence enzyme (β-lactamase), which is able to cleave open the β-lactam ring, thereby rendering the drug inactive.

Consequently, the bacterial strains that produce β-lactamases cannot be treated with penicillins, which are susceptible through this mechanism.