Search and rescue: tackling antibiotic resistance with chemistry
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Chapter 1

Introduction to Search and Rescue: tackling antibiotic resistance with chemistry

1. Antibiotics and the development of resistance

1.1 Brief history of antibiotics

Before the development and widespread use of antibiotics, bacterial infections were one of the leading causes of death.[1,2] With the discovery of penicillin, its subsequent isolation, and mass production, medical care improved dramatically.[3] Not only could bacterial infections be cured with ease, but medical procedures, such as invasive surgery, became safer to perform. At the same time, both quality of life and life expectancy have greatly improved.

The introduction of penicillin to the clinic in the 1940s kick-started the so-called golden age of antibiotic discovery in the decades that immediately followed. In fact, many of the antibiotic classes that are used in the clinic today were discovered between the 1940's and 1970's (**Figure 1**).[4,5] With the extensive arsenal of antibiotics developed during that time period, it was incorrectly assumed by many that bacterial infections were a threat of the past. However, the natural phenomenon of bacterial resistance to antibiotics means that the threat will never be completely eradicated.[6] Resistance to antibiotics is usually observed shortly after approval for use in a clinical setting.[7]

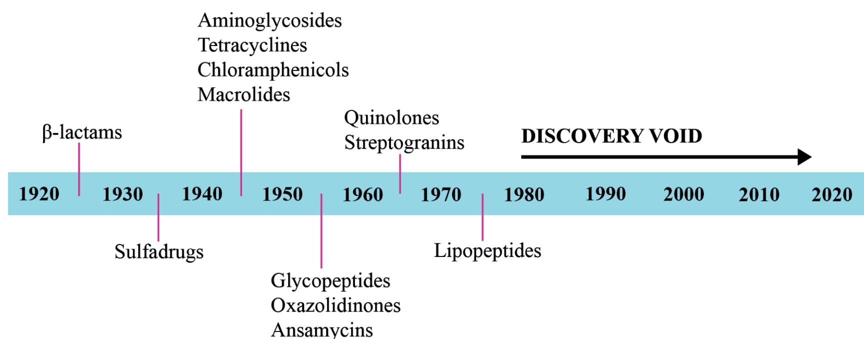

Figure 1. Timeline of antibiotic discovery over the decades. The discovery void refers to a gap in which no new antibiotic class has been found.

1.2 The rise of antibiotic resistance

Bacteria are under constant pressure from toxic compounds present in their environment, including foreign antibiotics. Emergence of resistance to such compounds can occur in several ways: a) by spontaneous mutation of gene(s) that code for the target(s) of antibiotics; b) by transfer of resistance genes from one organism to another and c) hostile environmental

conditions leading to selection pressure.[8] Mutations occur frequently in bacteria due to a short life cycle. Most mutations cause catastrophic changes in vital cell processes, leading to a non-viable cell. However, mutations can also occur in favourable positions such as genes which are involved in producing enzymes which antibiotics target. For example, a small change of just a single amino acid can greatly reduce an antibiotic's affinity for its target enzyme, leading to resistance to this antibiotic (**Figure 2A**). An example of this is one of the resistance mechanisms against the fluoroquinolone class of antibiotics, which can arise through a single mutation of the DNA sequence in the gene encoding for their enzyme target, DNA gyrase. Mutations which occur in the quinolone resistance-determining region result in an amino acid substitution in the enzyme, changing the protein structure and ultimately lowering the binding affinity of fluoroquinolone to the enzyme.[9]

Bacteria may also gain resistance by genetic exchange from other microorganisms. The most common method of transfer is via small circular fragments of DNA, known as plasmids, which are passed between bacteria by conjugation (**Figure 2B**).[10,11] This leads to the widespread distribution of resistance genes, an example being the distribution of plasmids that encode for the carbapenemase New Delhi metallo-ß-lactamase (NDM) enzymes which actively destroy most classes of ß-lactam antibiotics.[12,13] Lastly, selection pressure increases the spontaneous appearance of resistance. This provides an explanation for why there are more resistant bacteria in a hospital environment, where antibiotics are used every day, than in a less stressful environment.[8,14]

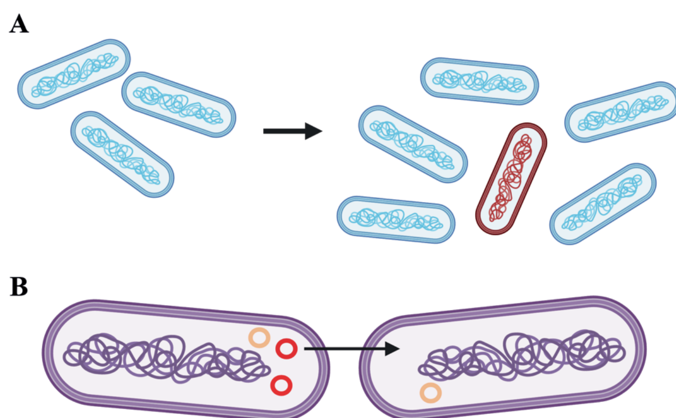

Figure 2. Common methods of resistance development. A) Cell division leading to random mutations inferring resistance. B) Horizontal gene transfer of resistance genes from one organism to another. Figure produced using BioRender.

There are several components of a bacteria cell that are essential for their survival in different environments. The bacterial cell envelope is one such feature and is made up of two components, the cell membrane and the cell wall. The cell membrane is made of a phospholipid bilayer which also contains many proteins that control the entry and exit of molecules often associated with the cell's metabolism.[15] The cell wall, made up of cross-linked peptidoglycan, is the primary stress bearing structure which also maintains the shape of the cell.[16] Most bacteria can be classified as either gram-positive or gram-negative, based on the structure of their cell envelope (**Figure 3**).[17] The cell envelope of gram-positive bacteria is comprised of a phospholipid bilayer cytoplasmic membrane, surrounded by a thick cell wall. This thick peptidoglycan layer is essential for the viability of gram-positive bacteria, and there are many antibiotics which exploit this by targeting the synthesis and the structural integrity of the cell wall.[18]

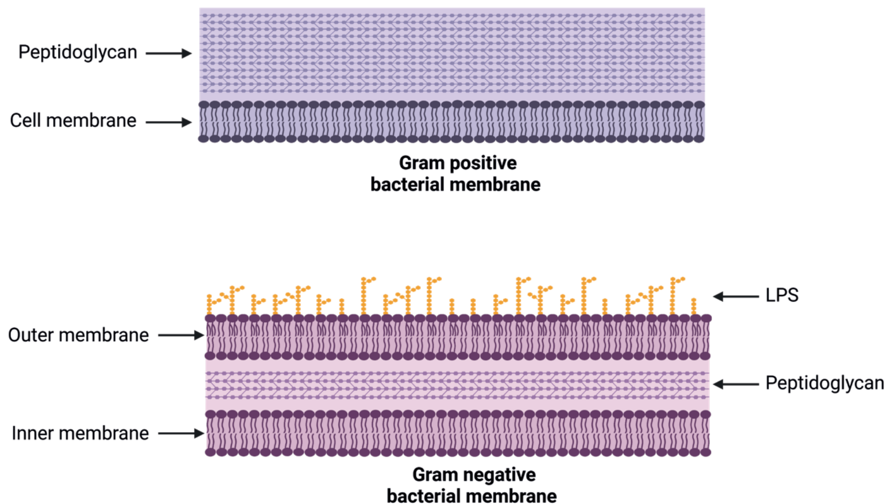

Figure 3. Cell envelope of gram-positive and gram-negative bacteria. Figure produced in BioRender.

Gram-negative bacteria contain an extra layer in their cell envelope: they consist of an outer and inner membrane sandwiching a much thinner cell wall. The outer membrane allows some small hydrophilic molecules to enter the cell envelope due to the presence of porin proteins.[19] However, it also acts as a stronger barrier to many larger hydrophobic molecules which may be toxic to the cell, such as antibiotics.[20] Many of the larger antibiotics which target intracellular pathways are ineffective against gram-negative bacteria for this reason. The outer

membrane also differs from the inner membrane as it is comprised of a lipopolysaccharide (LPS) layer as well as a phospholipid layer.[20,21] This outer layer is comprised of many proteins and structures which are essential to gram-negative bacteria. The resulting three-layered cell envelope is therefore much harder to penetrate compared with that found in gram-positive bacteria, providing a significant hurdle for antibiotic development.[19]

Along with the many ways that resistance can spread, there are many methods for the bacteria to become resistant to antibiotics. Several methods were mentioned previously which usually confer resistance to specific antibiotics, such as the modification of the enzyme target to reduce antibiotic affinity and antibiotic inactivation by bacterial enzymes.[11,22] However, there are some mechanisms which confer a more general resistance to multiple antibiotics. A particularly challenging method of resistance is the upregulation of efflux pumps, which act as an unspecific defence mechanism which span the entire cell envelope and actively work to excrete toxic substances including antibiotics.[23,24] If a bacterium is under attack by antibiotics, they will increase the rate at which the toxic substances are removed.[10,22] Bacteria can also modify the permeability of their cell membrane by modification of the porin proteins. These proteins allow entry of small hydrophilic molecules and are the main point of entry for some antibiotics (e.g. ß-lactams).[25] Bacteria can down-regulate the abundance of these porins or even replace them with other proteins which do not recognise the antibiotics as substrates.[11,22] Whether the mechanism of resistance is for a specific antibiotic or more general, all add to the major problem of antibiotic resistance.[26]

Given that resistance to antibiotics can develop via more than one pathway, bacteria can sometimes become insensitive to multiple antimicrobial agents, resulting in the difficult to treat multidrug resistant (MDR) bacteria.[26] The infections resulting from such organisms are particularly rife in hospitals, making generic surgical procedures much riskier in case of contamination. Over time, it has become increasingly difficult to treat infections with available antibiotics, and consequently deaths associated with antimicrobial resistance (AMR) have risen in the recent years. It has been estimated that in 2019 there were 4.95 million fatalities related to AMR.[27] If antibiotic resistance continues to spread at current rates and without the development of new antibiotics to counter the problem, the number of deaths related to AMR is projected to reach 10 million per year by 2050.[28] Gram-negative bacterial infections are a particular concern for public health due to their difficult-to-treat nature.[29] This is highlighted by the features of the ESKAPE pathogens, an acronym of six highly resistant pathogens of

which four are of the gram-negative type (*Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp*.).[30,31] It is therefore particularly imperative to develop strategies to overcome the resistance mechanism observed in these dangerous pathogens.

2. Strategies for overcoming resistance

There is a clear and desperate need for the rapid development of new antibiotics. However, compared to decades past, this is becoming increasingly more challenging. With the so-called 'low-hanging fruit' already discovered, today there are fewer options for antibiotic discovery. There are, however, strategies to overcome the resistance mechanisms described above. To this end, the work in this thesis focuses on developing strategies to combat resistance in bacteria, particularly the hard-to-treat gram-negative pathogens. One of the strategies used is to recover the activity of a class of potent clinical antibiotics, ß-lactams, which are targeted by resistance enzymes. Another route to combat resistance is the development of new antibiotics which target vital, unexploited pathways in bacteria. The background to these strategies will be discussed in the following sections.

2.1 Rescuing antibiotics

The ß-lactam class of antibiotics is a well-established and important clinical asset in the treatment of bacterial infections. They target penicillin binding proteins (PBPs), which are integral for the synthesis of the bacterial cell wall. The cell wall is built up of layers of peptidoglycan which are cross-linked together to produce a rigid structure which provides structural integrity to the bacterial cell.[16,32] PBPs process the final stages of peptidoglycan synthesis and are also involved with the crosslinking of the peptidoglycan layers.[33] Given their key roles in bacterial cell wall biosynthesis, inhibiting PBPs disrupts the integrity of the cell wall and leads to cell death.

The effectiveness of inhibiting these enzymes is clearly illustrated by the size of the ß-lactam antibiotic class. There are several subdivisions of antibiotics containing ß-lactam motifs, including the penicillins, cephalosporins, carbapenems, and monobactams.[34] Common to all is the highly strained four-membered lactam ring which mimics the D-Ala-D-Ala moiety found in the structure of many of the bacterial cell wall precursors, most notably lipid II (**Figure 4**).[35] Penicillins were the first antimicrobial agents to be discovered and continue to be used

frequently to treat minor bacterial infections, with well-known antibiotics such as penicillin and amoxicillin belonging to this subdivision.[3] The common structure of penicillins is a ß-lactam ring fused to a five membered ring containing a sulphur atom. In contrast, cephalosporin antibiotics contain a 6-membered ring containing sulphur fused to the ß-lactam ring. Cephalosporins were originally identified from *Cephalosporium acremonium*, a fungus isolated from sewers in Italy.[3] Carbapenem antibiotics are a very important class of antibiotic as they are frequently used as a last resort.[36,37] These antibiotics are the last line of defence we have against bacterial infections and are only used when all other options have failed to alleviate the infection. The simplest of all ß-lactam antibiotics is the monobactam subclass, which contains only the ß-lactam ring structure and only one area for diversification.[38] Due to the rise of resistance due to the spread of bacteria harbouring ß-lactamases enzymes, these important ß-lactam containing antibiotics, including those of last resort, are now under threat.[39]

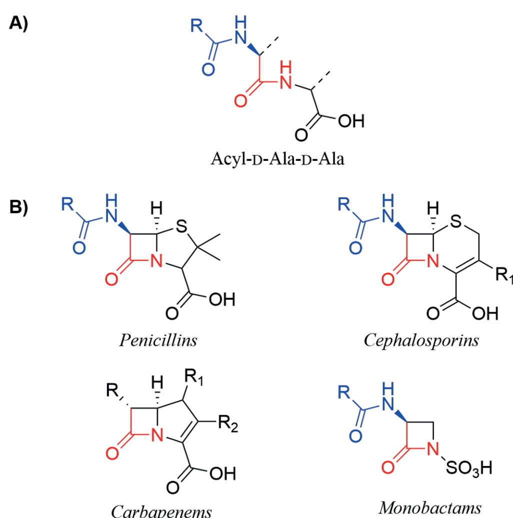

Figure 4. A) The native binding motif targeted by PBPs. B) The chemical scaffolds of the ß-lactam antibiotics which inhibit PBPs.

ß-Lactamase are resistance enzymes that catalyse the hydrolysis of the ß-lactam ring, the signature moiety of this class of antibiotic, leading to the loss of antibacterial activity. There are two main families of ß-lactam inactivating enzymes, serine-ß-lactamases (SBLs) and metallo-ß-lactamases (MBLs). As the name indicates, the activity of SBLs is dependent on an active site serine residue which contains a side chain hydroxyl group. This hydroxyl group acts as a reactive nucleophile in the context of the SBL active site and attacks the carbonyl group of the ß-lactam moiety.[40] This then undergoes a rearrangement which ultimately leads to the

opening of the four-membered ring and to the covalent attachment of the antibiotic to the serine side chain (**Figure 5A**).[41] The antibiotic/enzyme complex is subsequently hydrolysed by a water molecule to regenerate the enzyme which can then go on to further degrade other ß-lactams. The MBLs, on the other hand, do not contain an active site serine residue, but instead employ active site zinc ions to activate a water molecule that in turn attacks the carbonyl of the ß-lactam ring. [42,43] The amide bond breaks to give a carboxylic acid and secondary amine, and the inactivated antibiotic is released from the active site (**Figure 5B**).[41]

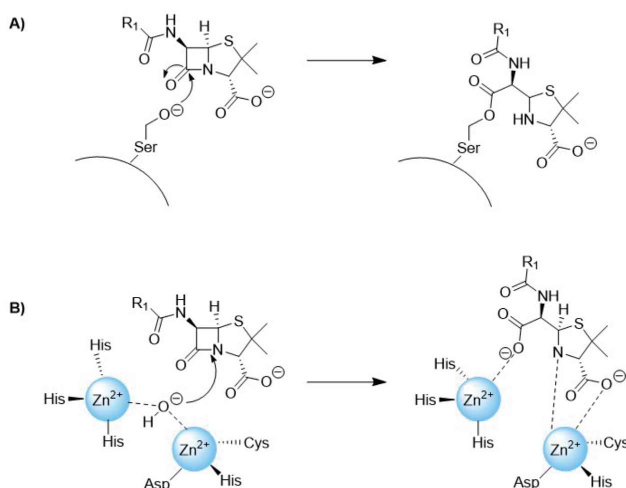

Figure 5. Mechanism of ß-lactam deactivation by A) serine-ß-lactamase and B) metallo-ß-lactamase enzymes.

Inhibitors of SBLs have been developed and a number are now used clinically in combination with ß-lactam antibiotics (**Figure 6A**). The first generation of ß-lactam inhibitors, clavulanic acid, sulbactam, and tazobactam, mimic the structure of the ß-lactam antibiotics and were formulated in combination with some of the penicillin class of antibiotics.[44] However, these combinations are not effective enough to overcome the carbapenem resistant bacteria. To address this need, vaborbactam, a compound characterised by a boronic acid functionality, has been brought to market and is used in combination with the carbapenem antibiotic meropenem.[45] Also of use against some carbapenem resistant strains are the diazabicyclooctane (DBO) class of SBL inhibitors including avibactam and the recently approved relebactam. Clinically, avibactam is combined with ceftazidime,[46] a third-generation cephalosporin, while relebactam is combined with imipenem/cilastatin.[47]

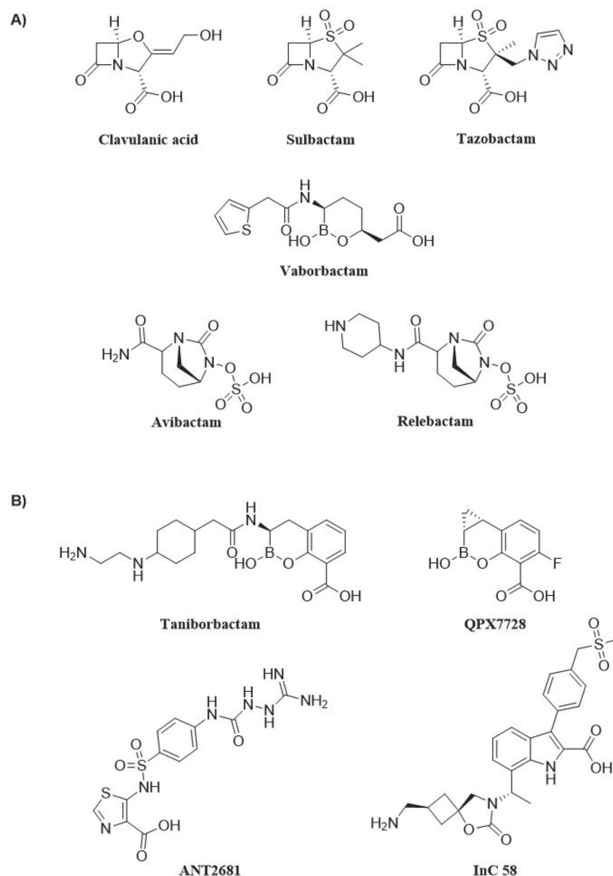

Figure 6. Chemical structures of A) clinically approved serine-ß-lactamase inhibitors and B) promising metallo-ß-lactamase inhibitors.

Unfortunately, inhibitors of SBLs are ineffective against MBLs. As part of their working mechanism, SBL inhibitors form a covalent bond with the active site serine, which is instrumental for the activity of the enzyme. However, this mechanism of action is not exploitable in the case of MBLs as an active site amino acid is not directly involved in the complexation of the ß-lactam antibiotics. Instead, a water molecule is activated by the zinc ions present in the active site which is readily replaced upon each turn of the catalytic cycle. Covalent inhibition is therefore not possible for inactivating MBLs. Impairing the enzyme's capacity to bind zinc in the active site is the most common route to MBL inhibition, with strong zinc chelating compounds capable of stripping the divalent metal from the active site offering

one means of inactivating the enzyme.[42] Recently, a more sophisticated method of MBL inhibition has been developed, based on compounds capable of coordinating the zinc cations within the active site, blocking substrate access in the process. Two such agents being developed are taniborbactam and QPX7728, which are currently undergoing clinical trials.[48,49] The structures of both these molecules are related to vaborbactam as they contain the same boronic acid functionality. Of note, these new molecules have the ability to inhibit both SBLs and MBLs, making them promising tools in the fight against ß-lactamase resistance. Other notable compounds which display an impressive ß-lactam recovery profile, and which are the result of extensive medicinal chemistry campaigns, are ANT2681 and InC 58.[50,51] The four compounds highlighted above represent the current most promising inhibitors in development with the potential to counteract the immense pressure that MBLs exert on the antibiotic resistance crisis (**Figure 6B**).

2.2 Identifying new targets

The ideal scenario for addressing antibiotic resistance would be the development of antibiotics against new bacterial targets which also have a low propensity to evade antibiotic pressure. These approaches should therefore rely on targeting unexploited bacterial mechanisms and key biological pathways for which resistance has not yet developed and for which it is unlikely to arise. Two such targets, essential for Gram-negative bacteria and for which there are no clinically used inhibitors, are the ß-barrel assembly machinery (BAM) complex and lipoprotein signal peptidase II (LspA). These enzymes are found in the outer- and inner-membrane respectively and present promising targets for antibiotic discovery and development (**Figure 7**).

2.2.1 The BAM complex

One of the greatest challenges associated with antibiotic resistance are efflux pumps in the bacterial membrane that actively remove toxic compounds from the cell.[23] For this reason, it would be beneficial to develop antibiotics which are impervious to efflux pumps. Designing such a compound is a challenging task, as the recognition process is complex and governed by various physiochemical properties such as hydrophobicity, aromaticity and ionisation state in the cell environment.[23] As a result, efflux pumps are fairly promiscuous and are able to recognise a wide range of substrates for removal. One approach that can both avoid the issues with efflux and the lack of permeability of the bacterial cell wall, is to develop antimicrobials that target essential bacterial structures present on the outside surface of the outer membrane.

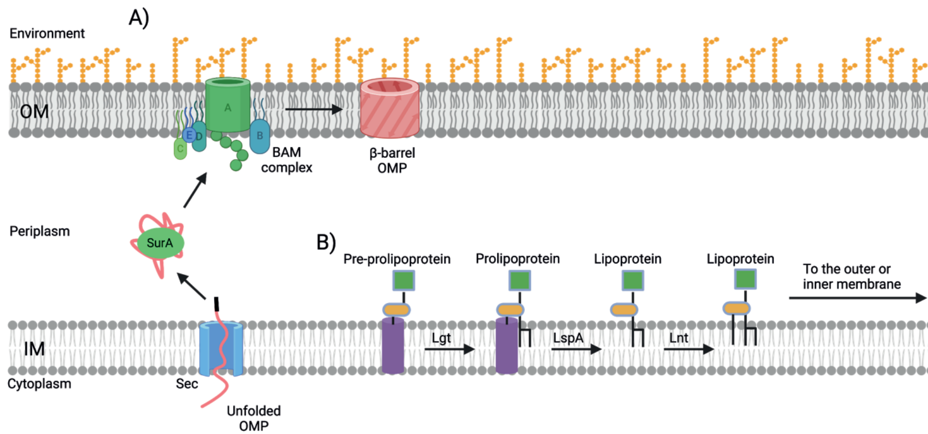

Figure 7. New targets for the development of antibiotics. **A)** The folding pathway for ß-barrel outer membrane proteins. The nascent protein is produced in the cytoplasm before being transported via various chaperon proteins to the BAM complex. The BAM complex is essential for processing the ß-barrel OMPs into their correct secondary structure. **B)** The lipoprotein processing pathway involves many inner membrane-bound proteins. The pre-prolipoproteins are acylated by Lgt before the membrane-anchoring signal peptide is cleaved by LspA. The lipoprotein can then be acylated by Lnt before being transported to its destination.

The gram-negative cellular envelope is rich in ß-barrel outer membrane proteins (OMPs) which are essential for the normal function of the cell. Since there is only one highly conserved pathway for the trafficking and folding of these proteins, enzymes involved in these key processes hold promise as target for novel antimicrobial agents. The formation of OMPs begins with the production of an unfolded protein in the cytoplasm which then travels through the inner membrane and periplasm with the assistance of chaperone proteins.[52–54] The folding and release of the protein as a mature ß-barrel OMP is carried out by the BAM complex.[55–57] This complex comprises five protein domains: BamA, itself a ß-barrel transmembrane domain, and four intracellular lipoprotein subunits (BamB-E) (**Figure 8**). BamA is connected to the other protein subunits by five polypeptide transport-associated (POTRA) domains. Despite the presence of many elements, only BamA and BamD are essential for the function of the complex.[58,59] The mechanism by which the BAM complex folds ß-barrel OMPs is still a matter of study, however it is understood that BamA plays a key role. BamA can exist in two conformations depending on the arrangement of a side loop often referred to as the lateral gate,

which can either be open or closed.[57] For the protein to be functional and able to engage in its role in the OMP trafficking, the lateral gate must be free to move between the two positions.[60]

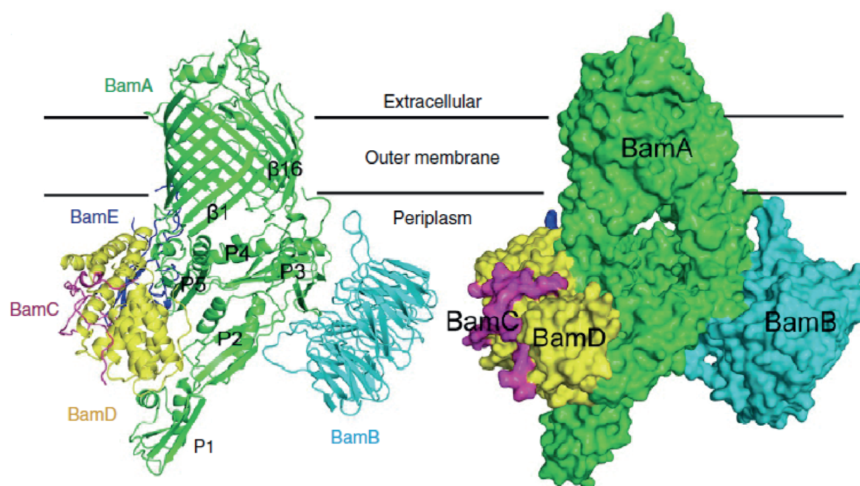

Figure 8. Crystal structure of the BAM complex, reported by Han et al.[61]

In recent years the BAM complex has attracted much attention as a novel target for antibiotic discovery, with a diverse range of inhibitors reported in the literature (**Figure 9**). In 2019, the natural product darobactin, a bicyclic heptapeptide, was reported to be BAM complex inhibitor. Discovered from bacteria found in the guts of nematodes, darobactin targets the complex by binding to the BamA ß-barrel.[62] Upon interaction with BamA, darobactin locks the ß-barrel in the "gate-closed" position which hinders the intake and the release of OMPs.[63,64] Blocking this essential protein-trafficking mechanism results in a reduced membrane integrity and eventually in cell death. In addition to darobactin, synthetic small molecule BAM inhibitors have also been described. Researchers at Merck recently reported MRL-494, discovered via a compound library screen, to be an inhibitor of BamA.[65] Three other structurally distinct small molecule inhibitors of the BAM complex were also reported by Steenhuis et al. by the use of live-cell fluorescence-based screen assays, which provide real time reporting on the activation of the σ^e and the Rcs stress response pathways, both of which are triggered by compounds that inhibit BAM complex.[66,67] Larger bicyclic peptides have also been shown to inhibit the BAM complex. Most notable in this regard is the recently reported chimera containing two macrocyclic peptides based on the known antibacterial agents murepavadin and polymyxin

B.[68,69] Interestingly, while the bicyclic construct targets BamA, neither of the component antibiotics is known to inhibit the BAM complex individually.

The essential nature of the BAM complex, along with its accessibility from the outside environment, makes the ß-barrel production pathway an interesting target for the development of new antibiotics.

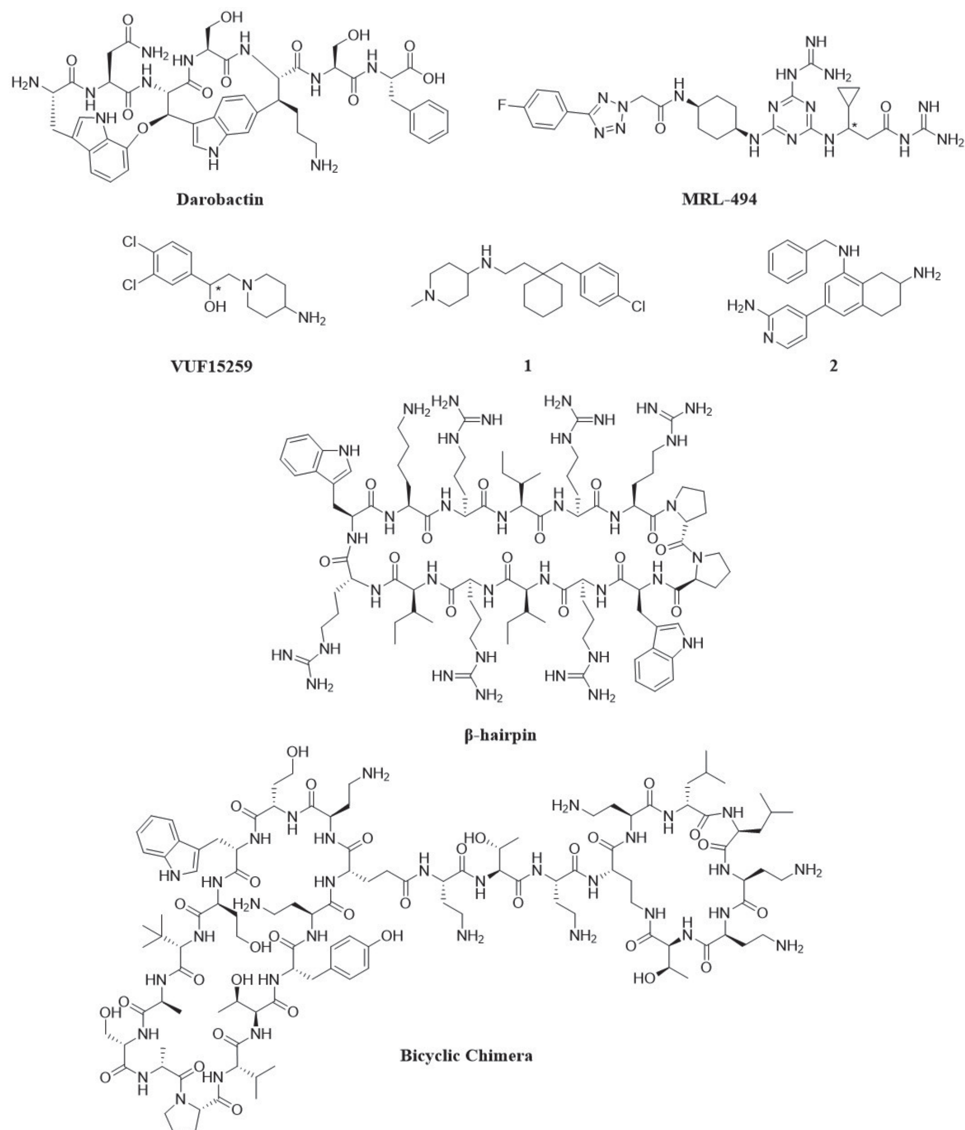

Figure 9. Chemical structures of reported inhibitors of the BAM complex.

2.2.2 LspA

Section 2.1 highlights the fact that many of the most heavily used antibiotics target a limited assortment of enzyme pathways and targets, in most cases associated with the bacterial cell wall and protein synthesis. This favours the rise of various mechanisms of resistance which reduce the activity of whole classes of antibiotics. To counteract this trend, it would be desirable to develop antibiotics against pathways which are yet unexploited and at the same time which are less likely to lead to resistance due to their highly conserved nature. An example of one such pathway is the bacterial route to producing lipoproteins. Lipoproteins are necessary for the survival of gram-negative bacteria, with vital roles such as cell signalling and facilitate nutrient uptake.[70] As illustrated above in **Figure 7B**, the processing of lipoproteins occurs via enzymes in the inner membrane and starts with preprolipoprotein precursors. The enzyme Lgt then acylates a specific cysteine residue located next to a transient signal peptide with a diacyl moiety (DAG), to give a prolipoprotein.[71,72] Following this, LspA cleaves the signal peptide to generate the lipoprotein.[73] This is an essential step in the pathway as the signal peptide acts as an anchor which binds the lipoprotein to the inner membrane. Following cleavage of the signal peptide by LspA, the resulting lipoproteins can then be further acylated by Lnt before they are transported either across the inner membrane or towards the outer membrane by the Lol sorting pathway. [70,74]

Given its key role in bacterial lipoprotein processing, LspA presents a promising target for antibiotic development. LspA is an aspartyl protease, meaning that its ability to catalyse signal peptide cleavage is facilitated by the two aspartic acid residues present in its active site. These aspartic acid side chains activate a water molecule, which subsequently performs a nucleophilic attack on the carbonyl of the target amide bond, forming a tetrahedral intermediate.[75] This intermediate then rearranges, resulting in a terminal carboxylic acid on the signal peptide and a primary amine on the lipoprotein (**Figure 10**).[76]

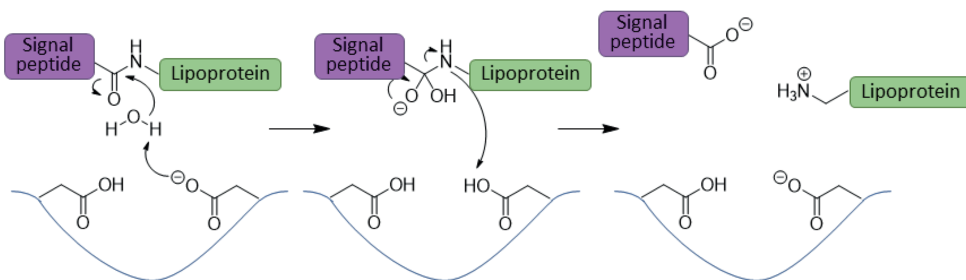

Figure 10. LspA mechanism of substrate cleavage. The aspartic acid residues activate a water molecule which attacks the carbonyl of the target amide bond. The tetrahedral intermediate rearranges, giving a C-terminal Signal peptide and N-terminal lipoprotein.

Further supporting the validity of LspA as an antibiotic target is the finding that two naturally occurring inhibitors have been discovered: the nonribosomal peptide globomycin, isolated from a species of *Streptomyces* and the hybrid nonribosomal peptide/polyketide myxovirescin which is produced by Myxobacteria.[77,78] Globomycin is a cyclic depsipeptide made up of five naturally occurring amino acids residues and one non-natural, making a 19-membered ring (**Figure 11A**).[79,80] Myxovirescin, however, has a rather unusual structure, containing a 28-membered ring with only one amide and one ester bond linkage. The rest of the compound has a lipophilic nature, consisting mostly of a carbon chain with a cluster of polar hydroxyl groups (**Figure 11B**).[78]

Recently, crystal structures of globomycin and myxovirescin complexed to LspA isolated from *Staphylococcus aureus* were reported by the group of Caffrey (**Figure 11C + D**).[81] These structures show that both globomycin and myxovirescin bind to the active site of LspA by interacting with the two catalytic aspartic acid residues via hydroxyl groups present in both antibiotics. Notably, while globomycin and myxovirescin bind the LspA catalytic dyad with a similar pharmacophore, the bulk of each molecule coordinates in different orientations within the binding pocket suggesting that there is ample exploitable space within the active site for the rational design of LspA inhibitors.

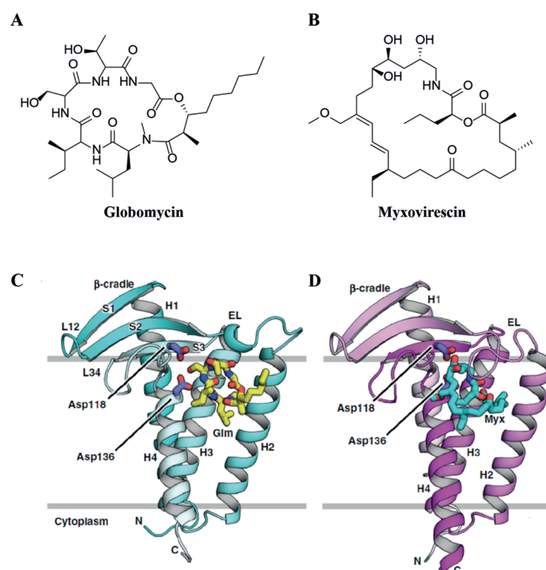

Figure 11. A + B) Chemical structures of known LspA inhibitors, globomycin and myxovirescin. **C)** Crystal structure of LspA and globomycin. **D)** Crystal structure of LspA and myxovirescin (images taken from Olatunji et al[81]).

To aid the search for LspA inhibitors, a fluorescence-based enzyme activity assay utilising Förster resonance energy transfer (FRET) has been developed independently by two research groups.[81,82] Both assays utilise a truncated version of the lipoprotein substrate with a fluorophore and quencher moiety at opposite ends of the compound's structure. While intact, the energy released from an excited fluorophore cannot be measured as it is accepted by the quencher moiety. Upon LspA mediated cleavage of the substrate and subsequent separation of fluorophore and quencher, the energy from the fluorophore can be measured and quantified.[83] In the event of LspA inhibition, the substrate will no longer be cleaved and a decrease in fluorescence will be observed.

With a highly conserved and essential nature, along with a well-functioning tool for inhibitory assays, LspA is a highly valuable target for the development of new antibiotics.

3. Outline of the thesis

This thesis contains research to address the problem of antibiotic resistance by a variety of approaches.

Chapter 2 describes an overview of the various compounds that act as MBL inhibitors. While a number of compounds have individually been reported as MBL inhibitors in different publications, they could not be reliably compared to each other. We therefore assembled a panel of MBL inhibitors from the literature and compared them head-to-head in a series of mechanistic investigations in order to provide a thorough assessment of their enzyme inhibition activity. This involved measuring their affinity to different divalent cations, determining the half-maximal inhibition constant (IC_{50}) for MBL enzymes, and comparing their ability to rescue meropenem's activity against carbapenem-resistant bacteria.

Chapter 3 focuses on the synthesis and evaluation of the reported BAM-complex inhibitor (MRL-494) and analogues thereof. Since the original publication did not disclose a synthetic route for this compound, an efficient and modular route to prepare MRL-494 was devised. This also allowed for the convenient preparation of MRL-494 derivatives aimed at probing the role of each of the two guanidine moieties present in MRL-494 in binding to the BAM complex. The resulting panel of compounds was assessed through minimum inhibitory concentration (MIC) and synergy assays. All compounds were also tested in a cell-based membrane stress assay, further confirming their mode of action via inhibition of the BAM complex.

Chapter 4 documents the implementation of a FRET assay to monitor inhibition of the bacterial aspartyl protease LspA, in addition to the synthesis of peptide substrates for the assay. The assay setup initially used was based on that reported by Olatunji et al.[81] Building from this, we synthesised a new substrate compatible with the assay, utilising a different FRET pair with distinctive excitation and emission wavelengths. This novel substrate was validated for the FRET assay and both substrates were employed to determine the IC_{50} of pepstatin A, a well-known pan-protease inhibitor, against LspA.

Chapter 5 describes the use of the LspA FRET assay described in Chapter 4 to evaluate a panel of peptidomimetic LspA inhibitors. The design of these inhibitors was based on LspA's natural substrate, using the same amino acid sequence but introducing simplified hydrophobic moieties

to mimic the acylated cysteine residue present in the native substrate. In addition, a number of different non-cleavable building blocks were incorporated in place of the glycine residue at the cleavage site in order to make these peptides impervious to LspA mediated hydrolysis. Each peptidomimetic was initially dosed at a single concentration to test the inhibition capabilities against LspA. The compounds which showed the highest percentage inhibition of LspA were further profiled in order to get an estimation of their IC_{50} value. These results will give an indication of which non-cleavable moieties perform better and will empower the search for more potent peptidomimetic inhibitors of LspA.

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