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Targeting Antibiotic Resistance

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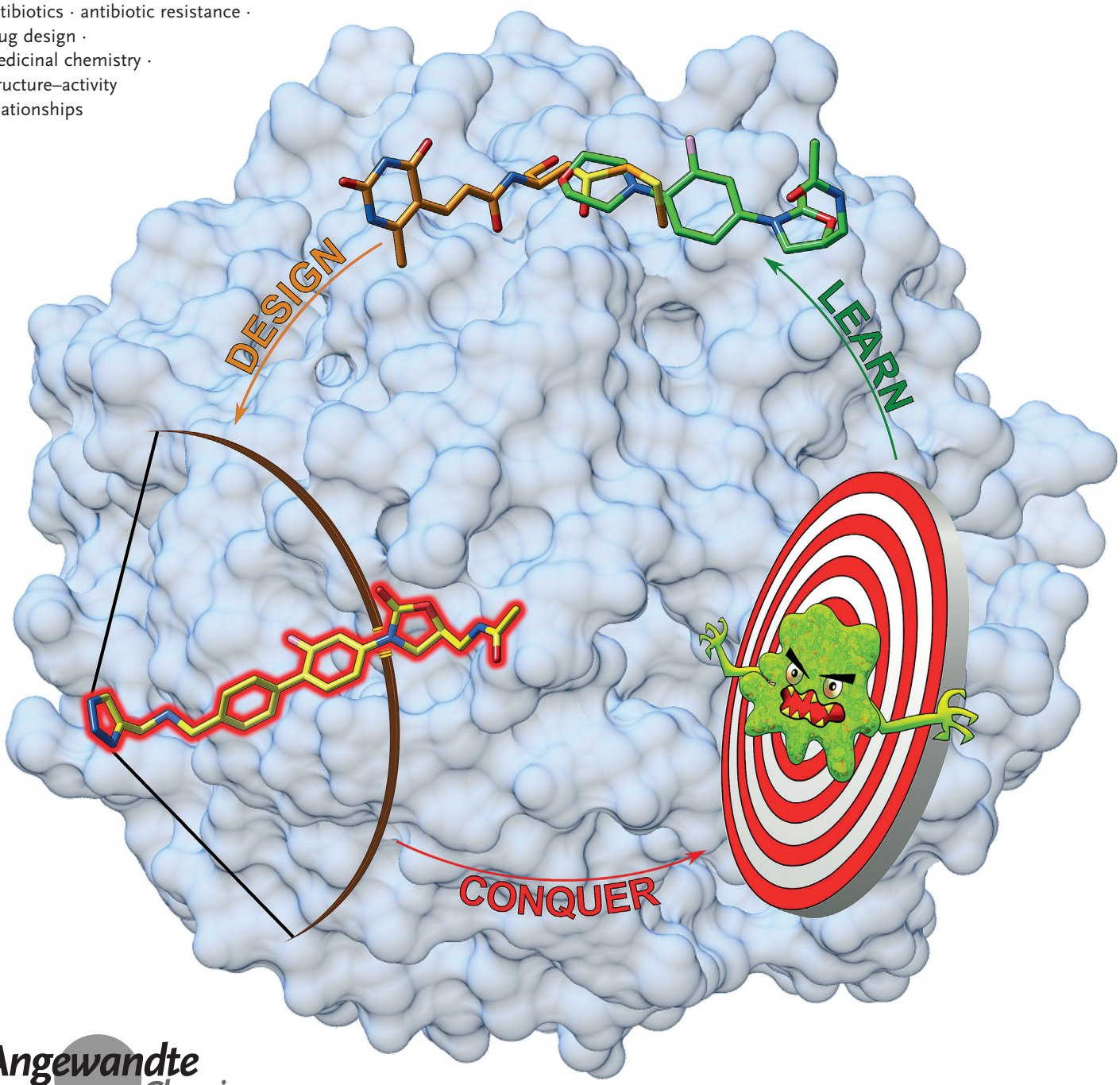

Finding strategies against the development of antibiotic resistance is a major global challenge for the life sciences community and for public health. The past decades have seen a dramatic worldwide increase in human-pathogenic bacteria that are resistant to one or multiple antibiotics. More and more infections caused by resistant microorganisms fail to respond to conventional treatment, and in some cases, even last-resort antibiotics have lost their power. In addition, industry pipelines for the development of novel antibiotics have run dry over the past decades. A recent world health day by the World Health Organization titled "Combat drug resistance: no action today means no cure tomorrow" triggered an increase in research activity, and several promising strategies have been developed to restore treatment options against infections by resistant bacterial pathogens.

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1. Introduction and Background

Since the pioneering work of Alexander Fleming, Paul Ehrlich, Gerhard Domagk, and others on antibiotics about 100 years ago, the benefits of these "miracle drugs" for the treatment of infectious diseases have been taken for granted in public health. Unfortunately, a dramatic change has taken place in recent years in terms of the efficacy of administered antibiotics. More and more bacteria have developed resistance against antibiotics, and these resistant microorganisms can withstand attack by antimicrobial drugs so that standard treatments become ineffective and infections persist, thereby increasing the risk of spreading to others.[1]

The evolution of resistant strains is a natural phenomenon that occurs through selection pressure on the microorganism population from the antibiotic. There are currently five main targets for antibiotics, and antibiotic resistance can essentially be acquired through four different pathways and expressed by four different mechanisms (Figure 1).

The use and misuse of antimicrobial drugs accelerates the emergence of drug-resistant strains. According to the World Health Organization (WHO), there were about 480000 new cases of multidrug-resistant tuberculosis (MDR-TB) in 2013 and extensively drug-resistant tuberculosis (XDR-TB) has been identified in 100 countries.[1] There are high proportions of antibiotic resistance in bacteria that cause common infections (e.g., urinary tract infections, pneumonia, bloodstream infections) in all regions of the world. A substantial percentage of hospital-acquired infections are caused by highly resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), or multidrug-resistant Gram-negative bacteria that are resistant to all β-lactam antibiotics.

Patients with infections caused by drug-resistant bacteria are generally at increased risk of worse clinical outcomes and death, and they consume more health-care resources than patients infected with the same bacteria that are not resistant.[1]

Owing to the discovery gap during the last decades for novel antibiotic chemotherapies in the pharmaceutical industry and to the occurrence of bacterial strains resistant to the current antibiotics, public health is running out of treatment options for dealing with infectious diseases. In order to respond to this emerging crisis, global organizations such as the WHO have urged the scientific community to search for new approaches to combat antibiotic resistance.

Antibiotic drug discovery is hampered by several intrinsic factors. For instance, the permeability barrier provided by the outer membrane of Gram-negative bacteria typically leads to more resistant strains compared to Gram-positive bacteria. Since the outer membrane hinders the antibiotics from gaining access to their targets inside the bacterial cell, the development of antibiotics to treat infections caused by Gram-negative bacteria remains a challenge. In this regard, cationic peptides can increase the permeability of the outer membrane and allow antibiotics to reach their target inside the bacterial cell.[2] Another important aspect of antibiotic drug discovery is the lack of novel antibacterial targets. In addition to the well-understood and heavily exploited antibacterial targets such as cell wall synthesis or protein synthesis, new targets or modes of action are desperately needed. Recently, targeting adenosine triphosphate (ATP) synthase has been a successful step forward for the treatment of drug-resistant strains of *Mycobacterium tuberculosis*.[3] More examples with novel modes of action, such as targeting antibiotic resistance at the genetic level, will be discussed in this Review.

A lot of the research for new antibiotics is still focused on developing improved versions of existing molecules. While some of these are simply "me-too" versions of drugs with only

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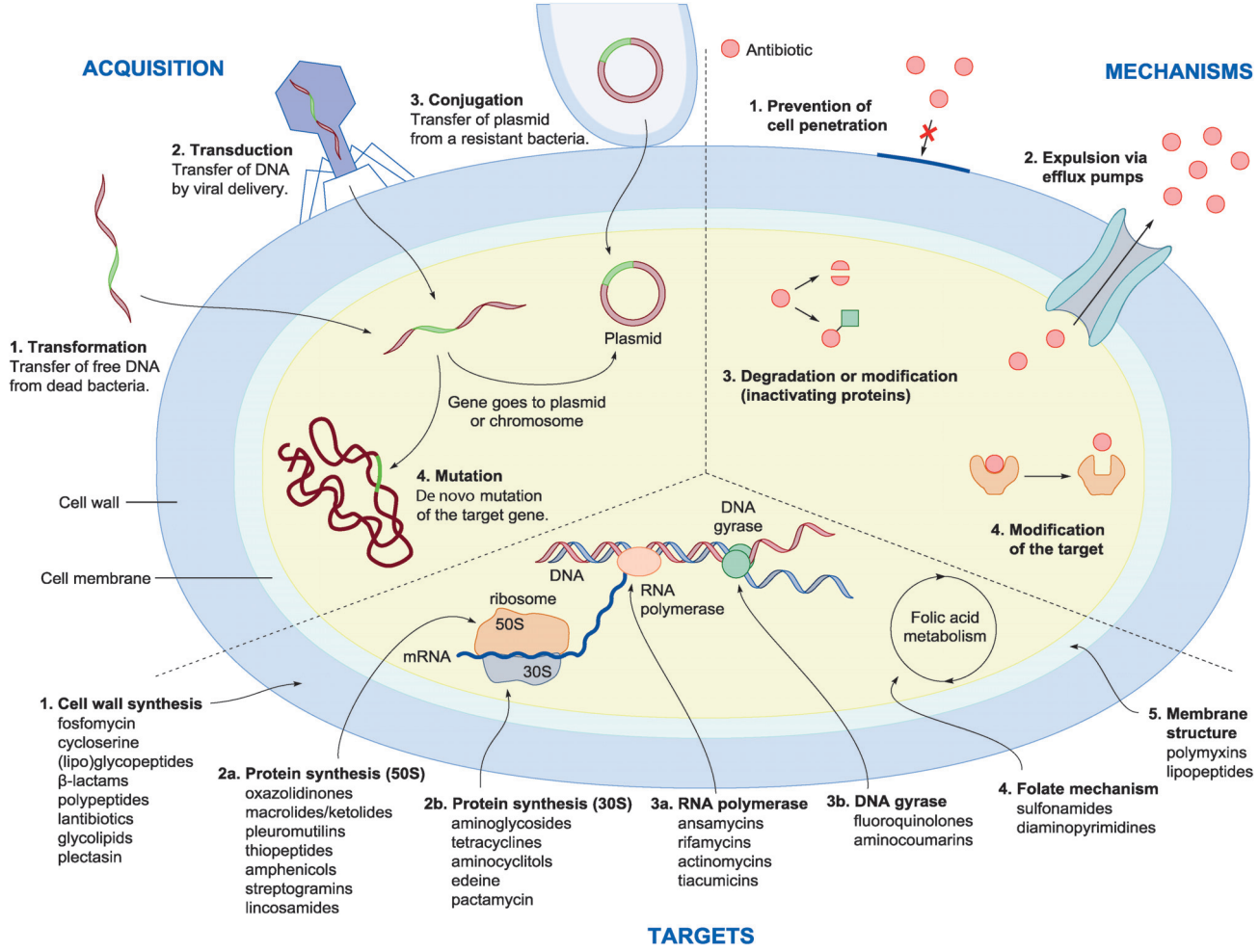
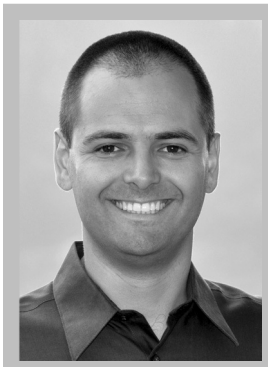


Figure 1. The four resistance acquisition pathways, the four main mechanisms of resistance, and the five main targets for antibiotics.

moderately better activity, some have been developed through logical and systematic changes that make them more efficient or less susceptible to resistance mechanisms. Screening for novel antibiotics from natural sources is another way to broaden our possibilities for treating infections, but this does not eliminate the intrinsic risk for the development of resistance to these novel antibiotics. A mechanistic and structural understanding of bacterial resistance offers much better opportunities for tackling this problem because this allows the origin of the problem to be addressed rather than simply generating additional resistance in the future. Combining mechanistic information on resistance with rational structure-based drug discovery approaches enables antibiotic resistance to be bypassed or even suppressed. This strategy has been applied lately with increasing success and will be the focus of this Review.

Structure-based design has developed into a mature technique in drug discovery. This has been fueled by



Mathieu Chellat obtained his M.S. in chemistry (CPE Lyon, France) in 2005. He then worked for Scynexis, Inc., NC, USA on the synthesis of antifungal agents. In 2006 he began his Ph.D. at The Ohio State University under the supervision of Prof. Stambuli, focusing on the synthesis of the marine macrolide leiodelide A. In 2011 he moved to the University of Geneva for postdoctoral work with Prof. Alexakis. Currently he is a research assistant for Prof. Riedl and works on novel compounds to overcome bacterial resistance.

Luka Ragužz received his BSc in chemistry in 2011 and his MSc in chemistry for the life sciences in 2013 from the Zurich University of Applied Sciences (ZHAW). For his master's thesis, he worked on the structure-based design and synthesis of novel protease inhibitors in the group of Prof. Riedl. After his studies, he continued in the group of Prof. Riedl as a research assistant to work on the development of novel strategies to combat antibiotic resistance.

a tremendous amount of crystallographic structural data for biological target macromolecules and backed up by genomics data from sequencing campaigns. Once the structure of a target or a co-crystal structure of a target together with a bound ligand is available, structure-based design can be applied both rationally and creatively in order to tailor the properties of a drug molecule. Fragment-based approaches are used for the identification of chemical starting points for lead optimization, virtual docking allows in silico screening, and de novo approaches generate new chemotypes for drug molecules.[4–8]

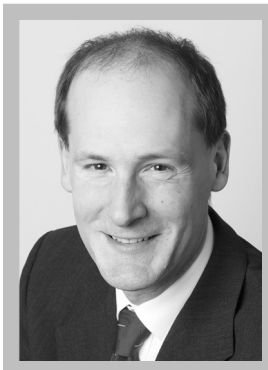
Several excellent and comprehensive review articles about antibiotics have appeared in this journal as well as others. Their focus has been, among other things, on the discovery and synthesis of antibiotics and on the evolution of antibiotic resistance.[9–13] In this Review, we highlight structure-based approaches that have successfully been applied to combat antibiotic resistance. This includes several target classes and different structure-based techniques in order to depict the broad applicability of this strategy. Our selection is predominantly based on examples that have already delivered clinical drug candidates and can serve as showcase projects for the antibiotic research community. In addition, we have also included the results of very recent collaborations in this field that have not yet delivered clinical candidates, but have delivered promising techniques and scientific concepts in order to stimulate the scientific discussion.

Owing to the high amount of structural data in this Review, the preparation of this manuscript required heavy use of graphics and modeling software. For the depiction of co-crystal structures and structural models, we used Chimera 1.10.1[14] along with Inkscape[15] for labeling. Molecular docking was done with AutoDock Vina 1.1.2.[16]

2. Protein Synthesis Inhibitors

The bacterial protein synthesis machinery is a major target for antibiotics and it has been used for efficient structure-based interventions against antibiotic resistance.[17,18] Protein synthesis, which is conducted by ribosomes, converts mRNA into the corresponding polypeptide chain.[19] This process can be divided into four steps: initiation, elongation, termination, and recycling. The first two are depicted in Figure 2 and will be described here briefly because they provide several targets for antibiotic intervention.

Bacteria have 70S ribosomes that are made up of two subunits.[19–22] The large 50S subunit, which includes the 23S and 5S rRNAs, binds aminoacyl tRNA (aa-tRNA), catalyzes peptidyl transfer, and controls the elongation process, while the smaller 30S subunit, which includes the 16S rRNA, binds mRNA and initiates protein synthesis. The initiation step involves assembling these two subunits around the mRNA and the initiator fMet-tRNA. This process is catalyzed by three prokaryotic initiation factors: IF1, IF2 and IF3. The resulting 70S initiation complex has three main tRNA binding sites, the A, P, and E sites. The A site is the site at which the charged aminoacyl-tRNA matching the mRNA codon enters the ribosome. The P site carries the peptidyl-tRNA, the tRNA carrying the growing peptide chain. The E site harbors the deacylated or uncharged tRNA before it exits the ribosome. The elongation step is the cycle that adds amino acids to the growing peptide chain in a stepwise manner and can be considered the heart of protein synthesis. In the first step, a ternary complex composed of aa-tRNA, the elongation factor Tu, and guanosine triphosphate (aa-tRNA•EF-Tu•GTP) binds at the A site. After successful decoding, the complex is hydrolyzed, thereby resulting in the departure of EF-Tu•GDP (GDP: guanosine diphosphate) and an inorganic phosphate (Pi), which allows the aa-tRNA to enter the A site and bind. Peptide bond formation then occurs through transfer of the entire peptide chain from the peptidyl-tRNA in the P site to the aa-tRNA in the A site. This pre-translocation ribosomal state (PRE state) is often referred to as a hybrid state since the tRNAs are moving back and forth between the A/A, P/P, A/P and P/E sites. In the next step, elongation factor G (EF-G) catalyzes translocation of the $tRNA_2$•mRNA complex by a distance of one codon (POST state). This results in the deacylated tRNA being moved to the E site, the peptidyl-tRNA being moved to the P site, and the A site becoming free to bind the next aa-tRNA. Until a stop codon enters the A site, this cycle continues, gradually building the full peptide chain. As seen in Figure 2, protein synthesis is targeted by a number of different classes of antibiotics at essentially every step of the process. While some classes of antibiotics such as macrolides, oxazolidinones, and pleuromutilins bind the large 50S subunit, others such as aminoglycosides and tetracyclines interfere with the smaller 30S subunit.



Rainer Riedl studied chemistry at the University of Heidelberg and received his Ph.D. in organic chemistry from the University of Cologne with Prof. Berkessel in 1998, before pursuing postdoctoral studies in chemical biology with Prof. Schultz as a Humboldt fellow at UC Berkeley and The Scripps Research Institute, La Jolla, CA, USA. He then joined Eli Lilly for drug discovery projects on cancer, inflammatory diseases, and diabetes. Since 2009, he has headed the Center for Organic and Medicinal Chemistry at ZHAW, focusing on structure-based treatment options for unmet medical needs with an emphasis on infectious diseases.

2.1. Oxazolidinones

Oxazolidinones are the only new class of synthetic antibiotics to be discovered and introduced into the clinic over the past 50 years.[23] They were first discovered in 1978 (E. I. duPont de Nemours & Co.)[24] but later abandoned owing to serious toxicity issues.[25] Research continued in this area (initiated by Upjohn, later Pharmacia, now Pfizer Inc.), and by 1996, two nontoxic oxazolidinones, linezolid and eperezolid, had been developed (**1** and **2**; Figure 3).[26]

These two molecules were the results of a wide structure–activity relationship (SAR) study that revealed the required

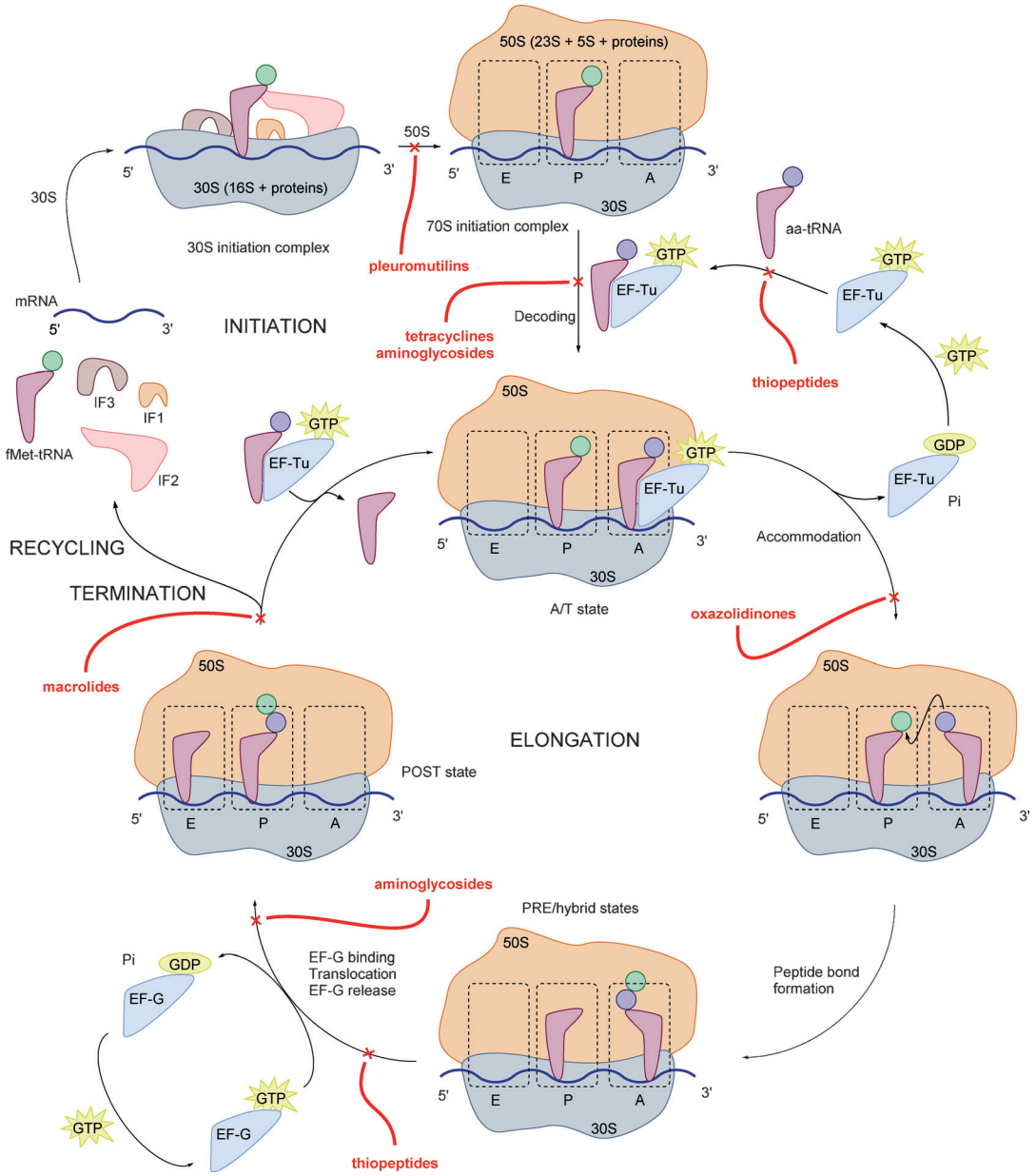


Figure 2. The mechanism of bacterial translation. Antibacterial targets highlighted.

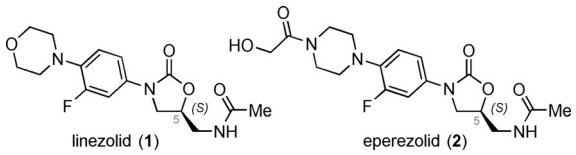


Figure 3. Structures of the first two oxazolidinones synthesized by Upjohn.

substitution on the central oxazolidinone core.[27] Essential factors for antibacterial activity were the *N*-aryl substituent, the 5*S* configuration, and the C5 acylaminomethyl group. The *meta*-fluoro substitution of the phenyl ring was not essential, but usually helped to increase activity, and the *para*-substitution could be varied to expand the antibacterial spectrum.

While oxazolidinones are protein synthesis inhibitors that bind to the ribosome, it has taken a number of years to identify the binding site and most likely mode of action.[28] In 2008, two reported X-ray co-crystal structures of linezolid bound to 50S ribosomal subunits confirmed the previously established site of action and suggested a mode of action (Figure 4).[29,30]

Linezolid binds to the A-site pocket of the 50S subunit at the peptidyl transferase center (PTC) in actively translating bacterial ribosomes and interferes with binding of the charged aminoacyl tRNA. Specifically, linezolid binds to a pocket formed by eight RNA residues, one of which, U2585Ec, is stabilized in a distinct conformation. By stabilizing U2585Ec in a nonproductive conformation, linezolid affects the binding and/or positioning of the initiator-tRNA and prevents the

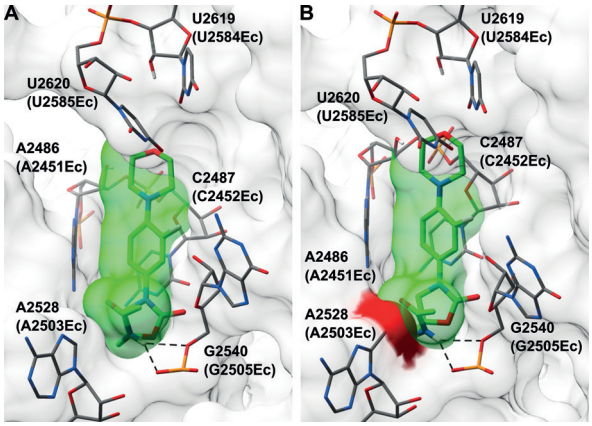


Figure 4. A) Co-crystal structure of linezolid (**1**; C green, F purple) in the 50S ribosomal subunit from *Haloarcula marismortui* (PDB ID: 3CPW). B) A model of linezolid (**1**) in the 50S ribosomal subunit from *H. marismortui* that has been methylated by Cfr at A2503Ec. The surface clash is highlighted in red. *Escherichia coli* numbering in parentheses. Model was generated with Chimera 1.10.1 according to K. J. Shaw et al.[14,35]

binding of tRNA at the A site, thereby halting the translation sequence (Figure 4 A).[29]

Resistance to oxazolidinones is still relatively rare. So far, three classes of oxazolidinone resistance mechanisms have been characterized.[31] The first involves mutations in the 23S rRNA central loop of domain V, the peptidyl transferase center. While some of the mutated residues interact directly with the oxazolidinone, many do not but are instead used to stabilize the region surrounding the oxazolidinone.[28] Mutations in these residues lead to small conformational changes of the linezolid binding pocket, which adversely affects drug binding. The second mechanism, which is less common, involves mutations in the genes *rplC* and *rplD*, which encode ribosomal proteins L3 and L4, respectively.[31]

Beyond these chromosomally encoded point mutations, the last mechanism involves acquisition of the ribosomal methyltransferase gene *cfr* (chloramphenicol-florfenicol resistance). This resistance is more worrisome than the mutation-based mechanism since it is horizontally transferable and carries a low fitness cost.[32,33] Mechanistically, the methyltransferase Cfr, through C8 methylation of the key residue A2503Ec in the 23S rRNA, greatly reduces susceptibility to a wide range of ribosome-targeting antibiotics, including amphenicols, lincosamides, pleuromutilins, streptogramin A, 16-membered macrolides, and linezolid.[34] As seen in Figure 4 B, the addition of a methyl group on A2503Ec leads to a steric clash with the acetamide group of linezolid, thereby causing a two- to eight-fold increase in the minimum inhibitory concentration (MIC).[36] Since the discovery of linezolid, over 30 companies have advanced more than a dozen candidates in clinical development. Unfortunately most of these have failed owing to issues related to pharmacokinetic (PK) properties, safety profile, solubility, or lack of improvement of antimicrobial activity over linezolid. Therefore the two main challenges for successful second-generation oxazolidinones are minimizing the myelosuppression safety signal and achieving adequate activity against linezolid-resistant strains of bacteria.[31] Two second-generation oxazolidinones that attempt to solve these problems in a rational way will be presented herein.

2.1.1. *Tedizolid*

Tedizolid phosphate (**3**; previously torezolid phosphate, TR-701, DA-7218) is the inactive prodrug of tedizolid (**4**; previously torezolid, TR-700, DA-7157, which was discovered by Dong-A Pharmaceuticals, and developed by Trius Therapeutics and Cubist Pharmaceuticals).[37,38] After two successful phase III trials, tedizolid was approved by the Food and Drug Administration (FDA) in June 2014 under the trade name Sivextro for the treatment of MRSA skin infections (Figure 5).

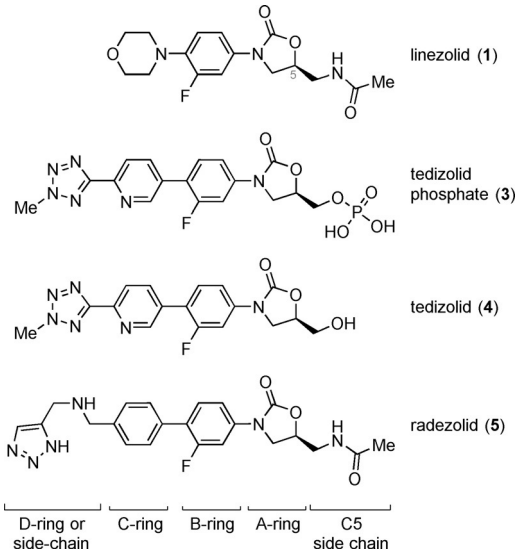


Figure 5. Structures of tedizolid phosphate, tedizolid, and radezolid compared to linezolid.

Structurally, tedizolid presents two main differences compared to linezolid: substitution of the acylaminomethyl group at C5 by a hydroxymethyl moiety and introduction of the C/D ring system, here a 6-(2-methyl-2*H*-tetrazol-5-yl)pyridine (Figure 5). The increase in lipophilicity brought by the addition of the C and D rings obliged the medicinal chemists to find an adequate prodrug that would solve the low aqueous solubility and oral bioavailability problems.[37] A series of formulations was evaluated and the monophosphate ester was found to have the best properties (high water solubility and improved bioavailability) while also masking the primary alcohol, which provided a greatly improved monoamine oxidase inhibition profile.[31]

The effect that these structural modifications bring to tedizolid can be clearly seen in Figure 6. At the bottom end of the binding site, the methylated A2503Ec is able to accommodate the sterically compact hydroxymethyl group while still maintaining the hydrogen bonding with G2505Ec. Additionally, the proposed binding model predicts two new

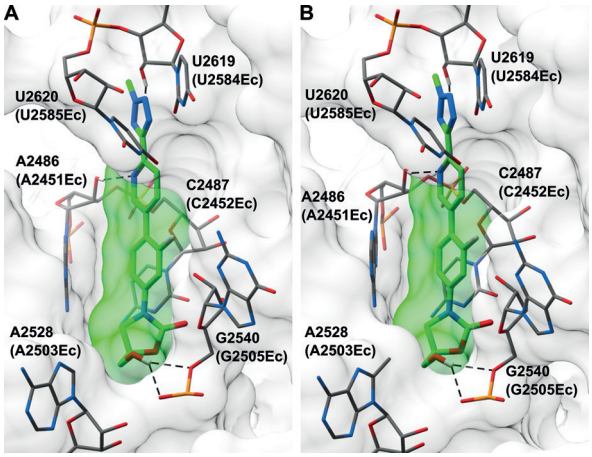


Figure 6. Models of tedizolid (**4**) (C green, F purple) in A) the 50S ribosomal subunit from *H. marismortui* (PDB ID: 3CPW) and B) the 50S ribosomal subunit from *H. marismortui* that has been methylated by Cfr. Even with methylation at A2503Ec, tedizolid (**4**), unlike linezolid, is able to bind to the ribosomal RNA. *E. coli* numbering in parentheses. Models generated with Chimera 1.10.1 and AutoDock Vina 1.1.2 according to K. J. Shaw et al.[14,16,35]

stabilizing hydrogen bonds between the C/D ring system and the backbone ribose sugars of A2451Ec and U2584Ec.

In a study of its activity against linezolid-resistant staphylococci, tedizolid showed a more than 16-fold improvement compared to linezolid.[39] It also maintained activity against most of the tested isolates, including multidrug-resistant ones, thus clearly demonstrating the benefit of the structure-based approach.

2.1.2. *Radezolid*

Radezolid (**5**; previously RX-1741 and Rx-01_667; Figure 5) is a fully synthetic oxazolidinone (developed by Melinta Therapeutics, formerly Rib-X) that has completed two phase II clinical studies.[40] Radezolid is the result of a program developed to expand the spectrum of oxazolidinones to Gram-negative bacteria and optimize drug-like properties.[41] The program started with the observation that linezolid and sparsomycin (**6**, a non-selective antibiotic) had overlapping binding sites within the peptidyl transferase center of the 50S ribosomal unit (Figure 7).[42,43]

The strategy was therefore to link the two molecules together via an adequate bridging element and make the required structural modifications on the sparsomycin side to

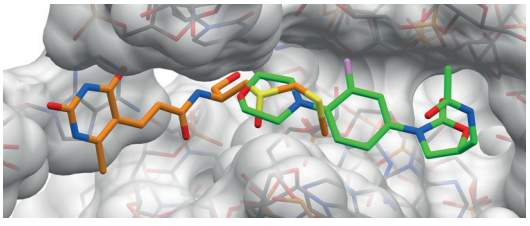


Figure 7. Overlay of sparsomycin (**6**) (C orange) and linezolid (**1**; C green, F purple) in the 50S ribosomal subunit of *H. marismortui* (PDB IDs: 1M90, 3CPW).

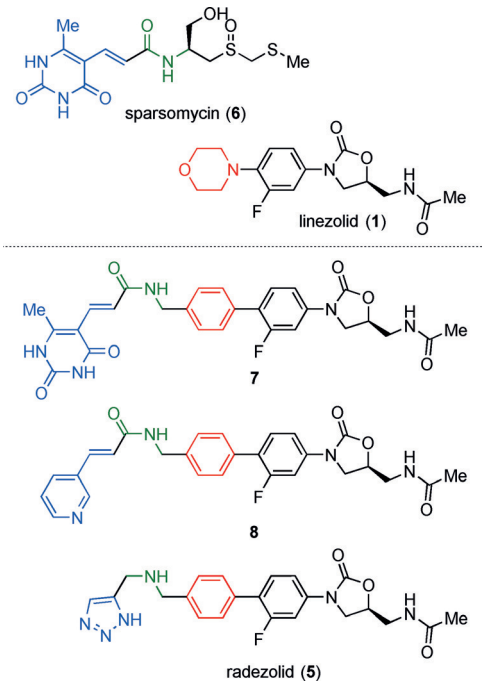


Figure 8. Structures of sparsomycin, linezolid, and molecules developed on the way to radezolid.

increase potency and selectivity.[41,44] Figure 8 shows two (**7** and **8**) of the numerous bridged antibiotics that were synthesized on the road to radezolid and clearly shows the evolution of the western half and the bridging unit.

Once the optimal structural elements were determined, the activity of radezolid was assessed and it was found to bind with higher affinity to the ribosome than linezolid, which gave it enhanced antibacterial activity (2–8-fold improvement over linezolid) against various Gram-positive pathogens.[45] Structure–activity studies of diverse oxazolidinones revealed that despite the presence of a C5 acylaminomethyl group in radezolid, it retains activity against the clinical *cfr*-positive CM05 strain of *S. aureus* with a minimal inhibitory concentration (MIC) value of 2 $\mu g\,mL^{-1}$, which is between those of tedizolid (0.5 $\mu g\,mL^{-1}$) and linezolid (8 $\mu g\,mL^{-1}$). This is most likely due to additional binding interactions of the C/D ring system with the PTC, far from the ribosomal modifications that lead to resistance to linezolid, including the *cfr*-mediated methylation of A2503Ec.[36,40,46] Most noteworthy is the fact that the spectrum could be expanded to Gram-negative organisms such as *Haemophilus influenzae* and *Moraxella catarrhalis*. Additional features of radezolid, besides overcoming the ribosomal mutation resistance, include a 100-fold decreased activity in inhibiting translation in rabbit reticulocytes than in *S. aureus* ribosomes and interaction with U2585Ec, as verified by biochemical assay.[40]

2.2. *Macrolides*

Macrolides are a family of 14-, 15-, and 16-membered polyketide lactone rings with one or more neutral or amino sugar substituents at various positions (Figure 9).[47] Eryth-

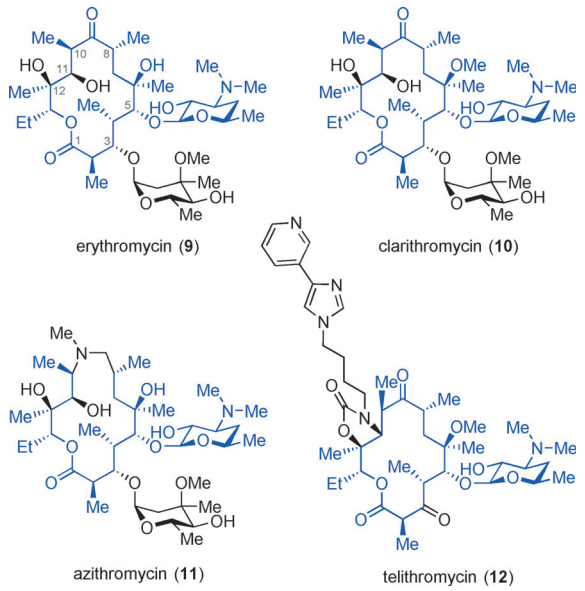


Figure 9. Structures of the three macrolides erythromycin, clarithromycin, and azithromycin, as well as the ketolide telithromycin.

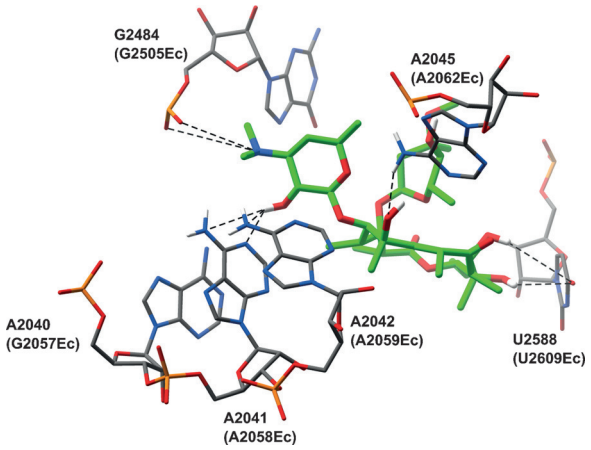


Figure 10. Overview of the binding mode of erythromycin (**9**; C green) to the 50S ribosomal subunit (PDB ID: 1JZY) from *Deinococcus radiodurans*. The nucleotides are labeled according to *D. radiodurans* (*E. coli* in parentheses).

romycin (**9**), the first and prototypical macrolide, was isolated from actinomycete bacteria in 1949.[48] It was first used clinically in the early 1950s, but owing to its acid instability, second-generation semisynthetic macrolides such as clarithromycin (**10**)[49] or azithromycin (**11**)[50] were developed (Figure 9). Subsequently, owing to the emergence of resistance to first- and second-generation macrolides, a third generation, coined the ketolides, was developed. In these 14-membered lactone rings, the cladinose sugar at C3 is replaced by a ketone moiety, C11 and C12 are now part of an oxazolidinone ring, and an alkyl-aryl side chain is appended to the macrolactone core. Telithromycin (**12**) is the only registered ketolide to date (Figure 9).

Like many other antibiotic classes, macrolides are bacteriostatic. They bind to the 50S ribosomal subunit in the vicinity of the peptidyl transferase center just above the constriction formed by the extended loops of ribosomal proteins L4 and L22 and were originally thought to inhibit protein synthesis by completely obstructing the ribosomal tunnel.[51] In fact, modeling studies have shown that even with a bound macrolide, the tunnel can still accommodate a nascent peptide chain. The macrolide nevertheless greatly hinders the progression of the peptide, which is usually dissociated by the peptidyl-tRNA drop-off mechanism before reaching its full size.[51,52] Macrolides have also been shown to block the formation of the large 50S ribosomal subunit by binding to its precursors.[53]

A number of co-crystal structures of macrolides bound to the ribosome have been published and they show that the main component in the binding pocket is A2058Ec.[54–56] Key interactions include polar contacts between the functional groups of the C5 desosamine sugar and residues A2058Ec and A2059Ec, as well as hydrogen bonds between the three lactone hydroxy groups and the 50S ribosomal subunit (Figure 10).

Bacteria have developed a number of mechanisms of resistance to macrolides.[57] Even though the majority of them involve alterations at the ribosomal target site, substrate inactivating enzymes (mainly esterases) and efflux mechanisms have also been reported. Alteration of ribosomal binding sites usually leads to failure of the antibiotic to bind, which in turn disrupts its ability to inhibit protein synthesis. In the case of macrolide resistance, these alterations mainly take place in one of two ways. The first, which has the smallest effect, is modification of the ribosomal proteins L4 and L22 at the end of their hairpin structures, close to the binding site.[58] Interestingly, mutations at L4 lead to large reductions in the binding affinity while the L22 mutants show no change in the binding constant but still confer resistance. This is explained by a widening of the tunnel, which allows passage of the peptide without affecting the binding.[59] The second and most common resistance mechanism is modification of the rRNA. This takes place, unsurprisingly, on A2058Ec, the key component of the binding pocket. Mono and dimethylation of A2058Ec are carried out by erythromycin ribosome methylation (Erm) methyltransferases.[60] While monomethylation confers only moderate resistance to macrolides and none to ketolides, dimethylation leads to complete blocking of the binding site and high resistance to both macrolides and ketolides.[18,61] Mutation of A2058Ec into G2058Ec also induces resistance owing to the similar increase in steric bulk. Notably, in archaea and eukaryotes, position 2058Ec is naturally a guanine, which explains the selectivity for bacteria.[62]

As mentioned earlier, ketolides were developed in an effort to counter resistance to macrolides. Their key features include a 14-membered macrolactone, the replacement of the C3 cladinose sugar by a keto group, a cyclic carbamate and an extended alkyl-aryl side chain (Figure 11). The C3 keto group gives rise to potent activity against strains with Erm-mediated inducible resistance and surmounts resistance through efflux.[63] It also removes some of the steric hindrance around the desosamine sugar, which allows it to reposition itself when binding to monomethylated ribosomes.[64] The

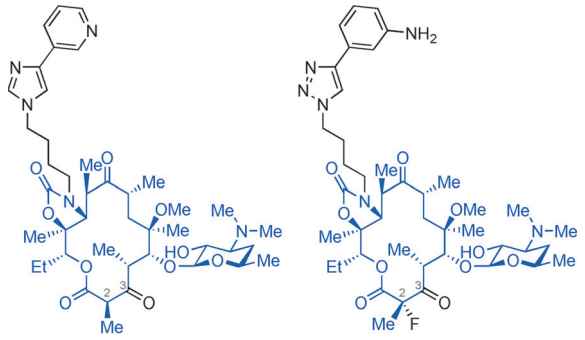


Figure 11. Structures of the ketolides telithromycin and solithromycin.

cyclic carbamate introduces additional interactions with the ribosome, which stabilize the conformation of the core macrolide and lead to potent antibacterial activity.[65] Crystal structures of ketolides in both *D. radiodurans* and in *H. marismortui* have been published and show notable differences in their binding modes.[51,56,66,67] Indeed, when complexed to the ribosome of *H. marismortui*, the side chain of telithromycin resides over the plane of the macrolactone ring (PDB ID: 1YIJ)[56] whereas with the ribosome of *D. radiodurans*, the side chain points away from the macrolactone core (PDB ID: 1P9X).[66] This clearly highlights the importance of crystallographic data and is a good reminder to exercise caution when using models. Nevertheless, the common feature is that the ketolides not only bind to domain V in a similar fashion as macrolides, but their elongated side chains engage in additional interactions in domain II. This results in tighter binding and allows them to compensate for modifications in domain V resulting from mutation or methylation.

Telithromycin (**12**) is currently the only ketolide on the market, but following safety controversies, it has been partially withdrawn.[68] Its use has been associated with severe hepatotoxicity along with blurred vision and serious cases of liver failure.[69] These are believed to result from inhibition of the nicotinic acetylcholine receptors. The pyridine ring in the extended side chain of telithromycin has been suggested to be the culprit.[70] New ketolides lacking this problematic pyridine ring are currently being investigated.

2.2.1. *Solithromycin*

Solithromycin (**13**; previously CEM-101, developed by Cempra) is a 2-fluoroketolide currently undergoing phase III clinical trials. As seen in Figure 11, solithromycin is very similar to telithromycin, with only two minor modifications. The first is replacement of the aforementioned problematic imidazolyl pyridine with a triazolyl aniline, and the second is the introduction of a fluorine atom at the C2 position. Removal of the imidazolyl pyridine resulted in a 30-fold reduction in the inhibition of nicotinic acetylcholine receptors compared to telithromycin.[70] As mentioned earlier, previous X-ray structures of ribosome-bound ketolides showed very different orientations of their alkyl-aryl side chains depending on the bacterial species. Therefore, questions remained as to the actual binding mode of solithromycin and telithromycin in pathogenic bacteria. In 2010, Cate, Mankin, and co-workers crystallized solithromycin as well as telithromycin in complex with the *E. coli* ribosome and were able to indicate that the placement of these ketolides most likely reflects the binding in *S. aureus* ribosomes given the sequence conservation of the A752:U2609 base pair among many eubacteria.[71,72]

These crystal structures delivered revealing insight into various aspects of the interaction (Figure 12). A stacking interaction can be seen with the A752:U2609 base pair, which is present in the ribosome of *E. coli* and many other pathogenic bacteria. The aniline moiety, which replaces the detrimental pyridine is shown to form additional hydrogen bonds, notably to A752 and G748, and results in tighter binding. Finally the fluorine atom at the C2 position was shown to contribute to drug binding as well as chemical properties such as solubility and cellular uptake.[73] Indeed, in comparison to non-fluorinated analogues, the fluorinated versions showed stronger inhibition of the growth of streptococci carrying the *erm* gene. Interestingly, for solithromycin, weak binding to ribosomes dimethylated at A2058Ec could be detected by chemical probing.[71] Key structural features of

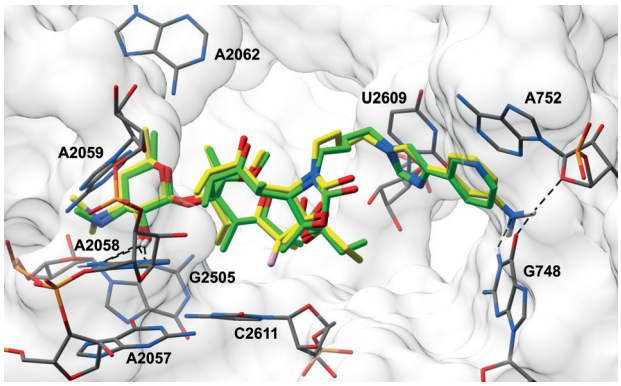


Figure 12. Overlay of telithromycin (**12**; C green, PDB ID: 4V7S) and solithromycin (**13**; C yellow, F purple) in the 50S ribosomal subunit (PDB ID: 4WWW) from *E. coli*. Nucleotides are labeled using the *E. coli* numbering system.

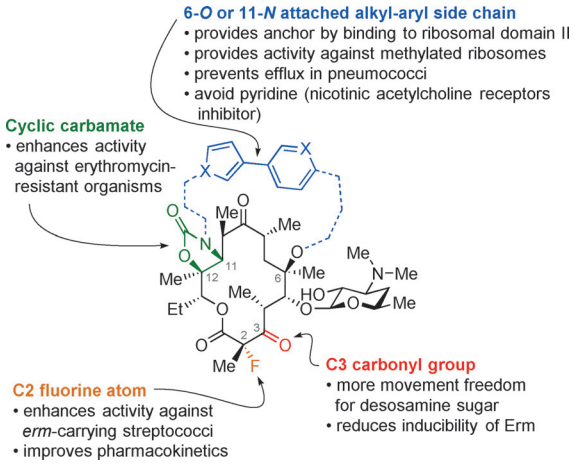


Figure 13. Structure–activity relationships of ketolides.

the ketolides are summarized in Figure 13 (for an example of a ketolide with a 6-*O*-attached side chain, see cethromycin).[74,75]

2.3. Thiopeptides

The thiopeptides are a family of highly modified sulfur-rich macrocyclic peptides (Figure 14, numbering according to thiomuracin A),[76] which have been isolated from diverse sources such as soil bacteria and marine samples.[77] While there are now over 100 known thiopeptides, the first member, micrococcin P1 (**14**), was isolated in 1948.[78] These molecules are of ribosomal origin, are highly posttranslationally processed, and feature a characteristic macrocyclic core consisting of multiple thiazoles and a 6-membered nitrogen-containing heterocycle, which can be found in different oxidation states. While thiopeptides are a new class of antibiotics with a novel mechanism of action, their use as an antibiotic treatment option has been hampered by their very large molecular size and poor aqueous solubility. Thiopeptides are inhibitors of protein synthesis, but their mode of action differs depending on the size of the macrocycle. Thiostrepton A (**15**),[79] the archetypal thiopeptide, and micrococcin P1 possess 26-membered macrocyclic cores and are known to bind to the GTPase-associated region of the ribosome/L11 protein complex.[80] Thiopeptides with 29-membered macrocyclic cores such as GE2270 A (**16**), on the other hand, interact with GTP-bound bacterial EF-Tu, preventing the formation of the ternary complex with aa-tRNA.[81]

GE2270 A was isolated in 1991 by scientists at Lepetit Research Institute.[82] Although the in vitro activity of this compound was shown to be excellent against MRSA, VRE, and streptococci, poor aqueous solubility prevented further development.[83] Two derivatives are currently being investigated.

2.3.1. LFF571

In order to increase the water solubility of GE2270 A, the unstable oxazoline side chain was replaced with solubilizing functional groups (Novartis). The 4-aminothiazolyl moiety was chosen as a starting point and a wide variety of amines and acids linked via different spacers were synthesized.[83–85] Guided by co-crystal structures with EF-Tu, this search led to the discovery of two potent analogues with cyclohexylcarboxylic acid side chains residing in proximity to the Arg223 residue of EF-Tu.

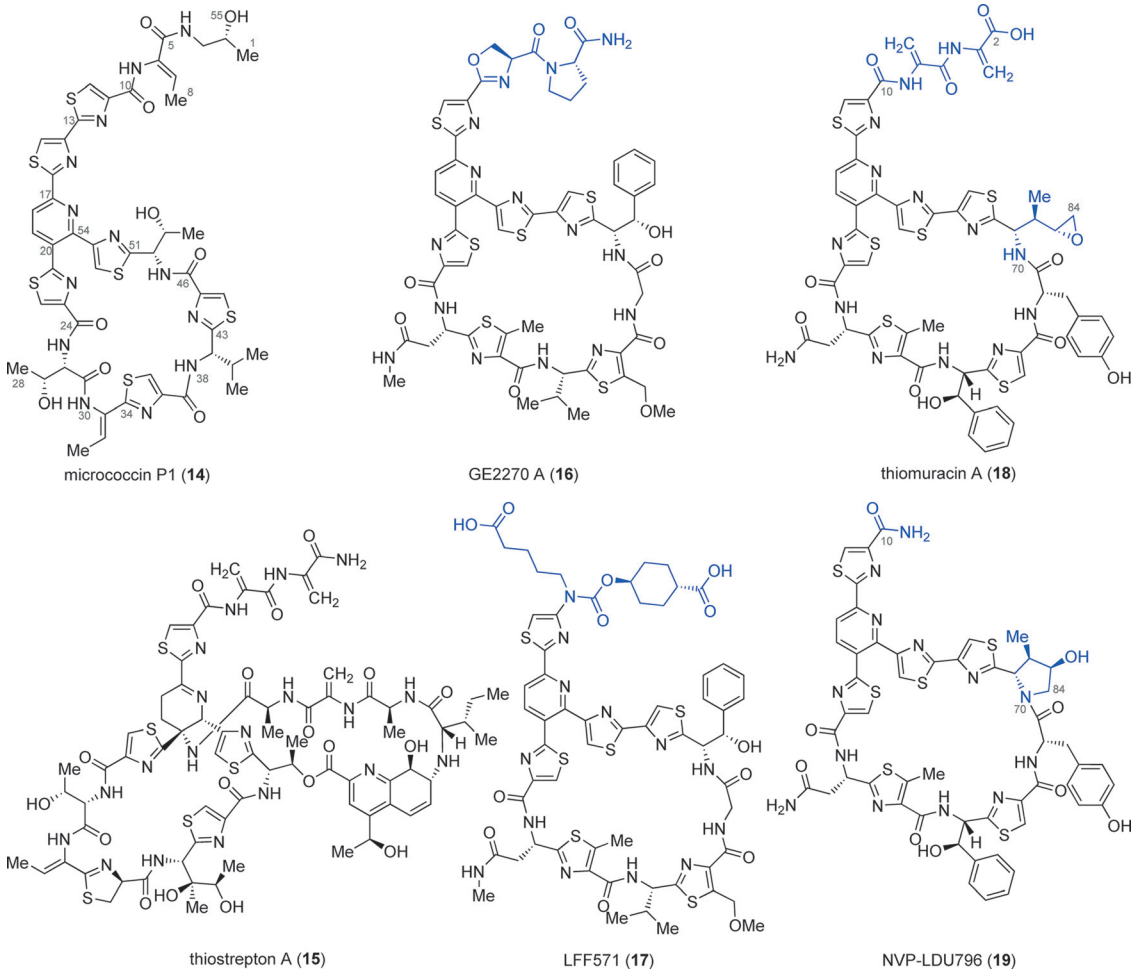


Figure 14. Structures of thiopeptides.

These compounds were then improved by the addition of a second solubilizing group.[86] Taking into account the position of Arg262, a large variety of differently linked acids were synthesized. It was found that a pentanoic acid residue appended onto the aminothiazole was the best since it placed the acid in proximity to this residue (Figure 15). The resulting compound, named LFF571 (**17**), also showed very high aqueous solubility ($>10\,mg\,mL^{-1}$). Triacid-containing analogues were also synthesized but these both failed to yield additional benefits and greatly increased the synthetic complexity, and they were therefore not pursued. LFF571 is currently undergoing phase II clinical trials against *C. difficile* infections.

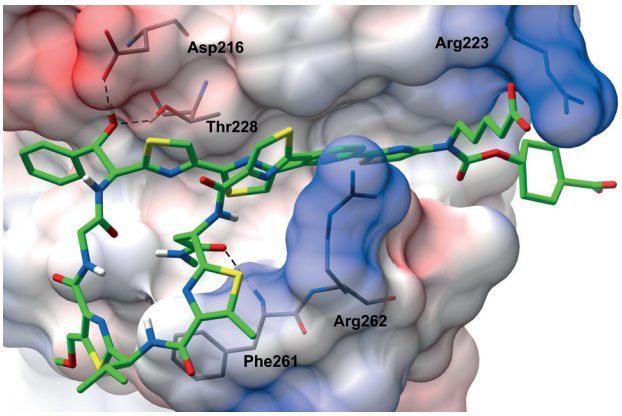


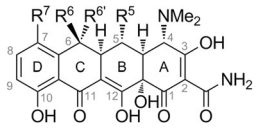
Figure 15. Co-crystal structure of LFF571 (**17**; C green) and EF-Tu (PDB ID: 3U2Q) from *E. coli*. The carboxylic acid groups of LFF571 are in proximity to Arg223 and Arg262.

2.3.2. NVP-LDU796

In 2009, the isolation of thiomuracins, a novel class of antibiotic thiopeptides, was reported (Novartis).[76] These secondary metabolites, which are produced by a strain of *Nonomuraea*, are structurally related to GE2270 A and share the same mechanism of action, namely binding to EF-Tu. The thiomuracins show potent antibiotic activity against MRSA and VRE, with minimum inhibitory concentrations below $1\,\mu g\,mL^{-1}$. Unfortunately, as with GE2270 A, these novel molecules are plagued by solubility and stability problems. In 2012, the same group reported the synthesis of a novel derivative of thiomuracin A (**18**), termed NVP-LDU796 (**19**), which retains antibacterial activity and shows improved chemical stability and physiochemical properties (Figure 14).[87] Key modifications include removal of the C2–C10 side chain and conversion of the C84 epoxide into an N70–C84 pyrrolidine ring. In co-crystal structures of NVP-LDU796 (**19**) with EF-Tu, the conformation adopted is very similar to the those of GE2270 A and LFF571, with key interactions still present (PDB ID: 4G5G).[87]

2.4. Tetracyclines

Tetracyclines are broad-spectrum antibiotics with activity against both Gram-positive and Gram-negative bacteria.[88] Chlortetracycline (**20**), the oldest member of this class, was discovered in 1945 and first used clinically in 1948 (Figure 16).[89] Since then, only three other naturally occurring tetracyclines have been discovered (tetracycline (**21**),[90]



chlortetracycline (**20**)	R^5 = H,	R^6 = OH,	$R^{6'}$ = Me,	R^7 = Cl
tetracycline (**21**)	R^5 = H,	R^6 = OH,	$R^{6'}$ = Me,	R^7 = H
oxytetracycline (**22**)	R^5 = OH,	R^6 = OH,	$R^{6'}$ = Me,	R^7 = H
demethylchlortetracycline (**23**)	R^5 = H,	R^6 = OH,	$R^{6'}$ = H,	R^7 = Cl
doxycycline (**24**)	R^5 = OH,	R^6 = H,	$R^{6'}$ = Me,	R^7 = H
minocycline (**25**)	R^5 = H,	R^6 = H,	$R^{6'}$ = H,	R^7 = NMe_2

Figure 16. Structures of representative tetracycline antibiotics.

oxytetracycline (**22**)[91] and demethylchlortetracycline (**23**)[92]), while countless others have been derived semi-synthetically, including doxycycline (**24**)[93] and minocycline (**25**).[94] Tetracyclines are easily recognizable by their four highly oxygenated fused rings and they show favorable antimicrobial properties along with an absence of major side effects, which has led to their extensive use in both human and animal infections. The third generation of tetracyclines, the glycylcyclines (Figure 17), were introduced 10 years ago, with the first and so far only clinically used member being tigecycline (**26**, previously GAR-936, discovered at Wyeth-Ayerst Research).[95]

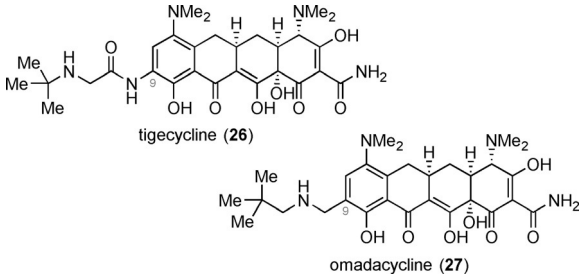


Figure 17. Structures of representative glycylcycline and aminomethylcycline antibiotics.

Glycylcyclines can be recognized by the glycylamido substituent on the C9 carbon. The *tert*-butylglycylamido moiety of tigecycline engages in stacking interactions with C1054 of the 16S rRNA, which leads to increased potency of tigecycline compared to tetracycline (Figure 18).[96]

Tetracyclines are primarily bacteriostatic. They penetrate the outer membrane of Gram-negative bacteria by passive diffusion through the OmpF and OmpC porin channels as divalent metal-ion chelates.[88] Once inside the periplasm, the liberated neutral tetracycline diffuses through the inner

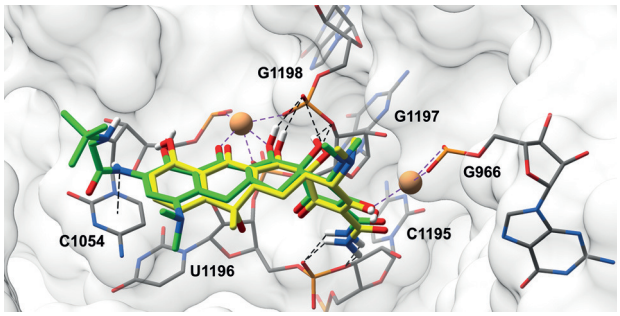


Figure 18. Overlay of tetracycline (**21**; C yellow; PDB ID: 4V9A) and tigecycline (**26**; C green, Mg^{2+} ions light brown; PDB ID: 4V9B) bound to the 30S ribosomal subunit (PDB ID: 4V9B) from *Thermus thermophilus*. Tigecycline makes additional stacking interaction with C1054 compared to tetracycline. *T. thermophilus* numbering is used for the nucleotides.

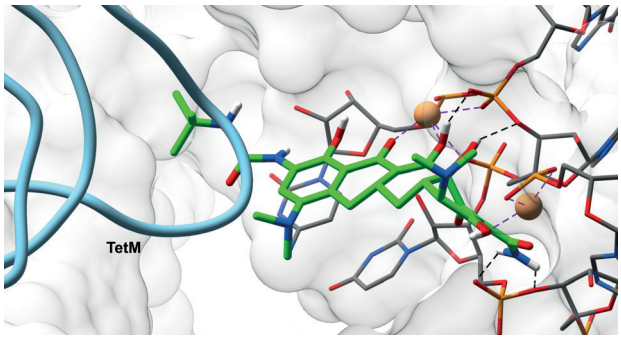


Figure 19. Overlay of TetM (blue ribbon; PDB ID: 3J25) and tigecycline (**26**; C green, Mg^{2+} ions light brown; PDB ID: 4V9B) bound to the 30S ribosomal subunit (PDB ID: 4V9B) from *T. thermophilus*. The superimposition was generated by means of the cryo-electron microscopy density map EMD-2183 of the TetM-70S complex from *E. coli* according to Jenner et al. and Dönhöfer et al.[96,99]

membrane in the same way that it penetrates Gram-positive bacteria and other organisms, namely by energy-dependent active transport. Once inside the cytoplasm, the higher pH and metal-ion concentration lead to the regeneration of a metal-ion/tetracycline complex that is postulated to be the active species. This complex then binds reversibly to the A site on the head of the 30S subunit (Figure 18).[96,97] This binding site has a slight overlap with the anticodon stem-loop, which correlates well with previous observations that tetracycline prevents binding of the aforementioned aa-tRNA•EF-Tu•GTP ternary complex to the A site.[98]

Resistance to tetracyclines can occur through four different mechanisms.[88] The proteins responsible for three of these mechanisms are encoded by the *tet* (tetracycline) and *otr* (oxytetracycline) genes, over 40 of which have been characterized. Of these, only three, namely *tet*(X), *tet*(34), and *tet*(37), lead to an enzymatic-alteration resistance mechanism where the encoded proteins chemically modify tetracycline.[88] Of the remaining genes, approximately two thirds encode efflux proteins and the others encode ribosomal protection proteins (RPPs). The efflux proteins occur in both Gram-positive and Gram-negative bacteria, but are more prominent in the latter. They are membrane-associated proteins and export tetracyclines from the cell, which effectively protects the ribosome by reducing the intracellular concentration of the antibiotic. Ribosomal protection proteins are cytoplasmic proteins that protect the ribosome from the action of tetracyclines by reducing their susceptibility. They are also found in both Gram-positive and Gram-negative bacteria, but they are usually more common in Gram-positive organisms, and *tet*(M) and *tet*(O) are the two most common genotypes. Mechanistically, these proteins cause allosteric disruption of the primary tetracycline binding site, which leads to the release of bound tetracycline molecules.[99] The ribosome is then able to return to its productive conformation and resume protein synthesis. The last and most recent mechanism of resistance involves mutations in the vicinity of the tetracycline binding site.[100]

The presence of the *tert*-butylglycylamido moiety in tigecycline (**26**) interferes with the binding of TetM to the ribosome and thereby enables the drug to overcome TetM-mediated resistance. As shown in Figure 19, tigecycline and TetM overlap in the ribosome owing to the bulky *tert*-butylglycylamido substitution of the drug molecule.[96]

2.4.1. *Omadacycline*

Omadacycline (**27**; previously PTK-0796, Paratek Pharmaceuticals, Figure 17) is a semisynthetic tetracycline derivative and the first member of the novel aminomethylcycline class. It is currently undergoing phase III clinical trials for the treatment of acute bacterial skin and skin structure infections (ABSSSI), community-acquired bacterial pneumonia (CABP), and complicated urinary tract infection (cUTI). Omadacycline has been shown to be active against strains that express either efflux proteins (*tet*(K)) or ribosome protection (*tet*(M)), while also exhibiting moderate inhibition of peptidoglycan synthesis.[101,102] The exact mechanisms by which omadacycline evades both efflux and ribosome protection are not fully known, but it is believed that omadacycline is a poor substrate for efflux transporters and that it binds in a unique way that circumvents the action of ribosome protection proteins.[101] Both of these factors almost certainly arise from the structural modification at C9 of the tetracycline core.

2.5. *Aminoglycosides*

Aminoglycosides are hydrophilic molecules comprised of a central aminocyclitol core linked to one or more amino sugars. In most cases, the aminocyclitol is streptamine or 2-deoxystreptamine (**28** and **29**, Figure 20). Depending on the substitution pattern, aminoglycosides can be grouped into four different subfamilies: monosubstituted (such as neamine (**30**)),[103] atypical, 4,5-disubstituted, and 4,6-disubstituted (Figure 20).[104] The aminoglycoside family of antibiotics is one of the oldest, with its first member, streptomycin (**31**), being discovered more than 70 years ago in 1944.[105] Despite their long history, widespread resistance, and possible safety issues such as nephrotoxicity and ototoxicity, aminoglycosides are still widely used.[106]

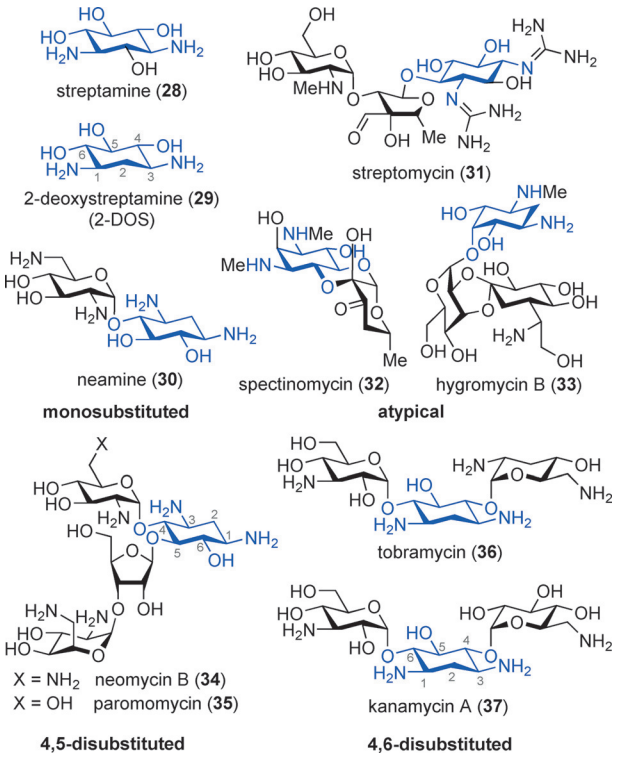


Figure 20. Structures of typical and atypical aminoglycosides. The aminocyclitol core is highlighted in blue.

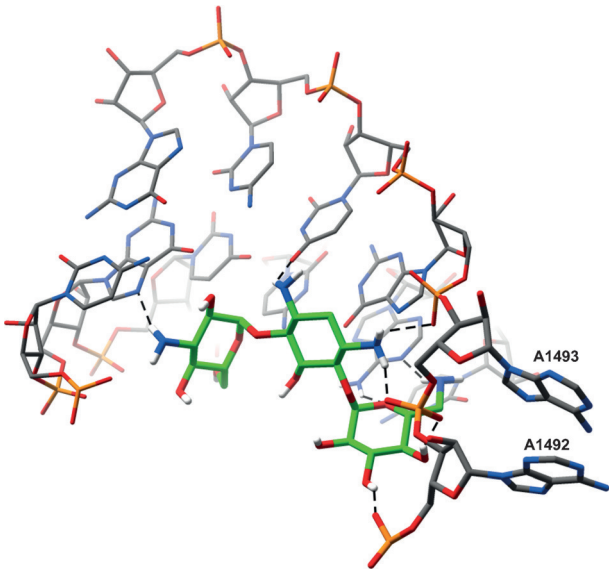


Figure 21. Kanamycin A (**37**; C green) in complex with a decoding A-site oligonucleotide (PDB ID: 2ESI). A1492 and A1493 are kept in the flipped-out position by kanamycin A. *E. coli* numbering is used for the nucleotides.

The aminoglycosides have two distinct main mechanisms of action. They are not only potent inhibitors of translocation but are also able to induce misreading by stabilizing the binding of near-cognate tRNAs and promoting their incorporation into peptide chains.[104] The high fidelity of translation (error frequencies ranging from 10^{-3} to 10^{-4} per codon)[107] is achieved by the ability of the ribosome to select the proper (cognate) tRNA over a wrong (non-cognate) one at the A site.

The structure of the 30S ribosome shows a "decoding" site within helix 44 (h44) of the 16S rRNA. In this asymmetric internal loop, two universally conserved adenine residues, A1492 and A1493, are directly involved in the decoding process, during which they flip out of the helix to analyze the codon–anticodon complex.[108] The energy needed for this flip is thought to be compensated by stabilizing interactions between the nucleotides and the codon–anticodon complex, but only in the case of a cognate tRNA. When a non- or near-cognate tRNA binds, the energy compensation is insufficient and the tRNA dissociates. Upon binding within the internal loop of h44, aminoglycosides induce a local rearrangement that flips A1492 and A1493 out of the helix and stabilizes them in this open conformation (Figure 21).[109] This results in near-cognate tRNA being fully accommodated in the A site, with the consequence that incorrect amino acids are incorporated into the peptide chain.

When the altered proteins are inserted into the cell membrane, the permeability is modified, which in turn leads to an increase in aminoglycoside uptake (hence their high bactericidal and concentration-dependent activity). Co-crystal structures of a variety of bound aminoglycosides have been published, including with streptomycin (**31**, PDB ID: 1FJG),[110] spectinomycin (**32**, PDB ID: 1FJG),[110] hygromycin B (**33**, PDB ID: 1HNZ),[97] neomycin B (**34**, PDB ID: 4V52),[111] paromomycin (**35**, PDB ID: 1FJG, 1IBK),[110,112] tobramycin (**36**, PDB ID: 1LC4),[113] and kanamycin A (**37**, PDB ID: 2ESI).[109]

Bacteria have developed three different mechanisms of resistance to aminoglycosides: uptake inhibition or efflux, ribosome modification, and aminoglycoside modification.[114] The first two mechanisms are relatively rare and have not been targeted yet. Aminoglycoside-modifying enzymes (AMEs), on the other hand, are widespread and the most common mechanism of resistance. These enzymes are described according to the modification they promote, along with the carbon atom at which the modification takes place.[115] The three known types of enzymes are aminoglycoside N-acetyltransferases (AACs), aminoglycoside O-nucleotidyltransferases (ANTs), and aminoglycoside O-phosphotransferases (APHs). Figure 22 shows the co-crystal structure of kanamycin A (**37**, Figure 20) with the aminoglycoside-modifying enzyme ANT(2″)-Ia.[116] The 2″ hydroxy group is complexed to the magnesium ion, ready to be modified by the enzyme.

A second generation of aminoglycosides called neoglycosides,[117] which maintain the potency of first-generation aminoglycosides while evading modification enzymes, is currently being researched.

2.5.1. Plazomicin

Two aminoglycosides, sisomicin[118] and amikacin[119] (**38** and **39**; Figure 23), were used as inspiration for the development of the semisynthetic plazomicin (**40**, formerly ACHN-

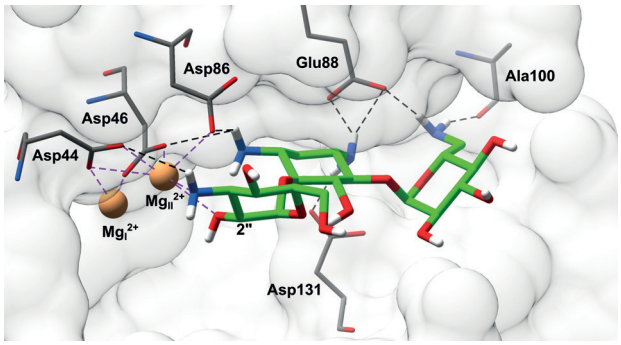


Figure 22. Co-crystal structure of kanamycin A (**37**; C green) and adenylyltransferase ANT(2")-Ia (Mg^{2+} ions light brown; PDB ID: 4WQL) from *Klebsiella pneumoniae*. Kanamycin A is kept in place by a hydrogen-bonding network.

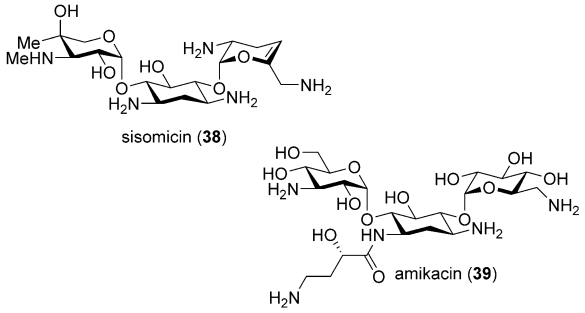


Figure 23. Structures of sisomicin and amikacin.

490, developed by Achaogen), which is the first molecule of this new family (Figure 24).[117]

The difference between sisomicin and plazomicin is the presence of two side chains on the nitrogen atoms at C1 and C6′ in plazomicin. As highlighted in Figure 24, the hydroxyethyl chain shown in red blocks AAC(6′), while the amikacin-derived hydroxyaminobutyric acid (HABA) chain shown in blue blocks AAC(3) along with ANT(2″) and APH(2″).[120,121] Compared to kanamycin A, plazomicin is also protected from APH(3′) and ANT(4′) by the absence of hydroxy groups at positions C3′ and C4′. The only aminoglycoside-modifying enzymes that plazomicin is still vulnerable to are AAC(2′) enzymes, but so far the expression of these enzymes has been detected only in *Providencia stuartii*. In MDR Enterobacteriaceae, including carbapenem-resistant Enterobacteriaceae (CRE), plazomicin remains active where most other antibiotics, including the commercially available aminoglycosides, show limited potency owing to resistance.

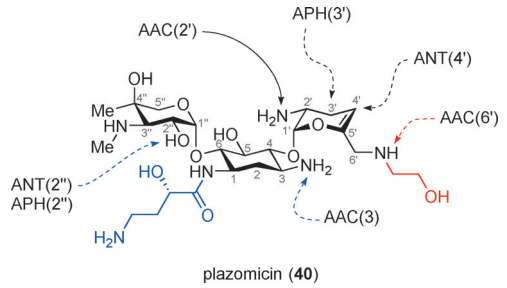


Figure 24. Structure of plazomicin and AME modification sites. The HABA chain (blue) blocks modifications at the 3-*N* and 2″-*O* positions, the hydroxyethyl chain (red) blocks modifications at the 6′-*N* position, and the absence of hydroxy groups at C3′ and C4′ prevents modification at these positions. Only the 2′-*N* position remains unblocked to AACs.

3. Cell Wall Synthesis Inhibitors

The bacterial cell wall is made of peptidoglycan, itself composed of strands of glycan units linked by peptides. Its synthesis takes place in three steps, starting in the cytoplasm, passing through the inner-membrane, and ending in the periplasm (Figure 25). The mechanism of this synthesis has been thoroughly studied and will only be briefly discussed here.[122] The initial precursor, UDP-MurNAc, is generated from UDP-GlcNAc by the action of the transferase MurA and the reductase MurB. To this glycan, five amino acids are then attached sequentially by the ligases MurC–MurF. The last two D-Ala residues are generated from L-Ala by the alanine racemase Alr and linked to the chain by the alanine ligase Ddl. The MurNAc pentapeptide is then transferred to an undecaprenyl pyrophosphate by the translocase MraY, followed by the addition of a GlcNAc unit by the glycosyltransferase MurG to give Lipid II. Finally five L-Gly units are attached to Lipid II by the acyltransferases FemX, FemA, and FemB. Lipid II is then transferred from the cytoplasm to the periplasm through the inner membrane by flippases such as FtsW and RodA. Once in the periplasm, Lipid II is incorporated into the peptidoglycan cell wall by the action of two successive penicillin-binding proteins (PBPs). The first, a transglycosylase, catalyzes polymerization of the glycan strands, while the second, a transpeptidase, crosslinks the glycan strands into a three dimensional mesh.

A large number of antibiotics target the synthesis of peptidoglycan or its precursors, which results in cell death (Figure 25). Eukaryotic cells do not possess a peptidoglycan cell wall, which makes these antibacterial agents selective for bacteria.

3.1. Glycopeptides and Lipoglycopeptides

Vancomycin (**41**, Figure 26), the prototypical glycopeptide, was isolated in the 1950s from microbes found in soil samples (Eli Lilly and Company)[123] and its structure was determined in the early 1980s.[124] It is a hydrophilic rigid glycopeptide with poor absorption in the gastrointestinal (GI) tract. It was first used in the clinic in 1959, but owing to its toxicity, it was usually only used as a last resort. While resistance was slow to develop, high-level resistance was reported in 1986 in enterococci.[124]

Glycopeptides all share a common mode of action, which consists of binding to the C-terminal D-alanyl-D-alanine of peptidoglycan precursors.[125] This was first discovered in vancomycin in the late 1960s and then confirmed by NMR studies by the Williams group in 1983.[126] More recently, these results have been confirmed by X-ray crystallography.[127–129]

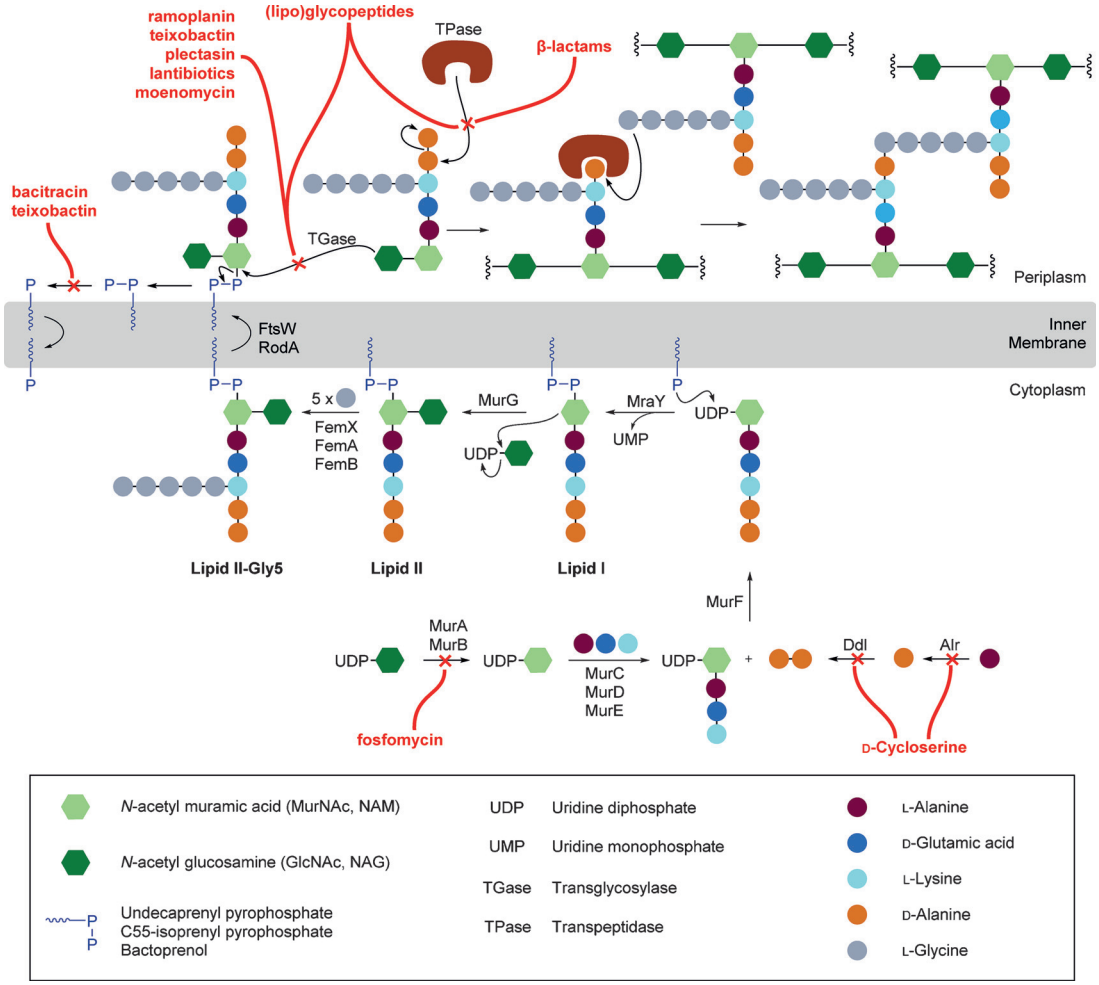


Figure 25. Cell wall synthesis. Antibacterial targets are highlighted.

The resulting steric hindrance from this binding inhibits the transglycosylation and transpeptidation steps in cell wall synthesis ultimately resulting in bacterial cell death (Figure 26).[125]

To circumvent glycopeptides such as vancomycin, bacteria have developed numerous mechanisms of resistance, the most common phenotype being VanA.[130] This phenotype shows substitution of the D-Ala-D-Ala terminus on peptidoglycan residues by D-Ala-D-Lac. Vancomycin can therefore only form four of the usual five hydrogen bonds with the resulting depsipeptide, the last one being replaced by a destabilizing lone-pair/lone-pair interaction.[130] This seemingly small change results in reduction of the binding affinity between the antibacterial agent and the target by up to 1000 fold.[130]

3.1.1. *Vancomycin Analogues*

The Boger group at Scripps has carried out a tremendous amount of work on the synthesis of vancomycin and its analogues, focusing on binding to D-Ala-D-Lac strands.[131] In resistant bacteria, peptidoglycans with the D-Ala-D-Lac terminus only make four hydrogen bonds with vancomycin, resulting in a 1000-fold loss in binding affinity. This figure can be divided into two factors: a 10-fold decrease as a result of the loss of a hydrogen bond, and a 100-fold decrease owing to repulsion of the lone pairs on the two nearby oxygen atoms.[132] In order to try and regain the lost binding affinity, a series of vancomycin analogues was synthesized with modifications at the carbonyl group of residue 4 (Figure 27 A).[131,133]

The thioamide equivalent proved to be completely inactive against both vancomycin-sensitive and vancomycin-resistant bacteria. This was postulated to be due to the increased bond length and size of the sulfur atom, which presumably prevents binding of the ligand. The reduced analogue, in which the carbonyl is replaced by a methylene group, did not reinstate the lost hydrogen bond but did remove the predominant lone-pair repulsion factor. This led to an increase in binding affinity for the mutated D-Ala-D-Lac strand. The best results were obtained when the amide was replaced by an amidine. Indeed, as seen in Figure 27 B, the amidine can serve a dual role. In the case of binding to D-Ala-D-Ala strands, the nitrogen atom can take the role of a hydrogen-bond acceptor, whereas in the case of binding to D-Ala-D-Lac strands, it can serve as a hydrogen-bond donor. This, in combination with the introduction of a lipophilic 4-(4-chlorophenyl)benzyl side chain derived from oritavancin[134] (which allows the antibiotic to anchor into the bacterial cell

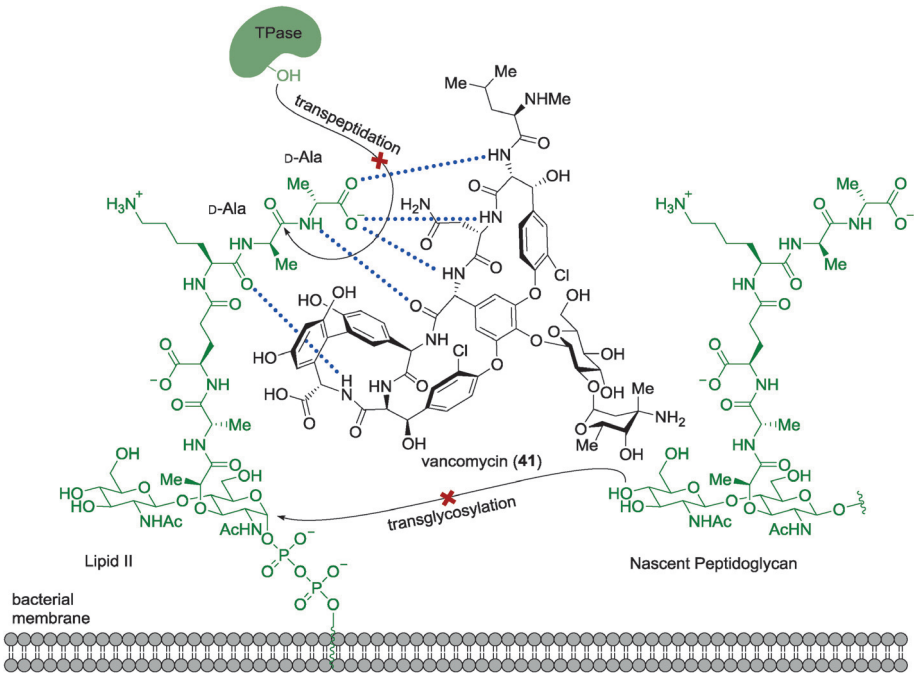


Figure 26. Diagram of the binding of vancomycin to Lipid II sterically prevents the transglycosylation and transpeptidation steps. Key interactions of vancomycin with the D-Ala-D-Ala fragment shown in blue and were identified from co-crystal structures of vancomycin with analogues of cell-wall precursors (PDB ID: 1FVM).[129]

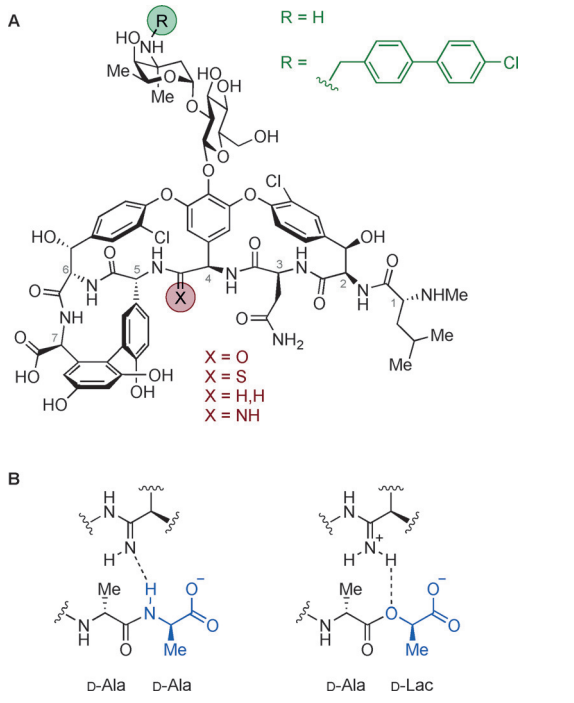


Figure 27. A) Modifications made by the Boger group to vancomycin. Addition of the lipophilic side chain from oritavancin is shown in green; modifications to the carbonyl of residue 4 are shown in purple. B) Dual binding behavior of the amidine functional group.

membrane, thus bringing target and host closer together), resulted in impressive antibiotic activities against both vancomycin-sensitive and vancomycin-resistant bacteria (MIC = 0.06–0.005 and 0.5–0.06 μg mL^{-1} for the amidine and methylene analogues, respectively).[133]

3.2. β-Lactams

In 1928, Alexander Fleming discovered the inhibitory effect of a *Penicillium* mold on the growth of bacteria.[135] Nevertheless, it wasn't until 1941 that penicillin was used for the first time to treat human patients.[136] To this day, β-lactam antibiotics are still widely used around the globe, and medicinal chemists have developed numerous variations and generations of this scaffold.[137] The mechanism of action of β-lactams involves covalent binding to transpeptidases, thereby inhibiting cell wall synthesis.[138] Indeed, β-lactams mimic the D-Ala-D-Ala terminal sequence of peptidoglycan chains, the natural substrate of transpeptidases (Figure 28, top right).

As seen in Figure 28, the distance between the two carbonyls of the peptidoglycan chain is very similar to the distance between the carboxylic acid and the β-lactam carbonyl. The antibiotic therefore acts as a competitive antagonist for the transpeptidase enzyme. Mechanistically, the hydroxy group of a serine residue reacts with the lactam carbonyl, which leads to opening of the four-membered ring (Figure 28, purple cycle).[136] The resulting acyl enzyme (Figure 29) is stable, and hydrolysis is very slow (low k_3),

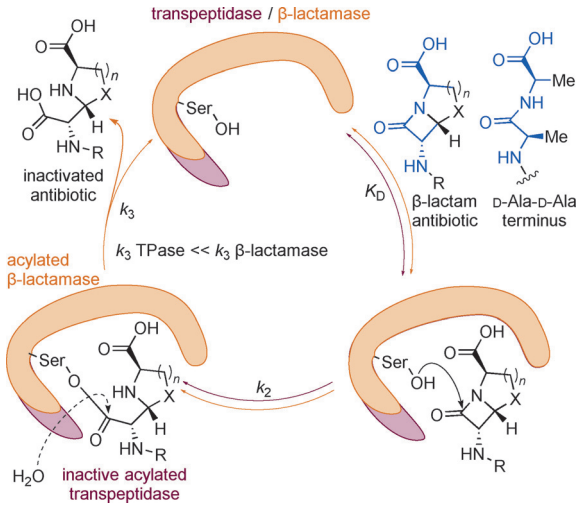


Figure 28. The structural similarities between the core of β-lactam antibiotics and the D-Ala-D-Ala terminal sequence of peptidoglycan chains (blue), and the general mechanism of action of transpeptidases (purple) and β-lactamases (orange).

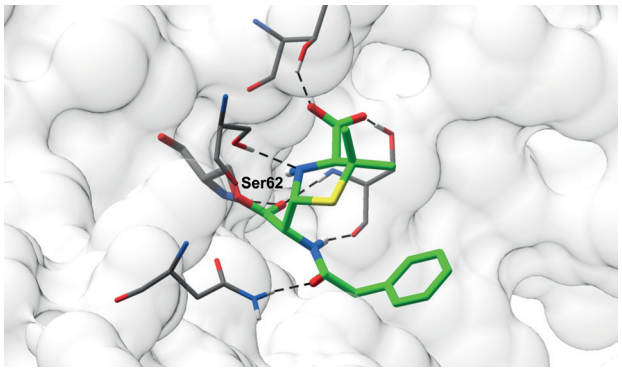


Figure 29. X-ray structure of penicillin G (C green) covalently bound via Ser62 to penicillin-binding protein 4 of *E. coli* (PDB ID: 2EX8).

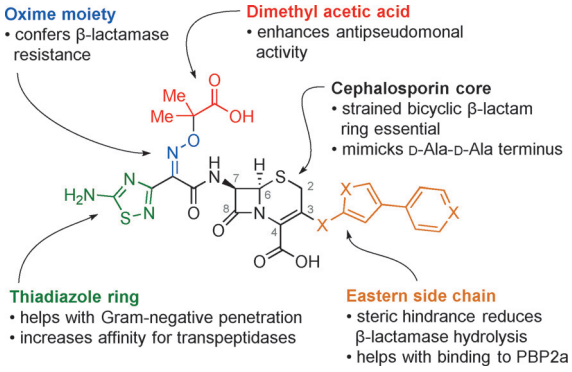


Figure 30. Summary of cephalosporin structure–activity and structure-property relationships.

thus rendering the enzyme inactive and ultimately leading to cell lysis and death.[139]

Resistance to β-lactams occurs through four mechanisms.[140] The first two are relatively rare and only occur in Gram-negative bacteria. One involves alteration of the porin channels, which prevents access to the transpeptidases, while the other involves efflux pumps that export the antibiotic. These mechanisms have not been specifically targeted in recent antibiotics. The third and most common mechanism of resistance is the production of β-lactam-hydrolyzing enzymes. These enzymes are structurally very similar to the transpeptidase enzymes and mechanistically function in a similar manner by opening the β-lactam ring (Figure 28, orange cycle).[140] What differentiates the two is the fact that while acyl transpeptidases were slow to hydrolyze, the acylated β-lactamases are hydrolyzed at a much faster rate (high k_3). This rapid hydrolysis results in regeneration of the β-lactamase and inactivation of the antibiotic. Originally, β-lactams were synthetically modified to resist and evade β-lactamases, which led to so many scaffolds and multiple generations of these types of antibiotics. The focus has nowadays shifted to the use of β-lactamase inhibitors co-administered with β-lactams (see Section 4).

The fourth mechanism of resistance, which is present in MRSA, is the presence of an exogenous β-lactam-resistant PBP called PBP2a. Studies on this PBP and the similar PBP2 have provided details on the resistance mediated by PBP2a.[141,142] The binding pocket for the antibiotic in PBP2a exists in an unreactive conformation since the active serine (Ser403) is misaligned and acylation does not take place. It was postulated that only under acidic conditions or if the β-lactam possesses a bulky hydrophobic substituent, would acylation take place.[143] Indeed, allosteric interactions are required to trigger a conformational change that opens the active site. Following these findings, three so-called "fifth-generation" cephalosporins, namely ceftaroline,[144] ceftobiprole,[145] and ceftolozane,[146] were developed by following the currently known SAR (Figure 30), and these show activity against MRSA.

3.2.1. *Ceftaroline*

Ceftaroline fosamil (**42**; previously PPI-0903, TAK-599, Forest Laboratories) is the prodrug of ceftaroline (**43**), a novel broad-spectrum cephalosporin approved by the FDA in 2010 (Figure 31). Like other β-lactams, it shows bactericidal activity against Gram-positive organisms but also against MRSA.

Ceftaroline was developed by modifying cefozopran (**44**), a fourth-generation cephalosporin. As seen in Figure 31, the western half remains mostly unchanged, with the 1,2,4-thiadiazole and oxime moieties still present for Gram-negative penetration and β-lactamase resistance, respectively.[144] Once bound, ceftaroline has been shown to undergo hydrolysis at a very slow rate.[144] The phosphono group in the prodrug ceftaroline fosamil is used to increase water solubility

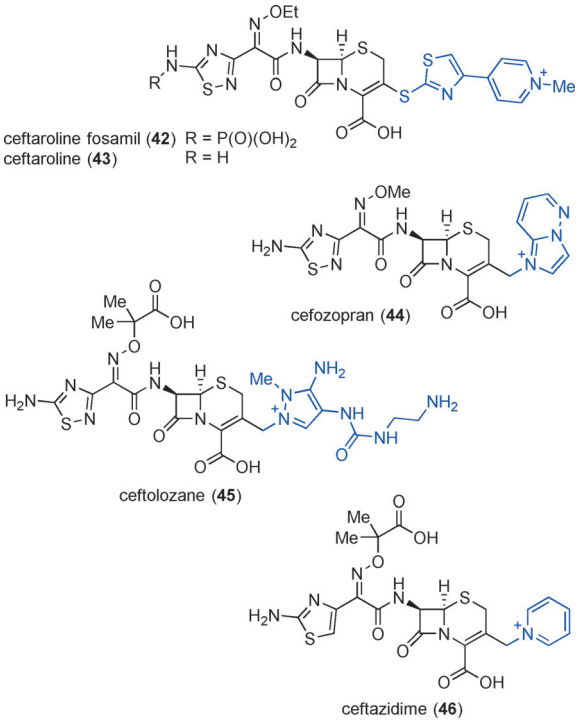


Figure 31. Structures of cephalosporins.

and is rapidly cleaved in the plasma to deliver the active drug ceftaroline.[147] For ceftaroline, an allosteric binding site at PBP2a has been identified by crystallographic analysis (Figure 32). The allosteric binding site (Figure 32C) is separated from the active site (Figure 32B) by a remarkable 60 Å distance (Figure 32A).[148] Crystallographic analysis also revealed the identity of other allosteric ligands for PBP2, such as muramic acid (a saccharide component of the peptidoglycan). Therefore, it has been proposed that the function of the allosteric domain is to sense nascent peptidoglycan and then open the active site to catalyze the transpeptidation.[148] The elucidation of the ability of the anti-MRSA β-lactam antibiotic ceftaroline and other molecules to trigger allosteric opening of the active site so that PBP2a can be inactivated by a second β-lactam molecule, should enable future structure-based design campaigns for β-lactam antibiotics.

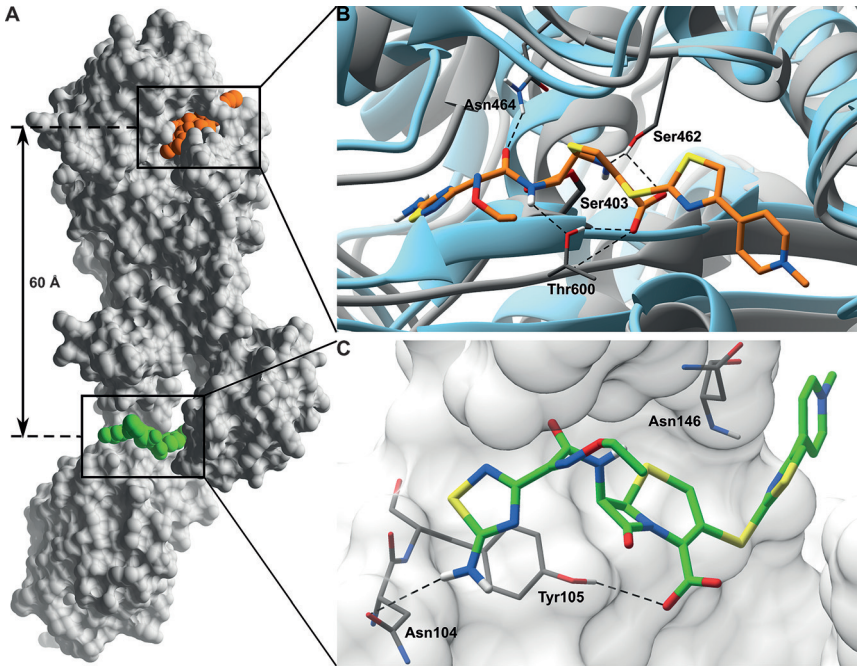


Figure 32. A) An overview of the two binding sites of ceftaroline (**43**; C green [allosteric site] and orange [active site]) in PBP2a from *S. aureus* (PDB ID: 3ZG0). B) Ceftaroline (**43**; C orange) covalently bound within the active site of PBP2a (gray ribbon, PDB ID: 3ZG0). Once ceftaroline is bound to the allosteric site, the active site opens up in comparison to the closed state (blue ribbon, PDB ID: 4BL2). C) Ceftaroline (**43**; C green) bound non-covalently to the allosteric binding site of PBP2a (PDB ID: 3ZFZ).

After the introduction of ceftaroline to the clinic, clinical strains resistant to this antibiotic were reported. Two sets of mutations within the PBP2a sequence were described (the double mutant N146K/E150K and the triple mutant N146K/E150K/H351N).[149] These mutations are distant from the active site of PBP2a, hence their functions cannot be understood by a loss of affinity of the antibiotic for the active site of the target protein. Interestingly, the N146K and E150K mutations are both within the allosteric domain of PBP2a, whereas the H351N mutation is outside of both the allosteric and active sites. By kinetic studies and by X-ray crystallography of the mutants, Mobashery, Hermoso et al. were able to rationalize that the clinically observed mutations interfere with triggering of the allosteric signal by ceftaroline along the interacting amino acid network between the two sites.[148,150] These mutations allow the mutant variants of PBP2a to manifest resistance to ceftaroline by two different mechanisms: the first is a modest increase in the dissociation constant for ceftaroline binding to the allosteric site, and the second is disruption of the transmission of conformational changes necessary for opening the active site. This results in resistance to ceftaroline by an unprecedented mechanism, namely interference with the fidelity of the allosteric response.[150]

3.2.2. *Ceftolozane*

Ceftolozane (**45**; previously CXA-101 and FR264205, discovered at Astellas Pharma, developed by Cubist Pharmaceuticals) is the β-lactam component of the cephalosporin/β-lactamase inhibitor combination Zerbaxa, which was approved by the FDA in December 2014.[151]

As shown in Figure 31, ceftolozane is derived from the third-generation cephalosporin ceftazidime (**46**). It maintains the aminothiadiazole ring and oxime functional groups of ceftaroline and ceftobiprole, which confer enhanced activity against Gram-negative bacilli and stability against β-lactamases, respectively.[151] Additionally the appended dimethylacetic acid moiety provides improved antipseudomonal activity.[146,151] On the eastern side, a highly substituted pyrazole ring provides the steric bulk necessary to reduce hydrolysis by β-lactamases.[151] The SAR of the substitution pattern of the pyrazole ring was studied in detail, and a 5-amino-1-methyl-1*H*-pyrazol-2-ium moiety was found to confer the highest antipseudomonal activity.[146] Additionally, the basicity of the substituent at the 3-position of the cephalosporin nucleus could be correlated with improved outer membrane permeability. Unfortunately, this also led to increased convulsion induction in mice. The solution was found by introducing basic side chains at position 4 of the 5-amino-1-methyl-1*H*-pyrazol-2-ium, with the 2-aminoethylureido group eventually found to have the best balance.

Ceftolozane shows broad-spectrum activity, including against drug- and multidrug-resistant *Pseudomonas aeruginosa*, along with potent antipseudomonal activity.[151,152] Unfortunately, like other oxyimino cephalosporins, it is sensitive to extended-spectrum β-lactamases (ESBL) and carbapenemases. For this reason, ceftolozane is marketed in conjunction with the β-lactamase inhibitor tazobactam (see Section 4), which broadens its spectrum to include ESBL-producing strains of *P. aeruginosa* and *Bacteroides fragilis*.[151,153]

3.2.3. BAL30072

Siderophores are small molecules that chelate iron ions, and they allow bacteria to acquire this essential element from the environment for vital cellular processes.[154]

The siderophore-containing β-lactam BAL30072 (**47**; Figure 33, developed by Basilea Pharmaceutica AG)[155] is

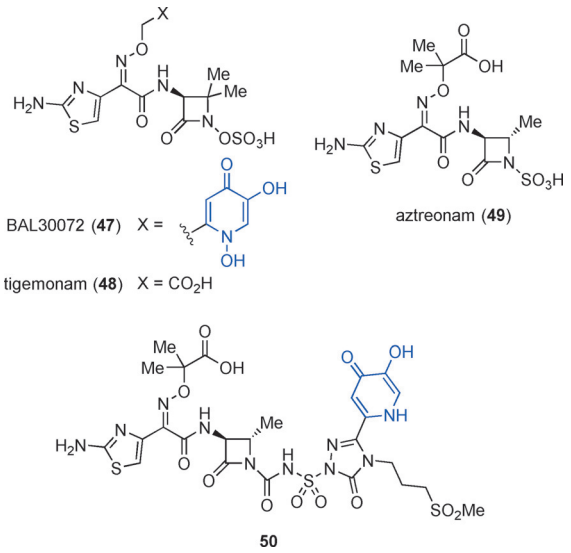


Figure 33. Monocyclic β-lactams with the siderophores highlighted in blue.

a siderophore sulfactam conjugate derived from tigemonam (**48**) with an additional dihydroxypyridinone moiety to chelate iron.[156] It serves as a bioisostere for catechol, and the BAL30072-Fe^{3+} complex can thus be actively transported through the outer membrane.[157] Inside the bacteria, it inhibits PBP1a, PBP2a, and PBP3 and acts as an inhibitor of class C β-lactamases.[155] BAL30072 shows good antibacterial activity in vitro against Gram-negative bacteria, is active in vivo against *Acinetobacter baumannii*, and features synergistic activity in combination with carbapenems.[155,158,159]

In 2014, Starr et al. reported PBP3/BAL30072 co-crystal structures, which showed that the siderophore moiety might not be at the ideal position. Indeed, compared to aztreonam (**49**; Figures 33 and 34A),[160] another similar monobactam, the molecule misses some favorable interactions, such as a salt bridge with Arg489 and a hydrophobic pocket interaction with the *gem*-dimethyl group of the antibiotic (Figure 34B).[161] Based on these structure-based findings, medicinal chemists tried to install the siderophore moiety at a more beneficial position.[161–163] Compound **50** (Figure 33, discovered at AstraZeneca) represents a most recent successful example of such a structure-based approach that allows the design of siderophore-conjugated monocarbams with optimized binding interactions to *P. aeruginosa* PBP3 (Figure 34C).[163] Besides reinstalling the strong interaction of the drug molecule with Arg489, it positions the polar siderophore in a more hydrophilic environment and allows the drug to make additional interactions with the target protein

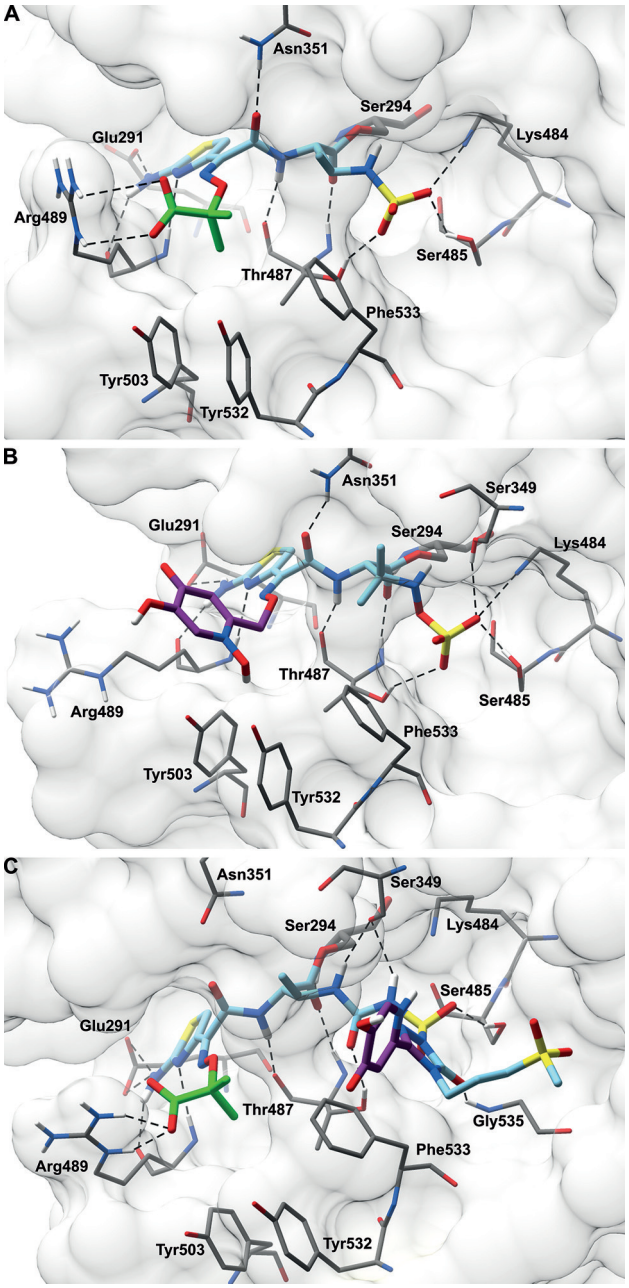


Figure 34. Comparison of siderophore-conjugated monocyclic β-lactam antibiotics. A) Aztreonam (**49**; C light blue) covalently bound to PBP3 (PDB ID: 3PBS) from *P. aeruginosa*. The carboxylic acid moiety (C green) of aztreonam engages in polar interactions with Arg489, and the gem-dimethyl group interacts with the hydrophobic pocket formed by Tyr503, Tyr532, and Phe533. B) X-ray structure of BAL30072 (**47**; C light blue) covalently bound to PBP3 (PDB ID: 4OOM) from *P. aeruginosa*. The siderophore moiety (C purple) of BAL30072 replaces the carboxylic acid and forces Arg489 into a non-binding orientation. C) The crystal structure of **50** (C light blue) covalently bound to PBP3 (PDB ID: 4WEL) from *P. aeruginosa* demonstrates optimized binding of the siderophore monocarbam conjugate (siderophore moiety: C purple; carboxylic acid moiety: C green).

PBP3 (Figure 34C). Compound **50** features excellent pharmacokinetic properties and strong cellular activity against *P. aeruginosa* (*P. aeruginosa* ARC545; MIC = 0.5 μg mL^{-1}).[163]

4. β-Lactamase Inhibitors

As discussed previously, β-lactam antibiotics are one of the oldest and most used classes of antibiotics. Resistance to these antibacterial agents predominantly occurs through the production of two different types of molecules: β-lactamases and β-lactam-resistant PBPs. β-Lactamases have been classified according to two schemes: the Ambler and the Bush–Jacoby classification.[164,165]

The first β-lactamase inhibitor to be used was clavulanic acid (**51**, discovered at Beecham Pharmaceuticals) in the mid-1970s,[166] followed over the next decade by sulbactam (**52**)[167] and tazobactam (**53**).[168] As seen in Figure 35, these β-lactamase inhibitors all possess a β-lactam core, but they have been shown to have only limited antibiotic activity. Their mode of action involves irreversible binding to β-lactamases, which prevents inactivation of the antibiotic (Figure 36).[169]

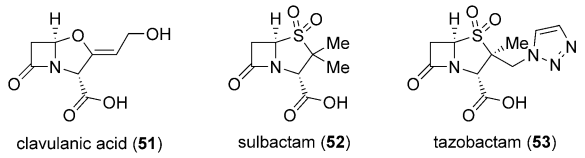


Figure 35. The three β-lactamase inhibitors with β-lactam cores that are currently in clinical use.

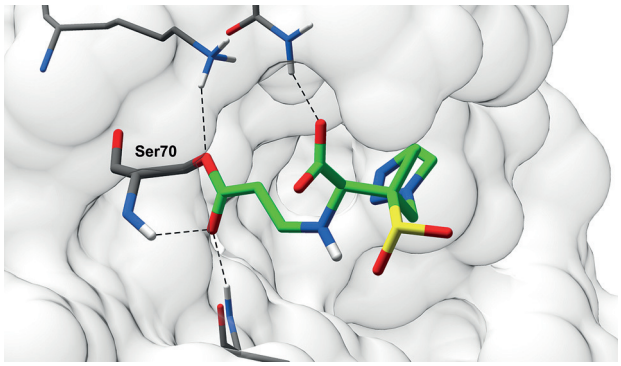


Figure 36. Crystal structure of tazobactam (**53**; C green) covalently bound via Ser70 to the β-lactamase SHV-1 (PDB ID: 2H10) from *K. pneumoniae*.

Unfortunately these three β-lactamase inhibitors are only effective against one of the four classes of β-lactamases, and the number of naturally occurring β-lactamases has risen exponentially over the last 20 years since the introduction of tazobactam. Novel β-lactamase inhibitors with extended spectra are now greatly needed. New β-lactamase inhibitors that are based on structural design and do not contain a β-lactam structure are currently being investigated.

4.1. Diazabicyclooctanes

The first class of these "non-β-lactam inhibitors" are the diazabicyclooctanes (DBOs), which were first proposed in the mid-1990s (Hoechst Marion Roussel).[170,171] These inhibitors are more potent and have a broader spectrum than current β-lactamase inhibitors. Mechanistically, they act in a similar manner to the traditional β-lactam inhibitors, with the carbonyl group of the cyclic 5-membered urea (imidazolidin-2-one) serving as the electrophile for the active-site serine of the β-lactamase. This structural similarity to β-lactams is highly important for rapid recognition by the β-lactamase (Figure 37). Unlike the traditional β-lactam inhibitors however, they are not subject to hydrolysis after acylation, but instead deacylate very slowly. Two of these novel DBOs will be discussed here.

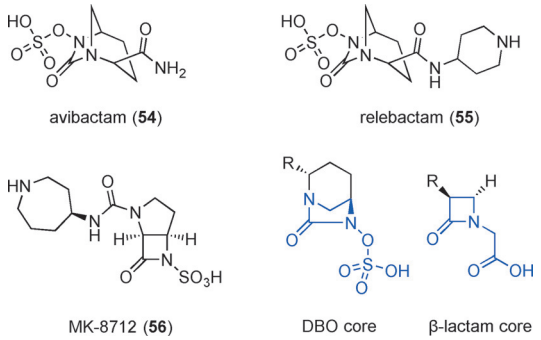


Figure 37. The diazabicyclooctane (DBO) non-β-lactam inhibitors and similarity with the β-lactam core (blue).

4.1.1. Avibactam

Avibactam (**54**; previously NXL-104, AVE1330A, developed by Actavis) is a DBO that was approved by the FDA in 2015 in combination with the β-lactam ceftazidime (**46**) and is still in clinical studies in combination with ceftaroline (**43**) and aztreonam (**49**).[172] It is a covalent, reversible inhibitor that has been shown to have a very fast "on" rate, as well as a very slow "off" rate for deacylation, with a half-life measured in days.[173] X-ray crystal structures of avibactam in complex with three different β-lactamases have provided important insight into the mode of action.[174–176] Avibactam interacts with conserved key residues in a fairly rigid conformation, and the highly polar sulfate group (which mimics the β-lactam carboxylic acid, Figure 37) engages in strong polar interactions with Arg261 (Figure 38).[177] Once covalently bound, avibactam remains in a similar conformation, which, along with the stability of the newly formed carbamoyl bond and the additional favorable interactions, explains the deacylation pathway over hydrolysis. Given the small size of avibactam and the efficient interactions with key catalytic residues near the active site, this should allow binding to nearly all β-lactamases. This could have positive implications for the suppression of emergence of new resistance in the clinic.

4.1.2. Relebactam

Relebactam (**55**; previously MK-7655, Merck and Company, Figure 37) is structurally very similar to avibactam, the only difference being the addition of a piperidine ring on the

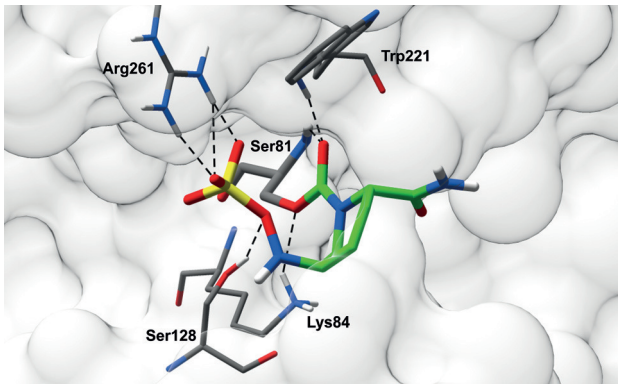


Figure 38. X-ray structure of avibactam (**54**; C green) bound via Ser81 to the β-lactamase OXA-24 (PDB ID: 4WM9) from *A. baumannii*.

amide. The choice of this heterocycle came from screening a variety of basic and neutral heterocyclic side chains, taking into account results obtained with a previous inhibitor MK-8712 (**56**).[178] It was found that the addition of a basic nitrogen atom greatly helped to reduce efflux from the bacterial cell.[178]

4.2. Boronic Acids

Boronic acids were already recognized as effective inhibitors of serine proteases in the 1970s.[179] Indeed, the boron atom can act as an electrophile, mimicking a carbonyl carbon atom, which can lead to the formation of a reversible covalent bond between the serine and the boronate moiety. Since Kiener and Waley described the use of phenylboronic acid as a β-lactamase inhibitor in 1978, a number of research groups have looked into this functional group to generate novel inhibitors.[180] Surprisingly, it is only very recently that the first boronate has been tested in an animal infection model.[181]

4.2.1. RPX7009

A cyclic boronic acid β-lactamase inhibitor is currently under development (Rempex Pharmaceuticals, a subsidiary of The Medicines Company). The original inspiration for its design comes from a report published in 2000 by Ness and co-workers in which compound **57** is described.[182] This led to the idea of using cyclic boronates as inhibitors. With the boronic ester locked in a ring, the inhibitor is constrained into a preferred conformation, thereby resulting in increased potency. Computational docking of several structures with different β-lactamase enzymes led to the discovery of the core structure shown in Figure 39. From this first hit, a large variety of compounds with varying N-acyl substituents were synthesized. The 2-thienyl acetyl analogue RPX7009 (**58**) was found to be the most potent derivative, while also displaying a broad spectrum of inhibition and high selectivity.[181] RPX7009 is a broad-spectrum inhibitor, notably restoring the activity of carbapenems against carbapenemase-producing *K. pneumoniae* strains. Combined with a carbapenem,

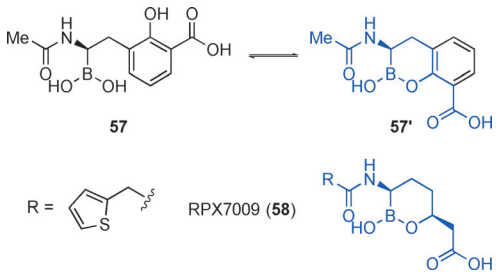


Figure 39. Inspiration for and structure of RPX7009.

RPX7009 is a promising candidate for the treatment of multidrug resistant Gram-negative bacteria.[183]

X-ray co-crystal structures of RPX7009 with β-lactamase enzymes confirmed the boron atom as the electrophilic site for the serine addition, as well as a hydrogen-bonding network between both the amide and the carboxylic acid groups and the enzyme backbone (Figure 40). This information could be used for further structure-based optimization of this novel chemotype of β-lactamase inhibitors.

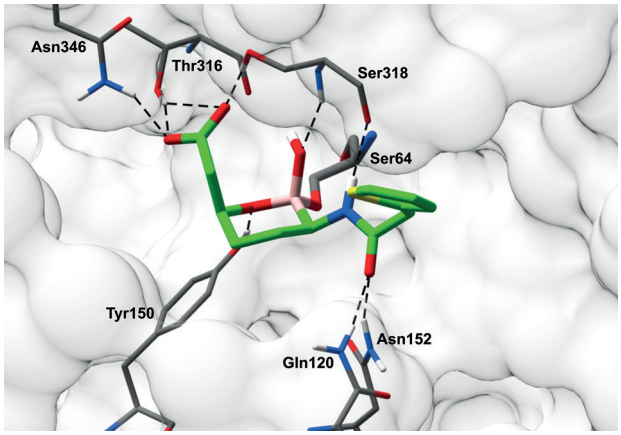


Figure 40. X-ray structure of RPX7009 (**58**; C green, B light magenta) bound to AmpC (PDB ID: 4XUX) from *Enterobacter cloacae*.

4.2.2. Oxaboroles

Recently, the design and synthesis of broad-spectrum boron-based β-lactamase inhibitors has been published (AstraZeneca).[184] The approach taken is a very nice and well described example of structure-based drug design that could potentially inspire future work in this field.

Verheijen et al. used two recent publications as starting points to come up with 2-(1-hydroxy-6-phenoxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-3-yl)acetic acid (**59**) as an initial scaffold (Figure 41).[185,186] An SAR study revealed the importance of the carboxylic acid and ether substituents. While the phenyloxy-substituted analogue showed slightly higher affinity against a panel of representative β-lactamases, the pyrazine analogues were synthetically less challenging and were therefore preferred for further optimization studies.

Docking studies against various β-lactamases revealed a lipophilic pocket near C4 and C5. This pocket was found to

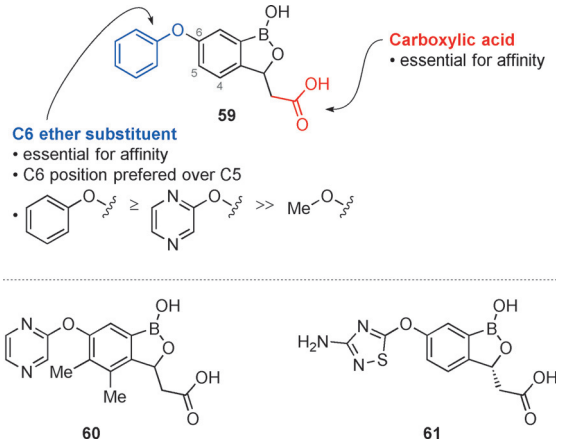


Figure 41. Structure–activity relationships contributing to the development of new boron-based β-lactamase inhibitors (**60**, **61**).

be only able to accommodate small methyl groups, which led to the advancement of compound **60**. At this stage, chiral separation also revealed that the *R*-enantiomer was the most active. Finally the pyrazine ring was replaced with a number of different heterocycles to try and increase the affinity for class A, C, and D β-lactamases. Eventually, compound **61** was found to have the highest overall affinity against various β-lactamases, with half maximal inhibitory concentration (IC_{50}) values in the nanomolar range. For ceftazidime (**46**), the application of **61** reduced the MIC in *E. coli* to 0.25 $\mu g\,mL^{-1}$. The predicted binding model was also confirmed by X-ray crystallography of **61** in co-crystal structures with different β-lactamases (PDB IDs: 4WZ4 [with AmpC] and 4WZ5 [with OXA-10]). Unfortunately, compound **61** produced only modest restoration of susceptibility to ceftazidime in a number of other Gram-negative pathogens such as *P. aeruginosa* and *A. baumannii*.

5. Intervention at the Genetic Level

Transcription factors are responsible for the regulation of gene transcription and are therefore essential in a wide variety of cellular processes.[187] They contain specific DNA-binding domains with which they bind to specific DNA sequences and thereby regulate gene transcription. Interference at the genetic level by using small molecules that target the transcription factor binding sites enables control of gene expression.[188,189] Since the ability of bacteria to develop antibiotic resistance is encoded in their genomes, targeting the relevant transcription factors offers a complementary approach to fighting antibiotic resistance, in addition to the development of new antibiotic compounds. This strategy benefits from recent genomic data as well as from detailed information about the molecular structure of transcription factors.

Several promising results were recently achieved by using either oligonucleotides (McArthur et al., Procarta Biosystems Ltd.)[190] or small drug-like compounds (Baulard et al., BioVersys AG, Riedl et al.)[191,192] as interfering molecules.

5.1. Targeting the Transcription Factor EthR

Baulard et al. delivered an excellent example of how to target a transcription factor by structure-based design in order to positively influence the genetic machinery of a bacterium with respect to its susceptibility against a given antibiotic agent.[191] They targeted the transcriptional repressor EthR, a member of the TetR family of repressors,[193] which blocks the transcription of *ethA*. This gene encodes the monooxygenase EthA, which catalyzes the activation of the prodrug ethionamide in *Mycobacterium tuberculosis* (Mtb).[194]

Ethionamide is recommended by the WHO for the treatment of multidrug-resistant tuberculosis (MDR-TB), but it has a low therapeutic index. Since most of the adverse effects are dose-related, an increase in the bioactivation of ethionamide should reinforce its benefit for the treatment of MDR-TB. The analysis of X-ray data for EthR bound to small-molecule ligands (PDB IDs: 1U9N, 1T56)[195,196] suggested that holoprotein conformations are not able to bind DNA. Selective binders to EthR would therefore inhibit the repression of *ethA* expression. This should in turn boost the activation of ethionamide and thus reduce the amount of prodrug which needs to be administered. The chemical structure and binding orientation of the initial ligands identified in the co-crystal structures (PDB IDs: 1U9N, 1T56; Figure 42) inspired the fragment-based design and synthesis of a focused library of drug-like small molecules.

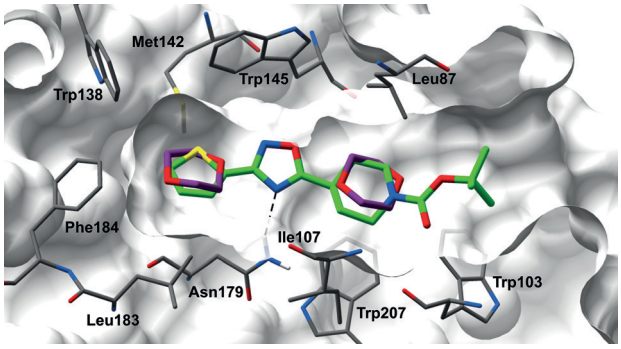
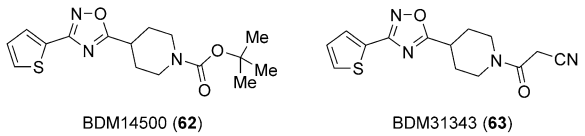


Figure 42. Overlay of the EthR crystal structure containing dioxane fragments (C purple; PDB ID: 1T56) and the co-crystal structure of BDM14500 (**62**; C green) bound to the transcription factor EthR (PDB ID: 3G1O), both from *M. tuberculosis*.

Out of this library, they could identify the initial leads BDM14500 and BDM31343 (**62** and **63**; Figure 43). These compounds inhibit the DNA-binding function of EthR.

Further medicinal chemistry optimization by a structure-based strategy led to compound **64** (Figure 43), which decreases the Mtb load of infected mice three times more effectively than ethionamide alone.[197] These boosters of ethionamide bioactivation are currently being developed toward preclinical testing.[198]



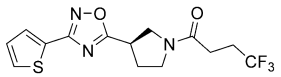


Figure 43. Boosters of ethionamide bioactivation.

5.2. Targeting the Transcription Factor TetR

The transcription factor TetR has been successfully targeted with drug-like small molecules in order to treat tetracycline resistant Gram-positive and Gram-negative pathogens (Bioversys AG in collaboration with our group). These molecules, called transcription regulator inhibitory compounds (TRICs) target transcription factors that are responsible for the development of resistance.[192] The aim is to use TRICs as adjuvants for restoring or potentiating the impact of antibiotics in order to overcome bacterial resistance.[198] Bacteria have developed extensive resistance mechanisms against tetracyclines, including tetracycline efflux, ribosomal protection, and tetracycline modification.[199] The two most dominant mechanisms in clinical settings are ribosomal protection and efflux.[199] The latter is mediated by the membrane transport protein TetA.[200] The expression of this protein is regulated by the Tet repressor (TetR), which binds to the DNA and prevents the transcription of *tetA*.[201] When tetracycline binds to TetR with the aid of Mg^{2+} (Figure 44), such as when it binds to its ribosomal target, the conformation of TetR changes and transcription of the resistance genes take place.[199,201,202]

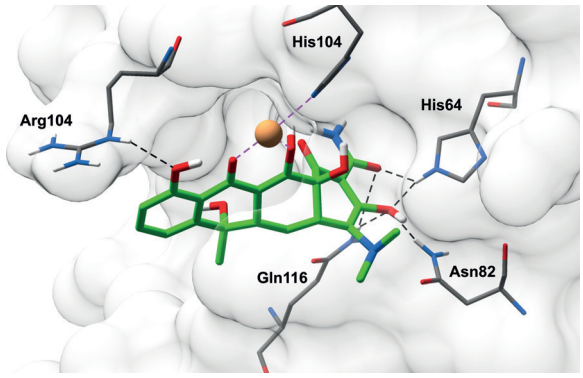


Figure 44. Co-crystal structure of tetracycline (**21**; C green, Mg^{2+} ion light brown) bound to the transcription factor TetR (PDB ID: 2TRT) from *E. coli*.

By following a structure-based approach based on the co-crystal structure of TetR with tetracycline in combination with a DNA/Protein (*tetO*/TetR) interaction assay, it was possible to design and develop compounds ZHAWOC1035 and ZHAWOC1132 (**65** and **66**; Figure 45), which specifically bind to TetR and prevent its release from the DNA in the presence of tetracycline.[192] In combination with tetracycline, these drug-like small molecules were able to inhibit the growth of tetracycline-resistant staphylococcal, enterococcal, enterobacterial, and *acinetobacter* isolates.[192]

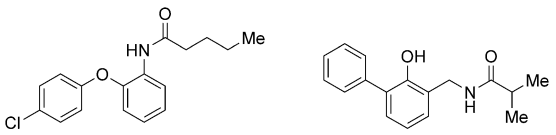


Figure 45. Transcription-factor-binding molecules for restoring tetracycline susceptibility.

6. Summary and Outlook

The alarming spread of antibiotic resistance has been recognized by relevant institutions such as the World Health Organization. After decades of declining efforts by the pharmaceutical industry in their search and development of new antibiotic treatment options, the latest wake-up calls have already brought some increase in activity in this exciting area of multidisciplinary research. Structural and mechanistic understanding of antibacterial targets and deciphering of the bacterial ability for developing antibiotic resistance provide a sound basis for rational campaigns against antibiotic resistance. Screening for new natural or synthetic compounds with antibiotic effects based on novel antibacterial targets still provide one route to outstanding discoveries in the field[203] but structural knowledge enables us to work directly on the resistance mechanisms with increasing success, as shown by some selected examples in this review. A multipronged strategy that involves targeting traditional and emerging antibiotic targets as well as pursuing adjuvant strategies at the genetic level with non-antibiotic compounds for potentiating known antibiotics gives rise to many opportunities to overcome antibiotic resistance. Beyond the basic considerations of the pharmaceutical industry concerning return on investments for the discovery and marketing of new antibiotic treatments, there is the invaluable power of antibiotics, which has helped to save a myriad of lives in the past and which nobody would like to see disappear. Since the level of resources required and the associated costs for developing a new drug molecule are very high and the expected financial gains from antibiotic treatments, which are typically only required for short periods, are relatively low compared to treatments for chronic conditions, the willingness for industrial players to start substantial efforts to discover new antibiotic treatment options remains low. Nevertheless, pharmaceutical companies are needed as strong partners in drug development programs, given their experience and knowledge of the highly complex endeavor of bringing a new drug molecule to the clinic. It is not sufficient to have a compound that kills bacteria on a petri dish. It takes a lot of complex medicinal chemistry optimization in order to be able to use the compound to treat bacterial infections in humans. Whereas the strength and mandate of academic institutions is

to develop novel strategies for ensuring antibiotic treatment options for the future, the final processing of new antibiotic drug molecules and their development through clinical studies is the domain of the pharmaceutical industry. A reasonable approach for integrating these two aspects is a closer interaction between industrial and academic partners in drug discovery for the mutual benefit of both partners and the patients. There are several examples in which major pharmaceutical companies have "opened their doors" and started cooperating very early on with academic groups on drug discovery projects. These "open innovation" collaborations provide easy reciprocal access to resources that the partners would not have otherwise. One characteristic of those open innovation approaches is that they are not focused on a specific drug development program from the beginning but rather screen the best scientific ideas and strategies for new therapeutic indications, including antibiotic resistance. More focused collaborations can then be defined later by research consortia between academic partners, biotechnology companies, and large pharmaceutical companies in order to develop clinical drug candidates. These translational research approaches and public–private initiatives such as the Innovative Medicines Initiative (IMI) can help to overcome the gap between basic research and a marketed drug. In particular, research into new strategies against antibiotic resistance should benefit from this new drug discovery concept, given the relevance of this challenge for public health.

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