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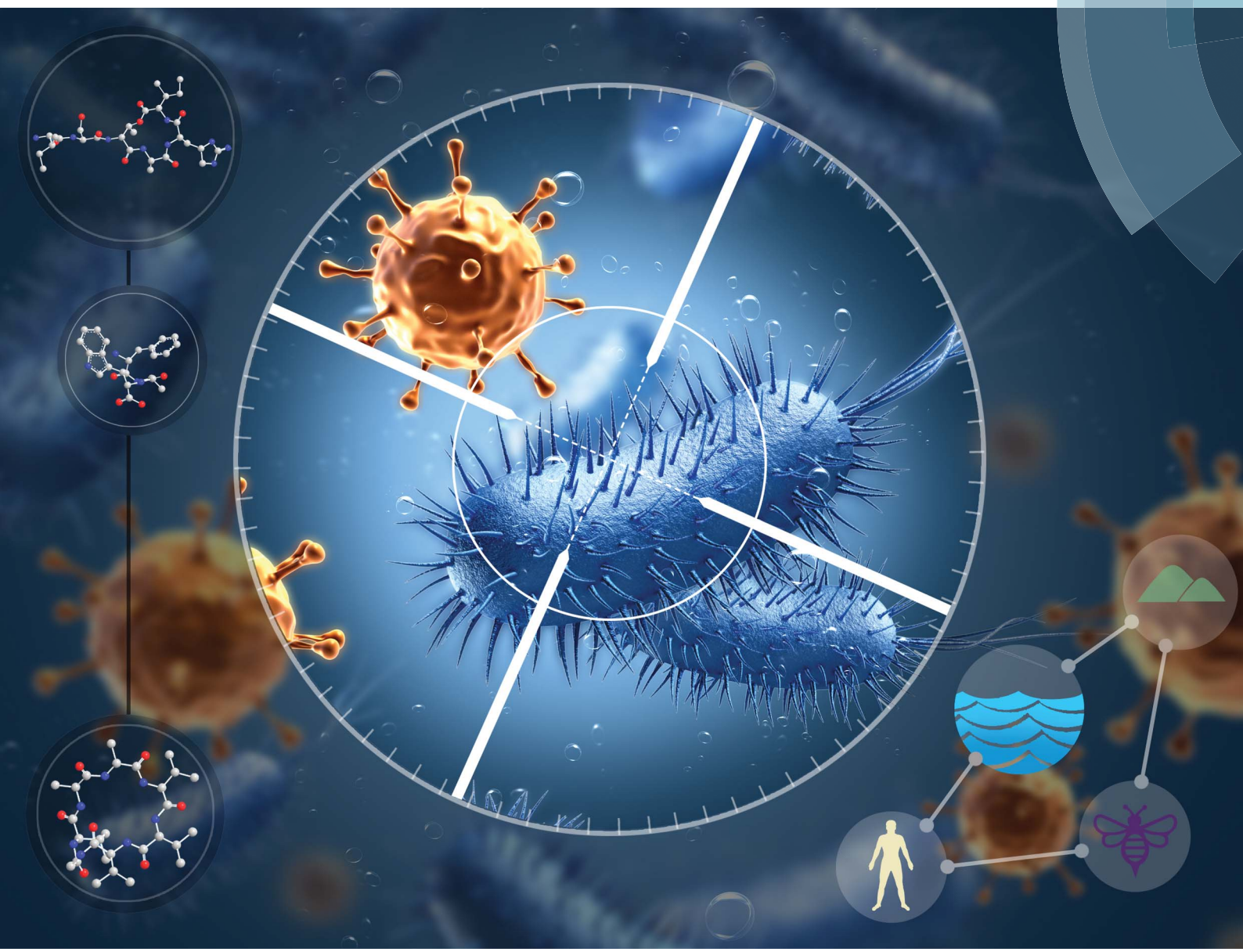

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Nonribosomal antibacterial peptides that target multidrug-resistant bacteria

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Covering: 2000 to 2018, particularly from 2010 to early 2018

The increase in the incidence of antibiotic resistant infections is threatening to overwhelm healthcare practices worldwide. Most antibiotics in clinical use are becoming ineffective, so therefore it is imperative to develop new antibiotics and novel therapeutic strategies. Traditionally, the chemical and mechanistic diversity of nonribosomal antibacterial peptides (NRAPs) as lead compounds have meant that their structures are ideal for antibiotic discovery. Here, we summarize the state of our current knowledge about the mechanisms of antibiotic resistance, which can be used to guide the development of new antibiotics. Furthermore, we provide an overview of NRAPs for treating multi-drug resistant bacteria, including innovative approaches for screening NRAPs from new sources and the underlying mechanisms of antibacterial activity. Finally, we discuss the design of NRAP scaffolds for precise medicine and combinatorial NRAP therapies with existing antibiotics to overcome resistance, which will help to control infections in the post-antibiotic era.

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1 Introduction

The rapid emergence and widespread distribution of antibacterial resistance is now recognized as one of the most serious global threats to human health.[1,2] Consequently, the Centers for Disease Control and Prevention (CDC) recently revealed that more than two million people suffer from antibiotic-resistant infections and at least 23 000 people die as a result per year in the United States alone.[3] Worryingly, the increase in the incidence of multi-drug resistant (MDR) Gram-negative bacteria, such as plasmid-mediated resistance to carbapenems[4] and colistin[5–7] in Enterobacteriaceae, is threatening to overwhelm healthcare practices worldwide. A similar situation has also been observed for Gram-positive bacteria, such as the notorious methicillin-resistant *Staphylococcus aureus* (MRSA)[8] and vancomycin-resistant enterococci (VRE).[9,10] Collectively, it means that no effective antibiotic is available for combating infections caused by either Gram-positive or Gram-negative superbugs. New antibiotics or alternative therapeutics are urgently required for clinical treatments.

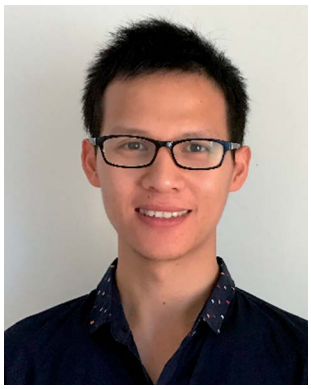
To accelerate the process of antibiotic discovery, we need to develop a mechanistic understanding of the diverse ways in which bacteria survive antibiotic treatments. Such an understanding is critical for developing new antibiotics and designing therapeutic approaches to revitalize existing antibiotics. For example, aspergillomarasmine A, a fungal natural product, selectively removes the zinc ion from metallo-β-lactamases such as NDM-1 and VIM-2, to restore their susceptibility to carbapenems in the treatment of Enterobacteriaceae.[11] Additionally, given that antibiotic-producers are always equipped with self-resistance to avoid suicide, inspired by the co-evolution between antibiotic-producers and diverse competitors in natural niches,[12–14] resistance-based approaches to mining novel antibiotic candidates will be more efficient.

In the golden era of antibiotics, the chemical and mechanistic diversity of antibacterial natural product lead compounds provided interesting and useful scaffolds for antibiotic discovery.[15–17] Heretofore, nonribosomal antibacterial peptides (NRAPs), such as penicillin (the first antibiotic introduced in modern medicine) are well-known as antibiotics in the clinical setting.[18–20] Additionally, vancomycin[21] and colistin[22] are recognized as last resort antibiotics against Gram-positive and Gram-negative pathogens, respectively. NRAPs are a subclass of nonribosomal peptides (NRPs) with antibacterial activities, produced by giant nonribosomal peptide synthetases (NRPSs).[23] In particular, NRPSs are composed of multiple modular sections, each of which is responsible for the incorporation of one defined amino acid (not limited to the 20 proteinogenic amino acids) into the final peptide-like products.[24,25] The flexible biosynthetic mechanism of NRAPs leads to compounds with structural diversity. Nevertheless, the dissemination of multiple resistant genes, such as β-lactamase associated genes and the *van* and *mcr* series of genes,[5,26] has ultimately paralyzed the use of such antibiotics in the clinical setting. Fortunately, new leads and NRAP scaffolds have been continually reported in the past decade, with potent antibacterial activity against MDR bacteria.

In this review, we will first provide a brief overview of the molecular mechanism of antibiotic resistance to better guide antibiotic discovery. Then, we will describe the recent progress on NRAPs, including new sources, methodologies, structure–activity relationships and modes of action. Finally, further perspectives on developing effective NRAPs and their derivatives will also be discussed.

2 Molecular mechanisms of antibiotic resistance

Antibiotics, used either for treating infections in human beings, as growth promoters in food-animal production, or as the waste



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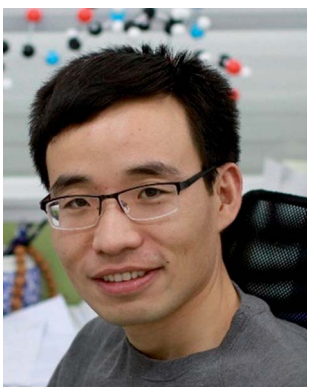

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of pharmaceutical plants, serve as a driving force to select antibiotic-resistant bacteria from the persistent coexistence of antibiotic-sensitive and antibiotic-resistant bacterial strains in natural environments. Bacteria have evolved multiple strategies to tolerate antibiotic treatments. Here, we will focus on the antibiotic resistance of individual cells, including the inactivation and modification of antibiotics, prevention of access to antibiotics and structural changes of antibiotic targets (Fig. 1A). Antibiotic resistance mediated by a bacterial population, such as quorum sensing and formation of biofilm, and by hosts (*in vivo*), such as hiding in the cytosols in the form of a "Trojan horse", will not be discussed here (Fig. 1B).

2.1 Inactivation and modification of antibiotics

Bacteria have evolved various strategies to impair or tolerate antibiotic assaults, of which bacteria can directly break and/or modify the structures of antibiotics to avoid growth inhibition or being killed. Enzymatic degradation and modification are effective means of antibiotic resistance that has brought about resistance to several major classes of existing antibiotics, including β-lactams and aminoglycosides.

The process of hydrolysis, carried out by a diverse range of hydrolases, has been identified to inactivate multiple antibiotics. The co-evolution of β-lactam antibiotics and β-lactamases is an excellent example to illustrate the arms race between antibiotics and antibiotic resistance. The β-lactamases serve as work horses to degrade β-lactam antibiotics, such as penicillins, cephalosporins and carbapenems, by breaking the core β-lactam ring open, through either serine nucleophilic attack or the metal-based activation of a water molecule. For example, the first β-lactamase discovered was penicillinase,[27] which was first isolated from *Escherichia coli* K-12 even before the introduction of penicillin in the clinical setting.

Compared to the β-lactams fused to five-membered rings in penicillins, in cephalosporins such as ceftiofur, the β-lactams fused to six-membered rings were developed to resist such β-lactamases. However, cephalosporins were challenged by the subsequent emergence of a new group of enzymes, the extended-spectrum β-lactamases (ESBLs). Fortunately, carbapenems were introduced for clinical use due to their high stability to ESBLs and other β-lactamases. In turn, the increasing numbers of clinical isolates carrying carbapenemases, such as serine *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM), were observed and became prevalent worldwide. For instance, NDM deactivates the activity of carbapenems by cleaving the β-lactam ring using a Zn^{2+}-activated water molecule. Nowadays, the *ndm* gene has been found to be widespread in the pathogens of Enterobacteriaceae since its first description in 2009.[28] Rapid dissemination of the *ndm* genes that often located on conjugative plasmids is assisted by the extreme mobility of IS *Aba125*, an element upstream of such genes.[29] Additionally, approximately 20 types of NDM variants have been reported from bacterial isolates of both human and animal origins. Recently, NDM-17 was discovered that has three amino acid substitutions (at the V88L, M154L and E170K positions) and was found to significantly confer enhanced carbapenemase activity to tested β-lactam antibiotics, including penicillin G, ceftazidime, ertapenem, imipenem, and meropenem.[30] However, it should be noted that NDMs confer resistance to all β-lactam antibiotics, except for aztreonam, which belongs to the monocyclic β-lactam family of antibiotics.[31]

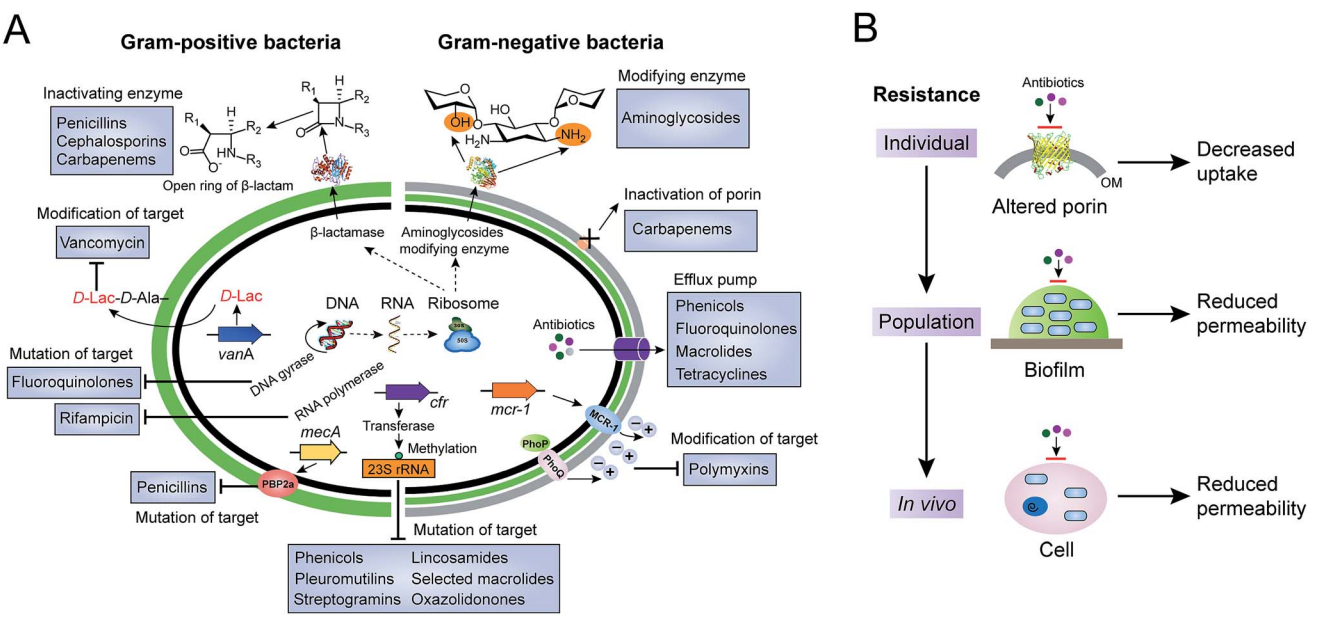


Fig. 1 Molecular mechanisms of antibiotic resistance in Gram-positive and Gram-negative bacteria. (A) Antibiotic resistance in single bacterial cells, including inactivation and modification of antibiotics by enzymes, reduced permeability due to inactivation or down-regulated expression of porins, increased efflux pumps, and mutation or modification of antibiotic targets. (B) Antibiotic resistance at the levels of individual cells, the population and *in vivo*. Bacterial population mediated resistance is known for the formation of biofilm, which serves as a barrier or unfavourable environment against antibiotic treatments. In addition, bacteria can invade and survive in host cells, to circumvent the use of antibiotics.

Unlike β-lactamases, bacterial enzymes can add different chemical groups to vulnerable sites of antibiotics, by preventing modified antibiotics from binding to the corresponding targets. Compared to other antibiotics, aminoglycoside antibiotics tend to be easily modified due to the abundant amide and hydroxyl groups on the surface of aminocyclitol nuclei linked to amino sugars. Nucleotidyl-transferases, phosphotransferases and acetyltransferases are three main classes of aminoglycoside modifying enzymes that can catalyze the modification at different amide or hydroxyl groups of 2-deoxystreptamine nuclei or sugar moieties.[32] Worryingly, a novel genomic island that can encode multiple aminoglycoside-modifying enzymes was found in *Campylobacter* isolates from a food-producing animal origin that conferred high-level resistance to gentamicin and kanamycin.[33] Meanwhile, new aminoglycoside-modifying enzymes are still being discovered, even in nonpathogenic susceptible bacterial species. For example, type VIII and type IX aminoglycoside 3′-*O*-phosphotransferases were reported in *Acinetobacter rudis* and *A. gerneri* in 2017,[34] respectively.

2.2 Prevention of access to antibiotic targets

The accumulation of enough antibiotic in bacteria is a prerequisite for antibacterial activity.[35] Decreasing the concentrations of intracellular antibiotics to increase resistance can be achieved through reduced permeability or enhanced efflux.

Reduced permeability of antibiotics in bacteria can be achieved either in an individual bacterium, by collective behavior or by host cells, as shown in Fig. 1B. Here, we concentrate on the prevention of access to targets in individual bacterial cells. In contrast to Gram-positive bacteria, Gram-negative bacteria are intrinsically resistant to many hydrophilic antibiotics due to the highly impermeable barrier of their outer membrane. Such antibiotics cross the outer membrane by harnessing the porin proteins anchored on the outer membrane.[36] Unlike the previous model of drug-binding sites in the channels of porins, reduced permeability of the outer membrane is modulated either by the down-regulation of porins or by the presence of more-selective porins. Therefore, expression of porin variants or reduced expression of porin related genes leads to antibiotic resistance. For instance, decreased expression of the outer membrane porin D (OprD) causes clinically high-levels of resistance to meropenem (a β-lactam antibiotic) in *Pseudomonas aeruginosa*, in the absence of carbapenemase production.[37] Likewise, inactivation of another main porin, CarO, contributes towards increased resistance to carbapenems in *A. baumannii*.[38]

Efflux pumps are active transporters that contribute to both intrinsic and acquired resistance to antibiotics,[39] particularly in Gram-negative bacteria.[40,41] Compared to efflux pumps with narrow substrate specificity (*e.g.* tetracycline-specific pumps),[42] MDR efflux pumps can transport a wide range of structurally diverse antibiotics.[43] Resistance-nodulation-cell division (RND) pumps are the most important transporters of mediated resistance.[44] A typical RND efflux transporter is located in the inner membrane, which interacts with a periplasmic fusion protein and an outer membrane channel protein to form a tripartite complex, to pump out antibiotics. Upregulation or overexpression of the efflux pump can enhance resistance to various antibiotics.[45] Intriguingly, functionally enhanced pumps with single or multiple amino acid substitutions have also been reported. For instance, a single amino acid substitution (G288D) in an AcrB transporter is sufficient to enhance the efficiency of the pump, resulting in clinically relevant resistance in *Salmonella*.[46] Similarly, a resistance-enhancing variant of the predominant efflux pump CmeABC (RE-CmeABC) was characterized in *Campylobacter*, which confers increased resistance to major classes of existing antibiotics, including chloramphenicol, ciprofloxacin, erythromycin, and tetracycline.[47]

2.3 Structural changes of antibiotic targets

As well as destroying and modifying antibiotics outside or preventing antibiotic access to intracellular targets, bacteria can form resistance by inducing structural changes of antibiotic targets. Such a strategy is a perfect example of the saying "*If you cannot change the world, change yourself*".

Bacteria can circumvent antibiotic therapeutics by altering the original targets, resulting in them being able to survive and cause infections. Similar to the direct modification of antibiotics by various modifying enzymes, protection by modification of antibiotic targets is also widely utilized by different bacterial species. Multiple types of antibiotics can target the ribosome to inhibit or block protein synthesis, such as the classes of phenicols, aminoglycosides and tetracyclines. Correspondingly, methylation of the ribosome by methyltransferases has been characterized in diverse bacterial species to resist their action. For example, wide dissemination of plasmid encoded chloramphenicol–florfenicol resistance (*cfr*) methyltransferase specifically methylates the adenine of position 2503 in the 23S rRNA,[48] which has been observed in the isolates of Gram-positive and Gram-negative pathogens from both human and animal origins.[49–52] Such an enzyme thereby confers resistance to a wide range of antibiotics, including phenicols, pleuromutilins, streptogramins, lincosamides, selected 16-membered macrolides and also oxazolidonones (such as linezolid).

Modifying enzymes play a crucial role in driving resistance to clinically relevant NRAPs, such as polymyxins and daptomycin. Polymyxins, consisting of polymyxin B and polymyxin E (also known as colistin), are positively charged cyclic NRAPs with hydrophobic fatty acid chains.[53] Although colistin has been reported to be responsible for serious toxicity,[54] it has become a last-resort antibiotic against MDR Gram-negative pathogens, particularly for carbapenem-resistant Enterobacteriaceae (CRE),[55] owing to a barren antibiotic development pipeline. The bactericidal activity of colistin is proposed to occur through the disruption of both the outer membrane and cytoplasmic membrane of the bacteria. Due to the wide use of colistin in the clinical setting and in food-producing animals, there has been a rise in colistin resistance. The first plasmid-mediated colistin resistant gene *mcr-1* in Enterobacteriaceae has been reported in China.[5] The *mcr-1* gene encodes phosphoethanolamine (pEtN) transferase in *E. coli*, to catalyze the addition of pEtN of lipid A

in lipopolysaccharides (LPS). As a result, the affinity between colistin and LPS significantly decreases, because the negatively charged lipid A becomes positively charged. Notably, the global distribution of *mcr-1* and a series of variants (*mcr-2/3/4/5/6/7/8*) has been reported,[56–62] which might be due to their high transferability among different bacterial species. Interestingly, a very recent investigation suggested that aquaculture is a potential reservoir of *mcr-1*.[63]

To protect critical antibiotic targets for physiological functions, bacteria may evolve different strategies by which to resist antibiotic stresses. Compared to the modification of lipid A with pEtN, recent studies have shown that reduced colistin binding to lipid A can be achieved through the addition of 2-hydroxymyristate, 4-amino-4-deoxy-L-arabinose and palmitate. For example, deletion of *mgrB* and overexpression of the PhoPQ two-component system increased resistance to polymyxins *via* different lipid A modifications in *K. pneumoniae*.[64,65] Importantly, these modifications in *K. pneumoniae* were accompanied by enhanced virulence through lowering the antimicrobial peptide susceptibility and attenuating the activation of early host defense responses. Additionally, despite daptomycin displaying remarkable selectivity against Gram-positive bacteria, the underlying mechanism of daptomycin resistance is not fully understood. The membrane protein multiple peptide resistance factor (MprF) plays a crucial role in the induction of daptomycin resistance, by transferring lysine to modify the membrane lipid phosphatidylglycerol (PG).[66] Meanwhile, both LiaF and a GdpD-family protein involved in the cell envelope and cell membrane events, also appear to contribute to daptomycin resistance.[67] These results suggest that there is an urgent need to develop new antibiotics to circumvent previously identified targets.

In addition, bacteria can produce alternative elements to mimic the primary targets, offering resistance. For example, MRSA always carries the *mecA* gene, encoding the penicillin binding protein 2a (PBP2a).[68] PBP2a has a low affinity for β-lactam antibiotics, which retains transpeptidase activity. Consistent with this notion, bacteria resist glycopeptide antibiotics such as vancomycin and semisynthetic derivatives by modification of the bacterial cell wall precursor lipid II. Vancomycin specifically binds to lipid II through the formation of a stable complex between the glycopeptide core and the acyl-D-Ala-D-Ala terminus of lipid II, to hinder subsequent building blocks from the penicillin binding proteins (PBPs), thereby inhibiting transglycosylation and transpeptidation.[69] As a consequence, bacteria replace D-Ala-D-Ala with D-Ala-D-Lac, D-Ala-D-Ser or other analogs,[70] with sharply reduced binding affinity, resulting in a corresponding 1000-fold loss in antimicrobial activity. For example, the genotypes of vancomycin-resistant enterococci (VRE) have been characterized, including the gene clusters of *vanA*, *vanB*, *vanC*, *vanD* and *vanE* in clinically relevant isolates.[71] Both *vanA* and *vanB* are two common phenotypes of acquired vancomycin resistance, and encode multiple enzymes to synthesize alternative dipeptide D-Ala-D-Lac replacing the original D-Ala-D-Ala in peptidoglycan synthesis.[72]

Collectively, elucidation of the resistant mechanisms and better understanding of the diverse ways by which bacteria resist clinically useful antibiotics, will shed light on the design of alternative therapeutic approaches and guide the development of new antibiotics.

3 Nonribosomal antibacterial peptides (NRAPs)

NRAPs possess versatile chemical scaffolds, suitable antibacterial activity and unique modes of action,[73] making them potent leads for antibiotic discovery. In the past decade, a diverse range of NRAPs have been identified, as shown in Table 1, which indicates that there are still untapped sources for discovering NRAPs with as-yet unknown functions. We will introduce these new compounds in terms of their sources, screening methods and structure–activity relationships (Fig. 2).

3.1 New sources

Most medically important antibiotics are isolated from terrestrial sources, and soil is still an intermittent source of surprise discoveries. Traditionally, the metabolites of many species of microorganisms in artificial media can be extracted with or without further modification to obtain antibiotic candidates.[74] Benefitting from the rapid development of biotechnology, more previously unidentified and uncultured bacteria from soil have become new producers for NRAPs, such as lysocin E,[75] teixobactin[76] (Fig. 3) and malacidins.[77] Lysocin E was isolated from *Lysobacter* sp. RH2180-5 using the silkworm infection model. Meanwhile, teixobactin was characterized from uncultured *Eleftheria terrae*. This indicates that uncultured bacteria are of importance in potent antibiotic discovery, because uncultured bacteria make up approximately 99% of all species in external environments.[78,79]

Given the tremendous biodiversity of organisms,[80] the marine environment actually represents another prominent source for antibiotic discovery. Sponges, corals and marine animals contain compounds with interesting scaffolds. For example, ilamycins were isolated and identified from deep sea-derived *Streptomyces atratus* SCSIO ZH16, of which the ilamycins E1/E2 (Fig. 3) were found to show highly potent anti-tuberculosis activity against *Mycobacterium tuberculosis*.[112] Furthermore, gageotetrins A–C, isolated from marine *Bacillus subtilis*, are a unique class of linear lipopeptides consisting of repeated Leu and Glu units alongside a fatty acid chain.[102] Most unexploited marine microorganisms have been isolated for a long time away from many bacterial pathogens that inhabit the earth, which may provide a solution to combat MDR pathogens.

Evolution between bacterial pathogens and microbial symbioses of insects and other arthropods, provides other sources for discovering new NRAPs. Paenilamicins are encoded by a hybrid NRPS/PKS biosynthetic gene cluster from the bee pathogen *Paenibacillus larvae*.[100] Paenilamicins are employed by producers to fight ecological niche competitors, resulting in American Foulbrood (AFB), the most destructive bacterial disease for honey bees. Similarly, the isolation and structure elucidation of other antibacterial metabolites from *P. larvae*,[116] such as bacillibactin (siderophores), paenilarvins (iturin-like

Table 1 Representative NRAPs from selected sources[a]

NRAPs	Years of discovery	Producers	Sources	Activity	Targets	Ref.
Penicillin	1928	*Penicillium*	Soil	G^+	PBP	18
Polymyxins	1947	*Paenibacillus polymyxa*	Soil	G^-	LPS	81
Vancomycin	1953	*Amycolatopsis orientalis*	Soil	G^+	Lipid II	82
Daptomycin	1987	*Streptomyces roseosporus*	Soil	G^+	Cell membrane	83,84
A54145	1990	*Streptomyces fradiae*	Soil	G^+	Cell membrane	85
Friulimicins	2000	*Actinoplanes friuliensis*	Soil	G^+	C_{55}-P	86
Bogorol A	2001	*Bacillus* sp.	Marine	MRSA, VRE	Unknown	87
Tolaasins	2004	*Pseudomonas tolaasii*	Soil	G^+	Unknown	88
Mannopeptimycins	2005	*Streptomyces hygroscopicus*	Soil	G^+	Lipid II	89
Bogorols B-E	2006	*Brevibacillus laterosporus*	Marine	MRSA, VRE, *E. coli*	Unknown	90
Tauramamide	2007	*Brevibacillus laterosporus*	Marine	*Enterococcus* sp.	Unknown	91
Sansanmycin	2007	*Streptomyces* sp. SS	Soil	*M. tuberculosis*, *P. aeruginosa*	Translocase I	92
PAX 3	2009	*Xenorhabdus nematophila*	Insect	*M. luteus*	Unknown	93
Entolysin	2010	*Pseudomonas entomophila*	Soil	*S. aureus*	Unknown	94
Pseudofactin	2010	*Pseudomonas fluorescens*	Water	G^+ and G^-	Unknown	95
Battacin	2011	*Paenibacillus tianmuensis*	Soil	G^-	Cell membrane	96
Paenibacterin	2012	*Paenibacillus* sp.	Soil	G^+ and G^-	Unknown	97
Pekiskomycin	2013	*Actinomycetes*	Soil	G^+	Lipid II	98
Taromycin A	2014	*Saccharomonospora* sp.	Marine	G^+	Cell membrane	99
Paenilamicin	2014	*Paenibacillus larvae*	Insect	*P. larvae*	Unknown	100
Sevadicin	2014	*Paenibacillus larvae*	Insect	*B. megaterium*	Unknown	101
Gageotetrins	2014	*Bacillus subtilis*	Marine	G^+ and G^-	Unknown	102
N-Acetylmureidomycins	2015	*Streptomyces roseosporus*	Soil	*P. aeruginosa*	Translocase I	103
Lysocin E	2015	*Lysobacter* sp.	Soil	G^+	Menaquinone	75
Teixobactin	2015	*Eleftheria terrae*	Soil	G^+	Lipid II and lipid III	76
Albicidin	2015[a]	*Xanthomonas albilineans*	Plant	G^+ and G^-	DNA gyrase	104
Cyclohexylgriselimycin	2015	*Streptomyces*	Soil	*M. tuberculosis*	DnaN	105
Tridecaptin A1	2016[b]	*Paenibacillus terrae*	Soil	G^-	Lipid II	106
Humimycins	2016	Unidentified	Human	G^+	Lipid II flippase	107
Telomycin	2016[c]	*Streptomyces canus*	Soil	*S. aureus, B. subtilis*	Cardiolipin	108
Lugdunin	2016	*Staphylococcus lugdunensis*	Human	G^+	DNA, RNA, protein and cell wall	109
Paenipeptins	2017	*Paenibacillus* sp.	Mushroom	G^+ and G^-	Unknown	110
Bacaucin	2017	*Bacillus subtilis*	Soil	G^+	Cell membrane	111
Ilamycins	2017	*Streptomyces atratus*	Marine	*M. tuberculosis*	Unknown	112
Ulleungmycins	2017	*Streptomyces* sp.	Soil	G^+	Unknown	113
Malacidins	2018	Unidentified	Soil	G^+	Lipid II	77
Octapeptin C4	2018	*Bacillus circulans*	Soil	G^-	LPS	114
Odilorhabdins	2018	*Xenorhabdus nematophila*	Nematode	G^+ and G^-	Ribosome	115

[a] a/b/c, albicidin/tridecaptin A1/telomycin were isolated in 1983/1978/1957 and re-elucidated in 2015/2016/2016, respectively. PBP, penicillin binding protein. LPS, lipopolysaccharide. C_{55}-P, undecaprenyl phosphate.

lipopeptides) and sevadicin (nonribosomal tripeptide), have also been reported. Notably, sevadicin (Fig. 3), D-Phe-D-Ala-Trp, is the shortest linear natural NRAP that acts upon bacilli.[101]

Similarly, very recent studies have revealed that human commensal bacteria produce NRAPs against bacterial pathogens, which has been previously reviewed (see ref. 117). For example, lugdunin (Fig. 3) from the human nasal bacteria *Staphylococcus lugdunensis*, has been shown to have selective antibacterial activity against MDR Gram-positive bacteria, including MRSA and VRE.[109]

Although we are facing the dilemma that it's difficult to identify novel compound structures due to increased re-discovery of known antibiotics or their analogues from soil microbes such as *Actinomycetes*, potent antibacterial compounds from other ecological niches may become valuable sources for new types of NRAPs. Taken together, these findings show how attractive NRAPs from new sources are in combating resistant pathogens and give deep insight into the underlying structure–activity relationships.

3.2 Screening methodologies

Screening methods play a critical role in mining potent bioactive compounds. The simplicity and effectiveness of the Waksman platform led to a golden era in antibiotic discovery, contributing to the discovery of most of the existing antibiotics used in the clinical setting. Due to the high re-discovery rates of previous antibiotics and their derivatives, more robust methods

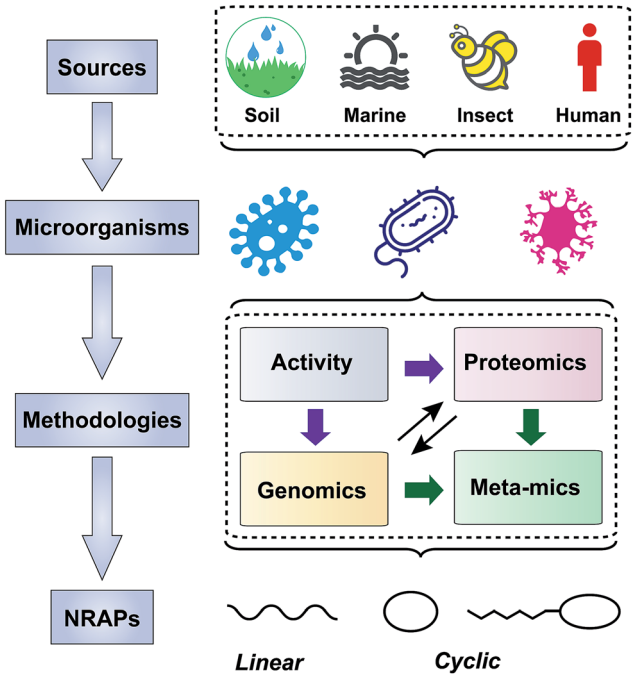


Fig. 2 Scheme of approaches taken to screen NRAPs for combating multi-drug resistant pathogens. The producers are mainly from soil, marine, insect and human microorganisms. Screening strategies are based on activity, genomics, proteomics and meta-mics.

are required to exploit antibacterial leads against antibiotic resistant pathogens from new sources.

3.2.1 Activity guided discovery. The traditional activity-guided approach is still full of vitality for screening new NRAPs. The discovery of lugdunin[109] and bacaucin[111] followed this classic protocol, including the isolation and identification of bacteria, antibacterial testing, separation and purification of active compounds, and structural elucidation. New achievements in biotechnology and chemistry boost such procedures. For example, a multichannel device (iChip) was designed and fabricated to simulate the natural environment of uncultured soil bacteria for antibiotic discovery.[76] Teixobactin produced by *Eleftheria terrae* was successfully identified in this way and was shown to have activity against both MRSA and VRE, and other Gram-positive bacteria. Compared to the small minority of culturable microorganisms *in vitro*, we expect that more NRAPs or other types of antibiotics will be found by culturing non-culturable species.

Importantly, NRAPs with antibacterial activity obtained by *in vitro* screening may be challenged by their inappropriate properties *in vivo*, such as poor activity, severe side effects and pharmacological drawbacks. Therefore, different animal infection models are directly utilized to evaluate the therapeutic effects of identified NRAPs. Insect infection models are more suitable for large scale screening due to low cost, fewer ethical concerns and adequate body size for handling.[118] For instance, lysocin E, a cyclic NRAP, was successfully obtained based on a silkworm infection model. Briefly, 2794 of 14651 (19%) culture supernatants showed inhibition of *S. aureus in vitro*, whereas only 23 of the 2794 (0.8%) supernatants showed therapeutic activity in a silkworm infection model, indicating the high efficiency of such a model in excluding candidates without therapeutic potential *in vivo*.[75]

3.2.2 Genomics driven screening. To obtain natural products of interest, sophisticated strategies are often involved to optimize the production or activation of the biosynthetic gene clusters (BGCs) of NRAPs in a diverse range of microorganisms. However, such approaches are always limited by the fact that only a small proportion of microorganisms can be cultured under artificial conditions, leaving territory that is yet to be explored. Meanwhile, extensive sequencing of bacterial genomics has revealed that the biosynthetic diversity traditionally accessed represents only a small fraction of what is offered by nature.[119] To address these limitations, the high efficiency of genomics driven screening has brought about a new age of NRAP discovery.

The rapid development of DNA sequencing technologies has greatly potentiated the acquisition of genomic data. Candidate BGCs can be identified and analyzed from draft genome sequences using widely used bioinformatics tools such as antiSMASH[120] (antibiotics and secondary metabolite analysis shell), PRISM[121] (prediction informatics for secondary metabolomes), NRPSPredictor2,[122] Minowa[123] and Stachelhaus.[124] These are open access algorithms for predicting genetically encoded NRPs. For example, a cyclic telomycin from *Streptomyces canus* was predicted by directly mining biosynthetic scaffolds using PRISM and characterized with a new antibacterial mechanism by being used to target cardiolipin.[108] Similarly, humimycin was synthesized by solid phase peptide synthesis (SPPS) based on the bioinformatic analysis of the human microbiome, with a unique antibacterial mechanism of targeting the lipid II flippase in MRSA and other Gram-positive bacteria.[107]

Another intriguing challenge is that most microorganisms produce far fewer metabolites of interest under artificial growth conditions than genomes suggest. Many specialized metabolite BGCs are poorly expressed, or not expressed at all, under laboratory growth conditions. Therefore, suitable methods for activating such silent BGCs are a prerequisite to enable the perspective of genomics-driven approaches for the discovery of NRAPs. Summarized strategies to induce the expression of silent BGCs to produce structurally diverse specialized metabolites have been reviewed elsewhere,[119] which are generally categorized into two groups including pleiotropic methods and pathway-specific methods. For example, two chlorinated cyclic hexapeptides (ulleungmycins A and B) were discovered from *Streptomyces* sp. KCB13F003 based on the presence of a cryptic gene cluster encoding NRPS and flavin-dependent halogenase and by manipulating the culture conditions.[113] Interestingly, subinhibitory concentrations of ribosome-targeting antibiotics (*e.g.* chloramphenicol) can enhance the production of NRAPs from *Streptomyces* through global metabolic perturbation.[125] Compared to pleiotropic strategies, pathway-specific approaches provide more genetically stable producers for predictable metabolites through sophisticated operations. Taromycin A, similar to clinically approved daptomycin, was obtained by manipulating pathway-specific regulatory genes in

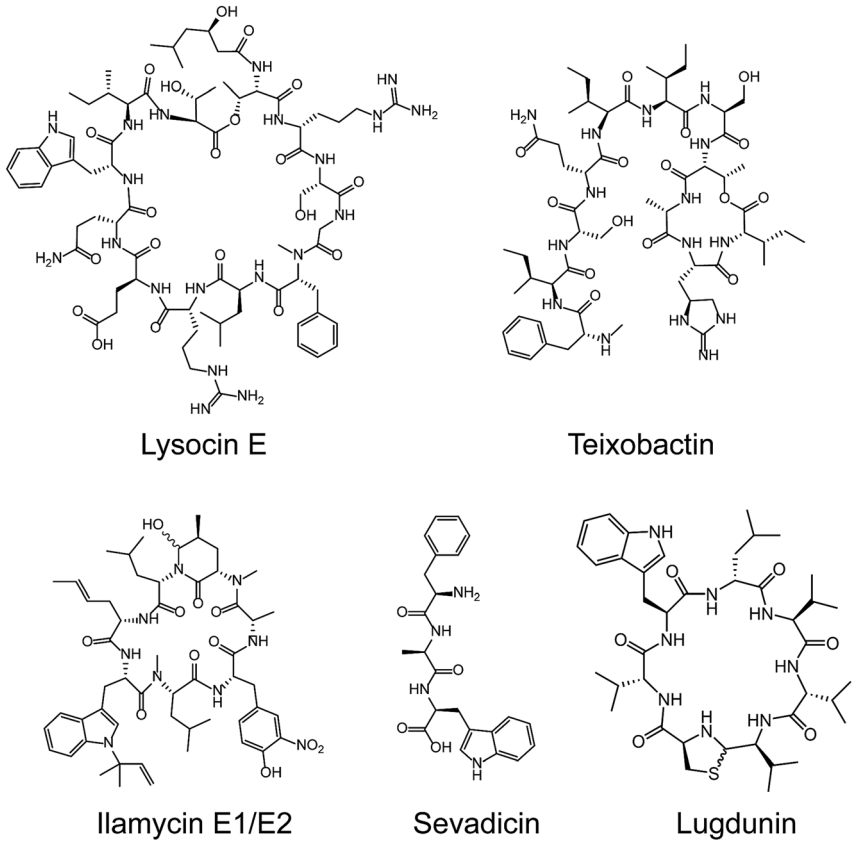


Fig. 3 Representative NRAPs from different sources of bacteria discovered since 2010. Lysocin E (culturable soil), teixobactin (unculturable soil), ilamycin (marine), sevadicin (insect) and lugdunin (human).

marine *Saccharomonospora* sp. CNQ490.[99] The development of gene editing techniques offers alternative methods to awaken silent BGCs, by engineering the transcription and translation machineries, manipulating regulators, replacing natural promoters and heterologous expression. For instance, a one-step robust CRISPR-Cas9 knock-in strategy was established to active multiple silent BGCs in *Streptomycetes* species.[126] Additionally, malacidins, a class of calcium dependent NRAPs, were discovered very recently to integrate the complete malacidin BGC for heterologous expression in *Streptomyces albus*.[77] These results indicate that genomics driven screening may enable the development of a remarkable range of NRAPs and open avenues for the discovery of new antibacterial compounds for applications in medicine and other fields.

3.2.3 Proteomics and meta-omics based discovery. Despite the great achievements made in genomics driven screening, identifying new NRAPs and their biosynthetic pathways still remains a challenge. Proteomics and meta-omics-based methods used as complementary approaches may accelerate the discovery of antibiotics. An intriguing application of proteomics technology is the proteomic investigation of secondary metabolism (PrISM),[127] which has been used to discover a lipoheptapeptide from the *Bacillus* strain NK2018 with a putative structure highly similar to that of the kurstakins[128] and unknown natural products from the hybrid NRPS-PKS zwittermicin A BGC. Meanwhile, an orthogonal active site identification system (OASIS) for the proteomic analysis of PKS/NRPS biosynthetic enzymes has been developed.[129] OASIS probes have proven to be successful for *Bacillus subtilis*, by targeting conserved active sites in NRPS/PKS systems, coupled with analysis through multidimensional protein identification technology (MudPIT) LC-MS/MS analysis. To some extent, PrISM and OASIS approaches represent innovative and complementary methods in NRAP discovery. However, PrISM can be affected by the unanticipated post-lysis proteolytic of NRPSs and its time-consuming and labor-intensive drawbacks limit the synthesis of OASIS probes.

Successes in multi-omics technologies mean that meta-omics is a promising approach for new NRAP discovery. Meta-omics combines the advantages of genomics, proteomics, metabolomics, and transcriptomics analysis tools. The construction of a metagenomics library, direct DNA sequencing and single cell technologies make the sequencing data significantly more available, while bioinformatics facilitates the rapid mining of BGCs.[130] Similarly, metaproteomics consistently provides insights into the discovery and analysis of NRPSs, providing the groundwork for NRAP development.

Inspired by versatile heterologous expression systems and achievements in synthetic biology,[131,132] meta-omic technologies will enable access to a range broad range of NRAPs that have therapeutic applications in medicine.

3.3 Structure–activity relationships

The diverse range of new NRAP scaffolds leads to comprehensive structure–activity relationships (SARs) and sheds light on the design of natural NRAP derivatives with improved antibacterial activity. We focus on the SARs of peptide scaffolds, amino acid types and chemical decorations in terms of antibacterial activity, in either newly identified or previously described NRAPs.

3.3.1 Diverse scaffolds. As discussed in Section 2.1, the evolution between β-lactam antibiotics and β-lactamases provides an excellent example to elucidate SARs and to guide the development of new β-lactam antibiotics. To inactivate the activity of various β-lactam antibiotics, MDR bacteria utilize a diverse range of enzymes to specifically catalyze the ring-opening of the core β-lactam ring, rendering the antibiotic useless. In turn, either five-membered or six-membered rings fused to all bicyclic β-lactams combined with the modification of side chains have been developed to resist hydrolysis, as shown in Fig. 4. However, it has been discovered that NDM overwhelms all bicyclic β-lactam antibiotics, while monolactams, such as aztreonam, are still effective. Meanwhile, the N-sulfonated monocyclic β-lactam ring has been well characterized in sulfazecin, due to the unprecedented activity of the thioesterase domain.[133] Nevertheless, it turns out that such balance is favorable for bacteria, as shown in a very recent report of an isolate of *E. coli* CCD1 of food-animal origin in China, which was found to be resistant to all β-lactams tested, including carbapenems and aztreonam.[134] Although the underlying mechanism of *E. coli* CCD1 resistance to aztreonam remains unclear, it indicates that the time where there are no available β-lactam antibiotics to treat CRE in the clinical setting may soon come.

A wide variety of NRAPs produced by microorganisms, that can be structurally categorized into cyclic and linear scaffolds, have been known for decades and show a wealth of activities. Unlike the ring opening of β-lactam antibiotics, which is ineffective, there has been an increasing number of reports that show that linear NRAPs and their analogs demonstrate comparable or even better antibacterial activity against MDR bacteria. Linear NRAPs represent a new class of antibiotic candidates that are structurally distinct from clinically used cyclic NRAPs, such as daptomycin, polymyxins and vancomycin, and may help to circumvent resistance. Linear NRAPs usually contain short peptide chains and different chemical accessories, in particular modified lipid tails, which have been reported for decades in compounds such as cerxins[135] and tridecaptins.[136] Recently, lots of linear NRAPs have been discovered from a diverse range of sources using different approaches, including lipopeptides such as humimycins, gageotetrins,[102] paenipeptins,[110] (Fig. 5) and peptides without any further modifications, such as bacaucin-1 and sevadicin. For example, the peptide skeletons of humimycins[107] were bioinformatically predicted from primary sequence data of human-associated bacteria and then chemically synthesized by SPPS, and showed moderate antibacterial activity against MRSA and a synergic effect with β-lactam antibiotics (such as carbenicillin and dicloxacillin). Similarly, antibacterial syn-BNP 1 (an N-acylated 13-mer linear peptide) and antifungal syn-BNP 2 (an N-acylated nonapeptide) can be obtained using the same synthetic-bioinformatic natural product (syn-BNP) approach.[137] Interestingly, linear heptapeptide bacaucin-1,[111] a ring-opened

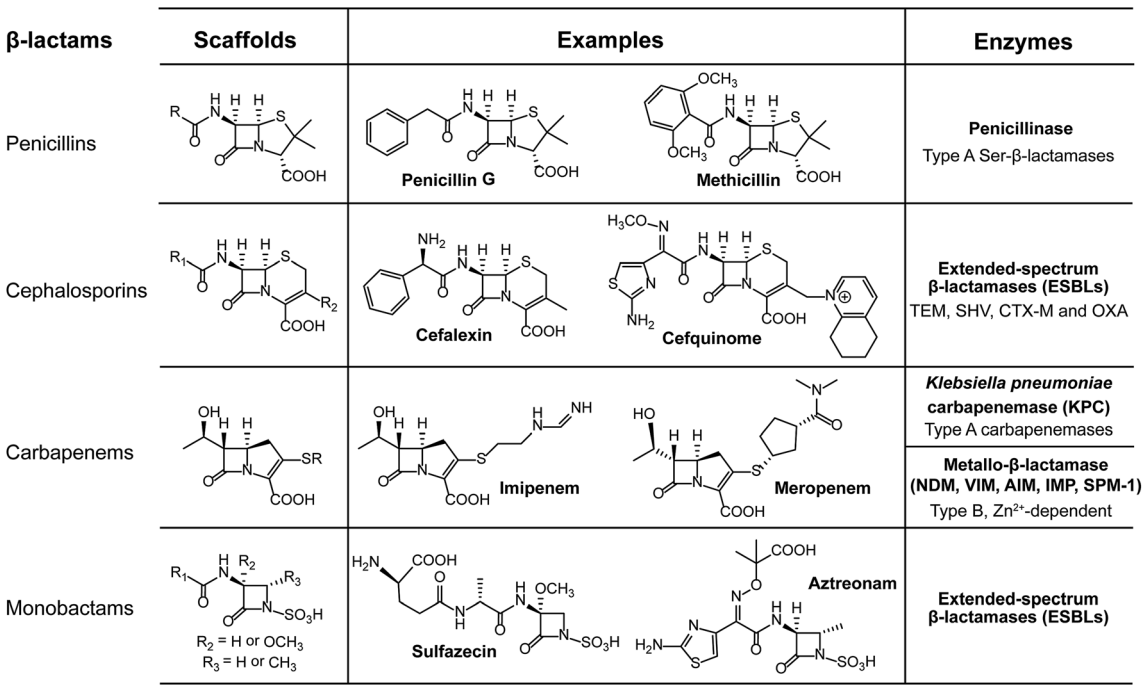


Fig. 4 Co-evolution of β-lactam antibiotics and β-lactamases.

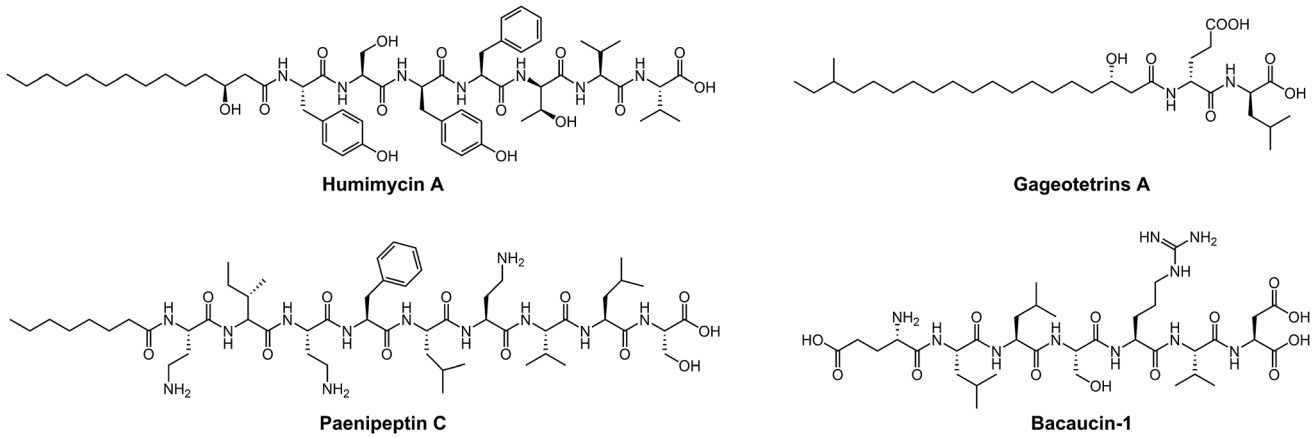


Fig. 5 Linear NRAPs and derivatives from a diverse range of sources. Humimycin A (human microbiome), paenipeptin C (mushroom), gageotetrins A (marine) and bacaucin-1 (chemically synthetic derivative of bacaucin) (soil).

NRAP of bacaucin without lipid modification, has shown specific antibacterial activity against MRSA in both *in vivo* and *in vitro* models. It demonstrates an elegant example of improving antibacterial properties by removing the fatty acid tail and opening the ring structure of the parent compound. Bacaucin-1 consists of all L-type amino acids and the cationic guanidino group under physiological conditions plays a crucial role in its selective activity, suggesting that the design of a linear peptide can be used as an alternative for next-generation precise antibiotics.[138] Notably, sevadicin (D-Phe-D-Ala-Trp), the shortest natural linear tripeptide, has been found, which shows activity against bacilli.[101] Collectively, the absence of a macrocycle within linear NRAPs makes them more easily accessible as they are easier to synthesize.

In addition, nonproteogenic amino acids can also be incorporated into the peptide skeleton, further expanding the diversity of NRAPs and enhancing their antibacterial activities. For instance, teixobactin is able to kill a series of clinically relevant Gram-positive bacteria, including MRSA, VRE, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Clostridium difficile* and *Bacillus anthracis*, with values of minimal inhibitory concentration (MIC) ranging from 0.005 to 0.5 μg mL^{-1}. Such ability is mainly due to the introduction of enduracididine, methyl-phenylalanine and four D-type amino acid residues.[76] Interestingly, the introduction of rare L-3-nitrotyrosine and L-2-amino-4-hexenoic acid endows ilamycins E1/E2 with highly selective anti-tuberculosis activity, with an MIC value of 9.8 nM.[112] Furthermore, lugdunin, the first example of a new class of macrocyclic thiazolidine NRAPs, has been shown to have potent antimicrobial activity against MRSA and VRE, and a wide range of Gram-positive pathogens with MIC values ranging from 1.5 to 12 μg mL^{-1}.[109] However, the detailed SARs of these new identified NRAPs remain largely unclear, and further studies are still required.

3.3.2 Modification and optimization. To optimize clinically used NRAPs, a better understanding of the SARs of such antibiotics will improve their therapeutic properties, either by enhancing their antibacterial efficiency or by decreasing their side effects. For example, vancomycin analog 18 was engineered to combat and improve durability against VRE using peripherally and binding pocket modified vancomycin,[139] which dramatically enhanced its activity, leading to a decrease in the MIC values from 250 μg mL^{-1} to 0.005–0.01 μg mL^{-1} (Fig. 6A). Compared to the approved agent oritavancin, that has the sole introduction of a (4-chlorobiphenyl) methyl (CBP) group to the disaccharide of vancomycin,[140] such modifications offer vancomycin analog 18 great advantages against MDR pathogens. Collectively, these modifications not only improve the capability to disrupt the cell membrane integrity, but also inhibit cell wall synthesis. Interestingly, combining C-terminal modification with peripheral CBP addition to the disaccharide in vancomycin achieves three independent and synergistic mechanisms of action, which significantly increases the fitness cost for VRE to obtain new resistance to fight back against such potent antibacterial leads.

Polymyxins including polymyxin B and polymyxin E (also known as colistin), are narrow-spectrum antibiotics that are used against MDR Gram-negative pathogens, in particular CRE. Importantly, the side effects of polymyxins involve nephrotoxicity and neurotoxicity in the case of long-term high-dose administration, that accompany their antibacterial activity. To reduce their intrinsic toxicity, systematic SARs have been performed to optimize and design new polymyxin analogs, mainly through the modifications of N-terminal fatty acyl chains and amino acid substitutions. For example, analog CB-182 804 (ref. 141) was obtained by deleting the fatty acid chain moiety and adding 2-chlorophenylisocyanate to the N-terminal free amino group in plymyxin B (Fig. 6B). CB-182 804 shows comparable antibacterial activity to that of plymyxin B/colistin, whereas decreased cytotoxicity in the kidney proximal tubule cells of rats was observed with a half maximal inhibitory concentration (IC_{50}) of more than 1000 μg mL^{-1}.[142] On the other hand, reduced cytotoxicity brought about by the replacement of Dab-3 with Gly and using an octanoic acid at the N-terminal was achieved in Analog 38,[143] which retained antibacterial activity with low cytotoxicity to HepG2 cells (IC_{50} > 300 μM). Detailed SARs of polymyxins have been emphasized and recently reviewed in ref. 144. Despite many efforts being made to obtain

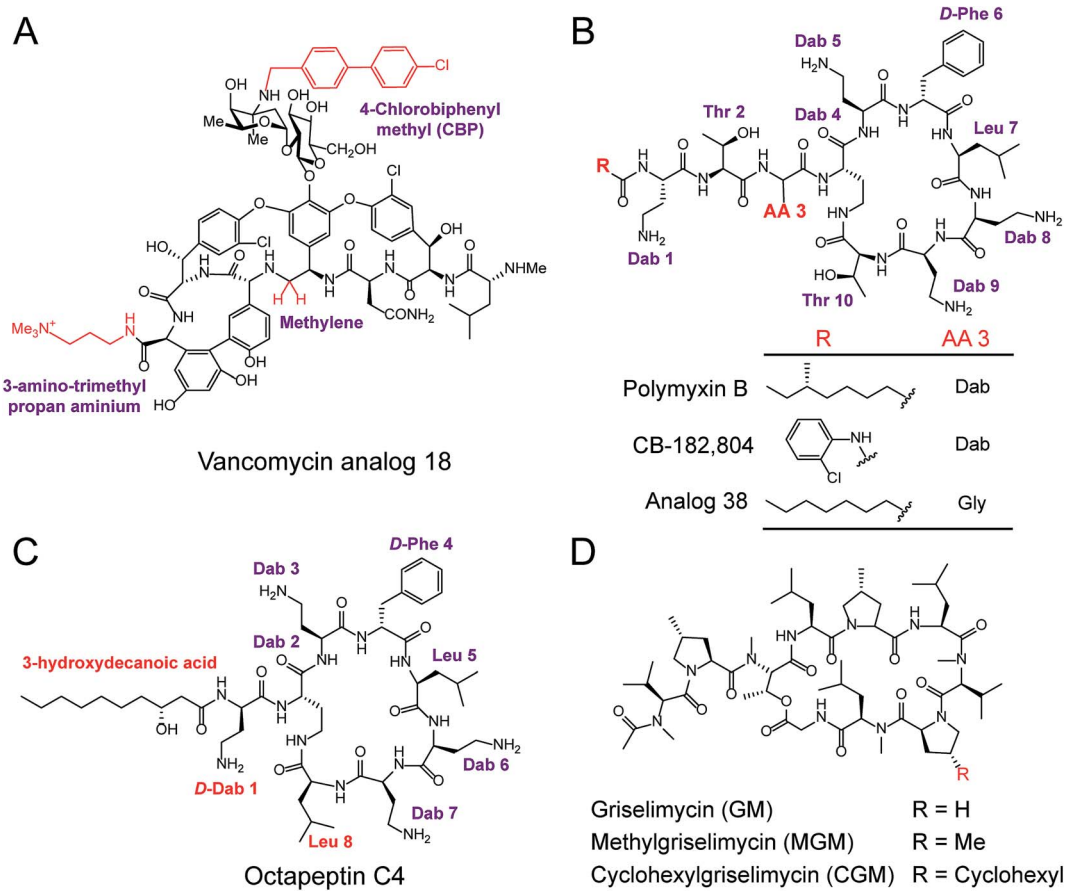
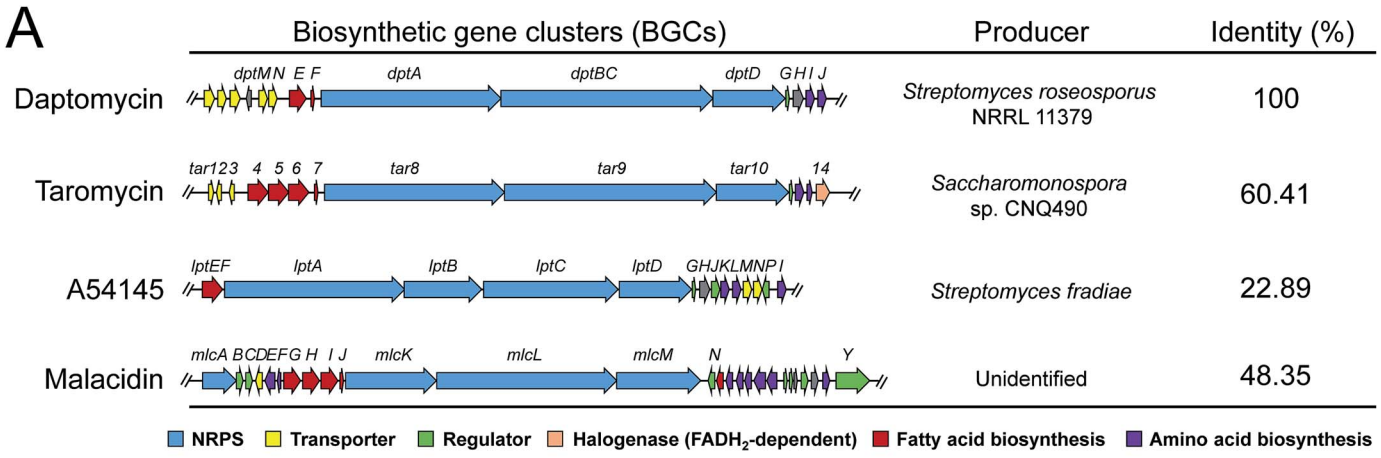


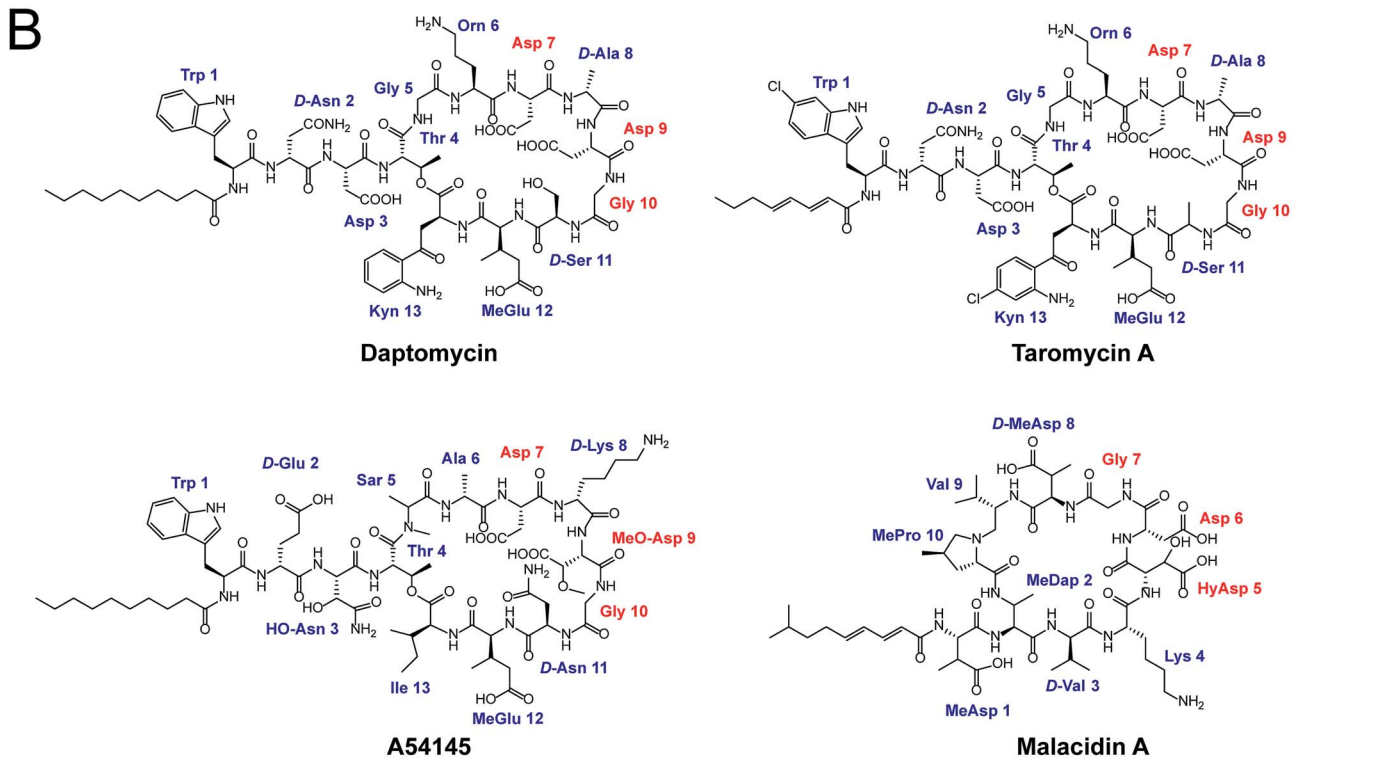
Fig. 6 Modification and optimization of NRAP antibiotics. (A) The peripheral modification of vancomycin exhibits enhanced activity against VRE. (B) Structures of polymyxin B and derivatives synthesized through the modifications of N-terminal fatty acyl chains and amino acid substitutions at the third amino acid residue (AA 3). Structures of octapeptin C4 (C) and the derivatives of griselimycin (D).

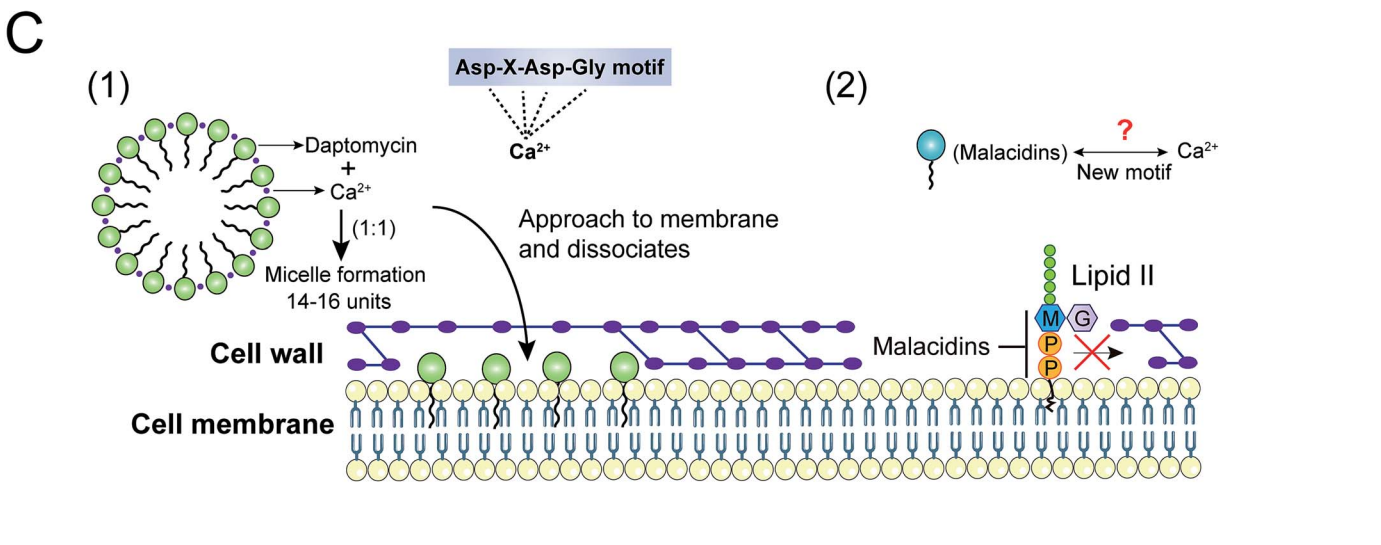
better analogs of polymyxin, unfortunately, no analog has been approved for clinical use. Therefore, further studies and alternative approaches are still required, especially in the mining of natural compounds. For example, octapeptin C4, a colistin-like NRAP, is active against MDR bacteria, including polymyxin-resistant bacteria (Fig. 6C),[114] and also exhibits reduced nephrotoxicity. Compared to polymyxins, octapeptin C4 and its analogs are N-terminally acylated with longer nonanoyl (C9) or decanoyl (C10) β-hydroxy fatty acyl chains. The change in Thr to Leu at position 8 in the heptapeptide ring of octapeptins is the significant difference between polymyxins and octapeptins.

Owing to the urgent need for novel antibiotics and advanced technologies, rich sources of antibacterial lead compounds have been re-discovered to revitalize previously neglected antibiotics. An exciting example of this is the optimization of the cyclic NRAP griselimycin (GM), which is used for tuberculosis therapy.[105] GM from *Streptomyces* with potential antibacterial and antimycobacterial activities has been known for decades,[145] but it still has unfavorable pharmacokinetic properties. Given its high activity, new studies have focused on GM and alkylation of the proline ring at position 8 has been shown to improve its pharmacokinetic properties (Fig. 6D). Attractively, the derivative (CGM) with the addition of a cyclohexyl group on the GM was found to be metabolically stable, and can penetrate the thick mycobacterial cell wall due to increased lipophilicity. CGM exhibited enhanced activity with a MIC value as low as 0.06 μg mL^{-1} and no cross-resistances with current anti-tuberculosis drugs, suggesting that such derivatives possess an unique mechanism of action differing from other mycobactericidal drugs in use.

3.3.3 Calcium-dependent NRAPs. The rich cationic amino acid residues in many NRPAs not only contribute to their antibacterial activity, but also cause unexpected pharmacological effects, such as toxicity, and high affinity to negatively charged serum proteins. The non-cationic scaffolds of NRAPs hold great promise to circumvent such disadvantages. Daptomycin is a cyclic lipopeptide antibiotic encoded by the daptomycin biosynthesis (*dpt*) gene cluster in *Streptomyces roseosporus* (Fig. 7A). Daptomycin was initially used for the treatment of severe skin associated infections caused by Gram-positive pathogens, including MRSA and vancomycin-resistant *S. aureus* (VRSA),[146] and was later extended to systemic and life-threatening infections[147] owing to the lack of available antibiotics in the clinical setting. However, this application is limited for external conditions, due to toxicity and calcium concentration dependent antibacterial activity. Similarly, taromycin A,[99] A54145,[85] (Fig. 7B) and friulimicins[86] are homologous structures of daptomycin, which also show calcium dependent activity







against Gram-positive bacteria. This notion is consistent with the observation that there is a sequence similarity between the *dpt* gene cluster and the *tar*/*lpt* gene clusters, although they contain additional biosynthesis genes in each cluster (Fig. 7A). The conserved peptide motif of Asp-X-Asp-Gly (DXDG)[148] has been proposed as a canonical calcium-binding motif to bind to bacterial membrane that is required for sequential oligomerization and pore formation (Fig. 7C1).

Intriguingly, a new class of calcium-dependent NRAPs, malacidins,[77] are challenging this model. The biosynthesis (*mlc*) gene clusters carry more accessories, suggesting structural differences between daptomycin, A54145 and taromycin A. Malacidins do not contain the DXDG motif incorporated from rare 3-hydroxyl aspartic acid (HyAsp) and also lack the variable spacer residue (Fig. 7B). Additionally, malacidins can specially inhibit lipid II, which indicates that malacidins may harness a new calcium-binding motif (Fig. 7C2). These observations again prove that natural products are, as always, extremely generous in supplying leads to achieve the same therapeutic purpose. Therefore, non-cationic and calcium-dependent NRAPs will be eagerly expected in the future to achieve robust antibacterial activity with reduced side effects.

4 Modes of action

Advanced technologies in chemical biology facilitate the understanding of the mode of action (MOA) of many old NRAPs, such as tridecaptin A_1 and GM, and recently identified ones, such as teixobactin, ilamycin and humimycin. We will summarize the validated targets (Table 1) for advancing hits in NRAP discovery programs, while the involved cellular pathways leading to bacterial death or growth inhibition are not discussed here. Meanwhile, Brown and co-workers have rigorously reviewed the strategies for target identification up to 2015.[149] Heretofore, many unique antibacterial targets have been identified, such as menaquinone, cardiolipin, flippase and translocase I, which open up new avenues to develop leads for combating resistant bacteria. Generally, inhibition of cell wall synthesis, membrane disruption and blocking critical intracellular processes are the major approaches utilized by NRAPs, as shown in Fig. 8.

4.1 Inhibition of cell wall synthesis

The cell wall provides bacteria with both structural support for performing sophisticated physiological tasks and protection against harsh environmental stresses and invaders, and is readily accessible in both Gram-positive bacteria and Gram-negative bacteria. The bacterial cell wall is composed of peptidoglycan, which is made of polysaccharide chains constructed from cross-linked peptides, as shown in Fig. 8. Lipid II and lipid III are two membrane-anchored precursors used for the biosynthesis of peptidoglycan and teichoic acid (a type of polysaccharide) in Gram-positive bacteria, respectively. Thus, different types of NRAPs predominantly target the bacterial cell wall biosynthesis machinery. For example, teixobactin inhibits cell-wall biosynthesis in *S. aureus* by binding to a highly conserved motif of lipid II and lipid III.[76] Given that lipid II is only present in bacteria, it serves as a specific antibacterial target in the absence of cytotoxicity to mammalian cells.[150] Additionally, no resistant *S. aureus* was obtained after serial passage at the sub-inhibitory concentrations of teixobactin. Similarly, malacidins bind to lipid II in a calcium-dependent manner,[77] which is different to the binding of previously characterized calcium-dependent NRAPs such as daptomycin (Fig. 7C).

Hydrophilic lipid II is synthesized in bacterial cytoplasm and is transported by lipid carrier molecules, such as undecaprenyl phosphate (C_{55}-P) and flippases, to cross the cytoplasmic membrane. Correspondingly, friulimicins bind to C_{55}-P to perturb cell-wall biosynthesis.[86] Humimycins have been demonstrated to specifically inhibit lipid II flippase, in particular for MRSA and other *Streptococcus* species.[107]

Although the single layer of peptidoglycan in Gram-negative bacteria is much thinner than that in Gram-positive bacteria, it still a potent target for many NRAPs. The lipopeptide tridecaptin A_1 was first isolated in 1978 and was elucidated in 2016. It exerts a bactericidal effect by selectively binding to lipid II in Gram-negative bacteria.[106] However, tridecaptin A_1 cannot interact with lipid II in Gram-positive bacteria. It remains unclear whether such difference is caused by the change of *meso*-diaminopimelic acid (DAP) in lipid II in Gram-negative bacteria, to lysine in Gram-positive bacteria. Akin to many other lipopeptides, the fatty acid tail, and D-Dab at position 8 are crucial for disrupting the proton motive force (PMF) and killing bacteria. Furthermore, nucleosidyl NRAP involved sansanmycin[92] can selectively target bacterial translocase I (phospho-MurNAc-pentapeptide translocase, also known as MraY), which is essential for the synthesis of lipid I, a key intermediate in mycobacterial peptidoglycan synthesis. And, novel mureidomycin analogues such as *N*-acetyl-mureidomycin are competitive inhibitors of MraY, exhibiting activity against *Pseudomonas aeruginosa*.[103]

4.2 Membrane disruption

Most cationic NRAPs can destroy the bacterial cell membrane by electrostatic interaction. After attachment, the intrinsic fatty acid chains of lipopeptides enable them to efficiently interfere with the most commonly found biological zwitterionic phospholipids in the cell membrane. Impressively, colistin is composed of five positively charged Dab residues and lipophilic moieties. The cationic and hydrophilic region can bind to the outer membrane, in particular lipid A of LPS in Gram-negative

Fig. 7 Biosynthetic gene clusters (BGCs) (A), structures (B) and modes of action (C) of four calcium-dependent NRAPs. The whole biosynthetic gene clusters of daptomycin (AY787762), taromycin (KF301601), A54145 (DQ118863) and malacidin (KY654519) were downloaded from GenBank, and the accession numbers are indicated in parentheses. The identities were analyzed against the daptomycin (*dpt*) gene cluster as a reference, by the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi).

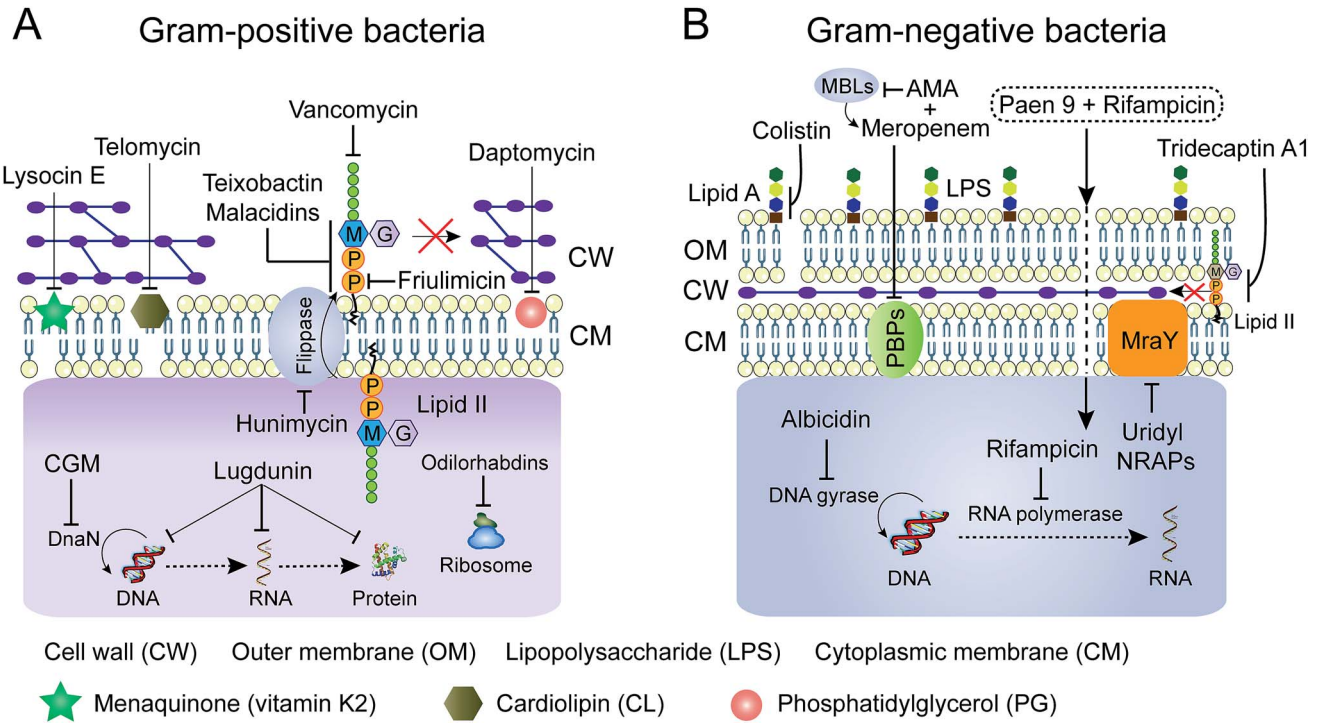


Fig. 8 Schematic representation of mechanisms of NRAPs against Gram-positive (A) and Gram-negative bacteria (B). MBLs, metallo-β-lactamases. AMA, aspergillomarasmine A.

bacteria, displacing the Ca^{2+} and Mg^{2+} ions and causing destabilization of the LPS layer. Furthermore, a new and ambiguous MOA of daptomycin suggests that moderately amphiphilic daptomycin builds up to 14 to16 units upon the addition of a 1 : 1 ratio of Ca^{2+} ions. Subsequently, the complex approaches the cell membrane, dissociates and inserts into the fluid membrane microdomains,[84] which might cause oligomerization and lead to the pores depolarizing the membrane, ultimately leading to bacterial death (Fig. 7C1).[151] Additionally, consistent with the notion that daptomycin targets phosphatidylglycerol (PG) and triggers membrane blebbing in *S. aureus*, it is interesting to observe that the release of abundant PG results in the inactivation of its antibacterial activity (Fig. 8A).[152] Therefore, other bacterial cell membrane phospholipids[153] such as phosphatidylethanolamine (PE) and cardiolipin (CL, also known as diphosphatidylglycerol) are potential candidates for screening new antibiotics. Analogously, telomycin, a predicted cyclic depsipeptide, and its natural analogues, possess a new antibacterial mode of action by inhibiting CL, causing rapid lysis of *S. aureus* and *B. subtilis*.[108,154] Meanwhile, cinnamycin, a tetracyclic antibiotic, binds to PE.[136] Compared to PE and CL, PG is more restricted to bacteria not found in mammalian cells,[155] thereby targeting PG would ensure the low cytotoxicity of antibacterial leads. Besides lipids, the bacterial membrane contains a variety of biological molecules. NRAPs can specifically target certain unique membrane components, to trigger inhibitory or killing processes. Unlike any other antibiotics, for the first time, lysocin E has been validated to target menaquinone to achieve potent bactericidal activity.[75] Menaquinone (vitamin K_2), similar to the electron carrier ubiquinone (coenzyme Q), plays a key role in the electron transport system of respiratory bacteria.[156]

4.3 Targeting intracellular bacterial components

By bypassing the cell wall and cell membrane barriers, the translocation of certain NRAPs into bacterial cytoplasm can disturb various intracellular machineries, which are responsible for crucial cellular processes such as molecular synthesis and enzymatic activity. For example, cyclohexylgriselimycin (CGM, Fig. 6D), a new derivative of griselimycin (GM), displays high activity against *M. tuberculosis* by inhibiting the DNA polymerase sliding clamp DnaN.[105] Binding of CGM to DnaN inhibits the interaction between DnaN and the α subunit of polymerase III (DnaE1), blocking NDA replication, leading to DNA strand breakages. Interestingly, albicidin, encoded by NRPS/PKS machinery from the sugarcane pathogenic bacterium *Xanthomonas albilineans*, is a potent DNA gyrase inhibitor for both Gram-positive and Gram-negative bacteria (Fig. 8B).[104] Albicidin affects the catalytic DNA cleavage–religation cycle, which is distinct from the widely used fluoroquinolones that act by forming complexes between bacterial DNA and gyrase or topoisomerase IV.[157] Furthermore, odilorhabdins, natural linear NRAPs produced by the nematode-symbiotic bacterium *Xenorhabdus nematophila*, exhibit broad bactericidal activities against Gram-positive and Gram-negative pathogens through binding to the new ribosomal site.[115]

In an attempt to reach intracellular components, many antibiotics cannot penetrate the thick cell wall in Gram-positive bacteria or the low permeability of the outer membrane in

Gram-negative bacteria. Intriguingly, several analogues of paenipeptin, such as paen 9 (Fig. 8B), have been shown to increase the activity of rifampicin and clarithromycin against carbapenem resistant and polymyxin resistant pathogens.[158] This indicates that many hydrophobic antibiotics such as rifampicin, which targets DNA-dependent RNA polymerase, can be revitalized in combination with NRAPs that disrupt the membrane integrity and destroy the thick cell wall.

In fact, NRAPs are often versatile enough to affect several cellular events. An exciting example is lugdunin,[13] a new class of macrocyclic thiazolidine NRAP produced by human nasal *S. lugdunensis*. Lugdunin simultaneously inhibits the biosynthesis of DNA, RNA, protein or cell-wall precursors and leads to the rapid collapse of bacterial energy resources. Such synchronous inhibition of multiple targets dramatically increases the fitness cost for bacteria to evolve resistance.

5 Future perspectives

5.1 Challenges and solutions

Although the discovery of NRAPs is blooming, the three crucial obstacles in the development of NRAP candidates for clinical trials are high cost, poor protease stability and nonspecific toxicity in both *in vivo* and *in vitro* models. Most NRAPs have unique structures with complicated decorations and tend to be very expensive drugs. Therefore, leads with simple and non-cationic scaffolds are promising candidates. As shown in Fig. 5, increased numbers of linear lipopeptides or their derivatives in the absence of accessories achieve potent activity against bacterial pathogens. The development of linear NRAPs or analogues *in lieu* of cyclic ones will be economically beneficial, because they can significantly accelerate the synthetic process and thus reduce the cost. Most importantly, a large number of such lead compounds can be produced using the standard protocol of solid-phase peptide synthesis (SPPS). Rationally engineered linear derivatives of paenipeptin A/B/C[110] and bacaucin-1 (ref. 111) reveal that the peptide cyclization is sometimes not essential for their antibacterial activity. Surprisingly, bacaucin-1, synthesized by all natural amino acids by SPPS without any fatty acid tail, shows even better antibacterial activity against MRSA than its cyclic parent bacaucin. Collectively, NRAP inspired synthetic linear peptides represent a new paradigm in the discovery of better antibiotics.

To improve the protease resistance of NRAPs, several approaches have been proposed, including the replacement of natural amino acids with mimics including D-type amino acids, non-natural amino-acid analogues and appropriate formulations, to render them protease resistant. For instance, a recent study showed that $_D(KLAKLAK)_2$, a membrane active all-D-enantiomer antimicrobial peptidomimetic, is resistant to proteolytic degradation and is a great prototype drug that targets certain Gram-negative pathogens.[159] Proteases or other polypeptidases usually recognize specific peptide sequences or certain side chains of amino acid residues to trigger hydrolysis of peptide bonds. For example, trypsin is the work horse in the digestive system of animals and human beings, cleaving peptide chains mainly at the carboxyl sites of lysine or arginine.[160] Thus, rearrangement of the peptide sequence based on the elucidated SAR may provide an alternative way of obtaining candidates.

In practice, the synthesis of most compounds with antibacterial activity is terminated due to their unfavorable pharmacokinetic properties or toxicity. Some antibiotics that are still in use have been continuously criticized for their toxicity, including hemolysis, cytotoxicity, apoptosis and degranulation of mast cells. Long-term and high-dose administration of colistin always results in nephrotoxicity and neurotoxicity.[54] To avoid such nonspecific toxicity, the most straightforward way is to reserve the active site by deleting toxic motifs. By removing the fatty acid chain of bacaucin, lead bacaucin-1 is successfully obtained with improved specificity to target MRSA and no detectable toxicity.[111] As discussed in Section 4.2, lipophilic fatty acid tails are utilized by many lipopeptides to disrupt the integrity of bacterial membrane. To reduce the side effects, nonspecific toxicity can be addressed by masking these tails based on advanced drug delivery systems or pharmaceutical techniques. For instance, 1-dodecanethiol functionalized gold nanodots with surfactin, a cyclic lipopeptide, can not only alleviate the nonspecific cytotoxic and hemolytic activity of surfactin, but also enhance the ability to treat wounds and skin infections caused by MDR bacteria.[161]

5.2 Precise antibiotics

Since the introduction of penicillin for treating bacterial infections, broad-spectrum agents have been advocated due to the lack of sensitive and reliable diagnostic methods and time pressures, which has led to the practice of empirical therapy and overprescription.[16] Nowadays, more and more studies are demonstrating that broad-spectrum antibiotics are being challenged in infection control and prevention, because they can result in serious side effects, including the triggering of hyper-inflammatory responses and, most notably, the disruption of the beneficial microbiome.[162] Therefore, the treatment of infections should be transformed into an era of precise medicine. Precise antibiotics or narrow-spectrum antibiotics, the cornerstone of precise medicine against infections, are urgently required to perform these tasks.

NRAPs and their derivatives are promising candidates for the next generation of precise antibiotics that can selectively target the bacterial pathogen of choice, without destroying the beneficial microbes in the hosts. For example, ilamycins,[112] heptapeptides from marine-derived *Streptomyces atratus*, show selective activity against two mycobacteria, including *M. smegmatis* and *M. tuberculosis*, while they fail to be effective against another six types of bacteria. This indicates that ilamycins are quite prominent as lead compounds for anti-tuberculosis agents. Similarly, bacaucin-1, a heptapeptide derivative of bacaucin, specifically targets MRSA and some *Staphylococcus* species.[111] These findings suggest that NRAPs hold great promise for generating fewer off-target effects on the gut microbiota and decrease the stress that results in the evolution of resistance.

Advanced achievements in the accuracy of point-of-care tests (POCT) and better understanding of the pathogenesis of bacterial pathogens, in particular resistant pathogens, will accelerate the screening, design and development of precise antibiotics. Most notably, the switch to the era of precise antibiotics is not limited to the targeting of specific bacterial components. Alternative strategies for combating bacterial virulent factors and biofilm are promising approaches to promote personalized therapies that exclusively prevent infections.

5.3 NRAP derived adjuvants

Combinatorial treatment consisting of an existing antibiotic and an adjuvant to potentiate antibacterial activity against MDR pathogens offers a potential approach to minimize the emergence of resistance. Consistent with the elucidation of the mechanism of resistance (Fig. 1A) and MOA of NRAPs (Fig. 8), potential adjuvants can be developed by designing inhibitors of enzymes that inactivate or modify antibiotics, by disruption of permeable barriers and by restoration of antibiotic-target affinity, based on a further understanding of the molecular mechanism of resistance. The use of adjuvants to revitalize antibiotics against resistant bacteria is exemplified by the extensive co-administration of β-lactamase inhibitors, such as clavulanic acid,[163] with β-lactam antibiotics, such as amoxicillin. Another exciting example is that of aspergillomarasmine A (AMA), a fungus-derived natural product, and potent inhibitor of metallo-β-lactamases (MBLs) through the chelation of Zn^{2+} ions from clinically relevant NDM and VIM.[11] The synergy between AMA and meropenem fully restores the activity against the enterobacteriaceae, *Acinetobacter* and *Pseudomonas* carrying either VIM or NDM-type alleles, in both *in vitro* and *in vivo* models. Additionally, hydrophilic antibiotics are intrinsically ineffective against Gram-negative bacteria due to their highly impermeable barrier. To reach intercellular components, adjuvants can be developed to tear or disrupt the outer membrane to facilitate the access of antibiotics to the cytoplasm, and ultimately treat infections. For example, paenipeptin analogues at sub-inhibitory concentrations can significantly enhance the antibacterial activity of rifampicin and clarithromycin against *A. baumannii* and *Klebsiella pneumoniae*.[158]

Studies of mechanisms and resistance evolution of new NRAPs and their derivatives are a mandatory requirement in the screening and development of NRAP-based antibiotics and/or adjuvants. Exposure of hidden targets also contributes to the re-sensitization of resistant bacteria to antibiotic therapy. For example, the activity of penicillin can be reversed to kill MRSA in the presence of statins, cholesterol-lowering drugs, through disassembly of penicillin binding protein (PBP2a) oligomerization.[164] In addition, some β-lactam antibiotics such as ceftaroline can specially target PBP2a to destroy the cell wall, which augments the activity of daptomycin against resistant strains in the clinical setting.[165] This indicates that knowledge about the underlying mechanisms of resistance and synergy will certainly facilitate the development of new therapeutics to revitalize existing antibiotics and minimize the emergence of resistance. Such a strategy is crucial in the near future, as new antibiotics or solutions are not likely to enter the clinical setting immediately.

6 Conclusions

The evolution, dissemination and accumulation of multi-resistant pathogens pose a severe threat to human health, and calls for the development of new antibiotics or novel antibacterial strategies. The achievement and development of antibiotics from a diverse range of sources offers promising alternatives to tackle resistance and to treat resistant bacterial pathogen associated infections. Innovative approaches such as high-throughput screening in genome sequencing and bioinformatics tools accelerate the discovery of new NRAPs. Compared to conventional antibiotics, NRAPs are less prone to causing resistance due to their unique mechanisms of action. Furthermore, advances in the understanding of antibiotic resistance at the molecular level will shed light on how to design new scaffolds of NRAPs as precise antibiotics to generate fewer off-target effects on the host microbiome and to minimize the stress that facilitates resistance. Finally, the combination of NRAP based antibiotic adjuvants with existing antibiotics will enhance antibacterial activity and reverse resistance. Such strategy will be well positioned to fill the gap before new antibiotics are introduced into the clinical setting, in particular for infections caused by Gram-negative pathogens, because many NRAPs can disrupt the impermeable outer membrane and increase the accumulation of intracellular antibiotics. Collectively, NRAPs provide highly potent leads for the production of next-generation antibiotics against bacterial pathogens in the resistance era.

7 Conflicts of interest

There are no conflicts to declare.

8 Acknowledgements

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9 References

1 K. Kupferschmidt, *Science*, 2016, **352**, 758–761.
2 J. O'Neill, *Antimicrobial resistance: tackling a crisis for the health and wealth of nations, Review on antimicrobial resistance*, 2014, http://archive.wphna.org/wp-content/uploads/2015/06/2014-UK-paper-on-superbugs-projected-to-2050.pdf.
3 M. McKenna, *CDC Threat Report: We will soon be in a post-antibiotic era*, 2013, https://www.wired.com/2013/09/cdc-amr-rpt1/.
4 T. R. Walsh, J. Weeks, D. M. Livermore and M. A. Toleman, *Lancet Infect. Dis.*, 2011, **11**, 355–362.

5 Y.-Y. Liu, Y. Wang, T. R. Walsh, L.-X. Yi, R. Zhang, J. Spencer, Y. Doi, G. Tian, B. Dong, X. Huang, L. Yu, D. Gu, H. Ren, X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J. Liu and J. Shen, *Lancet Infect. Dis.*, 2016, **16**, 161–168.
6 Y. Wang, G.-B. Tian, R. Zhang, Y. Shen, J. M. Tyrrell, X. Huang, H. Zhou, L. Lei, H.-Y. Li and Y. Doi, *Lancet Infect. Dis.*, 2017, **17**, 390–399.
7 Y. Wang, R. Zhang, J. Li, Z. Wu, W. Yin, S. Schwarz, J. M. Tyrrell, Y. Zheng, S. Wang and Z. Shen, *Nat. Microbiol.*, 2017, **2**, 16260.
8 H. F. Chambers and F. R. DeLeo, *Nat. Rev. Microbiol.*, 2009, **7**, 629–641.
9 R. D. Gonzales, P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten and J. P. Quinn, *Lancet*, 2001, **357**, 1179.
10 E. Tacconelli and M. A. Cataldo, *Int. J. Antimicrob. Agents*, 2008, **31**, 99–106.
11 A. M. King, S. A. Reid-Yu, W. Wang, D. T. King, G. De Pascale, N. C. Strynadka, T. R. Walsh, B. K. Coombes and G. D. Wright, *Nature*, 2014, **510**, 503–506.
12 V. Tracanna, J. A. De, M. H. Medema and O. P. Kuipers, *FEMS Microbiol. Rev.*, 2017, **41**, 417–429.
13 N. Ziemert, M. Alanjary and T. Weber, *Nat. Prod. Rep.*, 2016, **33**, 988–1005.
14 V. M. D'Costa, C. E. King, L. Kalan, M. Morar, W. W. Sung, C. Schwarz, D. Froese, G. Zazula, F. Calmels and R. Debruyne, *Nature*, 2011, **477**, 457–461.
15 A. L. Harvey, R. Edrada-Ebel and R. J. Quinn, *Nat. Rev. Drug Discovery*, 2015, **14**, 111–129.
16 E. D. Brown and G. D. Wright, *Nature*, 2016, **529**, 336–343.
17 J. Clardy, M. A. Fischbach and C. T. Walsh, *Nat. Biotechnol.*, 2006, **24**, 1541–1550.
18 A. Fleming, *Br. J. Exp. Pathol.*, 1929, **10**, 226–236.
19 G. Banko, A. L. Demain and S. Wolfe, *J. Am. Chem. Soc.*, 1987, **109**, 2858–2860.
20 A. R. Awan, B. A. Blount, D. J. Bell, W. M. Shaw, H. Jch, R. M. Mckiernan and T. Ellis, *Nat. Commun.*, 2017, **8**, 15202.
21 *Antibiotic resistance threats in the United States*, CDC webpage, 2013, https://www.cdc.gov/drugresistance/threat-report-2013/index.html.
22 A. Corona and D. Cattaneo, *Clin. Infect. Dis.*, 2017, **65**, 870.
23 M. Strieker, A. Tanović and M. A. Marahiel, *Curr. Opin. Struct. Biol.*, 2010, **20**, 234–240.
24 M. Winn, J. Fyans, Y. Zhuo and J. Micklefield, *Nat. Prod. Rep.*, 2016, **33**, 317–347.
25 C. Walsh, *Nat. Prod. Rep.*, 2016, **33**, 127–135.
26 D. Hughes, *Nat. Rev. Genet.*, 2003, **4**, 432–441.
27 B. Jaurin and T. Grundström, *Proc. Natl. Acad. Sci. U. S. A.*, 1981, **78**, 4897–4901.
28 L. Dortet, L. Poirel and P. Nordmann, *BioMed Res. Int.*, 2014, **2014**, 249856.
29 P. Nordmann, L. Poirel, T. R. Walsh and D. M. Livermore, *Trends Microbiol.*, 2011, **19**, 588–595.
30 Z. Liu, Y. Wang, T. R. Walsh, D. Liu, Z. Shen, R. Zhang, W. Yin, H. Yao, J. Li and J. Shen, *Antimicrob. Agents Chemother.*, 2017, **61**, e02233-16.
31 A. Saxon, A. Hassner, E. A. Swabb, B. Wheeler and N. F. Adkinson Jr, *J. Infect. Dis.*, 1984, **149**, 16–22.
32 M. Ramirez and M. Tolmasky, *Drug Resist. Updates*, 2010, **13**, 151–171.
33 H. Yao, D. Liu, Y. Wang, Q. Zhang and Z. Shen, *Antimicrob. Agents Chemother.*, 2017, **61**, e00112–e00117.
34 E.-J. Yoon, C. Grillot-Courvalin and P. Courvalin, *J. Antibiot.*, 2017, **70**, 400–403.
35 M. F. Richter, B. S. Drown, A. P. Riley, A. Garcia, T. Shirai, R. L. Svec and P. J. Hergenrother, *Nature*, 2017, **545**, 299–304.
36 S. Cowan, T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R. Pauptit, J. Jansonius and J. Rosenbusch, *Nature*, 1992, **358**, 727–733.
37 H. Chalhoub, Y. Sáenz, H. Rodriguez-Villalobos, O. Denis, B. C. Kahl, P. M. Tulkens and F. Van Bambeke, *Int. J. Antimicrob. Agents*, 2016, **48**, 740–743.
38 X. Wu, J. D. Chavez, D. K. Schweppe, C. Zheng, C. R. Weisbrod, J. K. Eng, A. Murali, S. A. Lee, E. Ramage and L. A. Gallagher, *Nat. Commun.*, 2016, **7**, 13414.
39 L. J. Piddock, *Nat. Rev. Microbiol.*, 2006, **4**, 629–636.
40 M. Webber and L. Piddock, *J. Antimicrob. Chemother.*, 2003, **51**, 9–11.
41 H. Nikaido, *J. Bacteriol.*, 1996, **178**, 5853–5859.
42 M. Linkevicius, L. Sandegren and D. I. Andersson, *Antimicrob. Agents Chemother.*, 2016, **60**, 789–796.
43 J. M. Blair, G. E. Richmond and L. J. Piddock, *Future Microbiol.*, 2014, **9**, 1165–1177.
44 E.-J. Yoon, Y. N. Chabane, S. Goussard, E. Snesrud, P. Courvalin, E. Dé and C. Grillot-Courvalin, *mBio*, 2015, **6**, e00309–00315.
45 Z. Shen, X.-Y. Pu and Q. Zhang, *Appl. Environ. Microbiol.*, 2011, **77**, 7128–7133.
46 J. M. Blair, V. N. Bavro, V. Ricci, N. Modi, P. Cacciotto, U. Kleinekathöfer, P. Ruggerone, A. V. Vargiu, A. J. Baylay and H. E. Smith, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 3511–3516.
47 H. Yao, Z. Shen, Y. Wang, F. Deng, D. Liu, G. Naren, L. Dai, C.-C. Su, B. Wang and S. Wang, *mBio*, 2016, **7**, e01543-16.
48 J. Shen, Y. Wang and S. Schwarz, *J. Antimicrob. Chemother.*, 2013, **68**, 1697–1706.
49 G. Morales, J. J. Picazo, E. Baos, F. J. Candel, A. Arribi, B. Peláez, R. Andrade, M.-Á. de la Torre, J. Fereres and M. Sánchez-García, *Clin. Infect. Dis.*, 2010, **50**, 821–825.
50 L. Diaz, P. Kiratisin, R. E. Mendes, D. Panesso, K. V. Singh and C. A. Arias, *Antimicrob. Agents Chemother.*, 2012, **56**, 3917–3922.
51 Y. Liu, Y. Wang, C. Wu, Z. Shen, S. Schwarz, X.-D. Du, L. Dai, W. Zhang, Q. Zhang and J. Shen, *Antimicrob. Agents Chemother.*, 2012, **56**, 1650–1654.
52 Y. Wang, T. He, S. Schwarz, D. Zhou, Z. Shen, C. Wu, Y. Wang, L. Ma, Q. Zhang and J. Shen, *J. Antimicrob. Chemother.*, 2012, **67**, 1094–1098.
53 T. Velkov, P. E. Thompson, R. L. Nation and J. Li, *J. Med. Chem.*, 2009, **53**, 1898–1916.
54 T. Velkov, C. Dai, G. D. Ciccotosto, R. Cappai, D. Hoyer and J. Li, *Pharmacol. Ther.*, 2018, **181**, 85–90.

55 D. Van Duin, K. S. Kaye, E. A. Neuner and R. A. Bonomo, *Diagn. Microbiol. Infect. Dis.*, 2013, **75**, 115–120.
56 W. Yin, H. Li, Y. Shen, Z. Liu, S. Wang, Z. Shen, R. Zhang, T. R. Walsh, J. Shen and Y. Wang, *mBio*, 2017, **8**, e00543-17.
57 A. Carattoli, L. Villa, C. Feudi, L. Curcio, S. Orsini, A. Luppi, G. Pezzotti and C. F. Magistrali, *Eurosurveillance*, 2017, **22**.
58 M. Borowiak, J. Fischer, J. A. Hammerl, R. S. Hendriksen, I. Szabo and B. Malorny, *J. Antimicrob. Chemother.*, 2017, **72**, 3317–3324.
59 B. B. Xavier, C. Lammens, R. Ruhal, S. Kumar-Singh, P. Butaye, H. Goossens and S. Malhotra-Kumar, *Eurosurveillance*, 2016, **21**, 30280.
60 M. Abuoun, E. J. Stubberfield, N. A. Duggett, M. Kirchner, L. Dormer, J. Nunezgarcia, L. P. Randall, F. Lemma, D. W. Crook and C. Teale, *J. Antimicrob. Chemother.*, 2017, **72**, 2745–2749.
61 Y. Q. Yang, Y. X. Li, C. W. Lei, A. Y. Zhang and H. N. Wang, *J. Antimicrob. Chemother.*, 2018, **73**, 1791–1795.
62 X. Wang, Y. Wang, Y. Zhou, J. Li, W. Yin, S. Wang, S. Zhang, J. Shen, Z. Shen and Y. Wang, *Emerging Microbes Infect.*, 2018, **7**, 122.
63 Y. Shen, H. Zhou, J. Xu, Y. Wang, Q. Zhang, T. R. Walsh, B. Shao, C. Wu, Y. Hu, L. Yang, Z. Shen, Z. Wu, Q. Sun, Y. Ou, Y. Wang, S. Wang, Y. Wu, C. Cai, J. Li, J. Shen, R. Zhang and Y. Wang, *Nat. Microbiol.*, 2018, **3**, 1054–1062.
64 T. J. Kidd, G. Mills, J. Sá-Pessoa, A. Dumigan, C. G. Frank, J. L. Insua, R. Ingram, L. Hobley and J. A. Bengoechea, *EMBO Mol. Med.*, 2017, **9**, 430–447.
65 A. Cannatelli, T. Giani, M. M. D'Andrea, V. Di Pilato, F. Arena, V. Conte, K. Tryfinopoulou, A. Vatopoulos, G. M. Rossolini and C. S. Group, *Antimicrob. Agents Chemother.*, 2014, **58**, 5696–5703.
66 A. S. Bayer, T. Schneider and H. G. Sahl, *Ann. N. Y. Acad. Sci.*, 2013, **1277**, 139–158.
67 C. A. Arias, D. Panesso, D. M. Mcgrath, X. Qin, M. F. Mojica, C. Miller, L. Diaz, T. T. Tran, S. Rincon and E. M. Barbu, *N. Engl. J. Med.*, 2011, **365**, 892–900.
68 D. Lim and N. C. Strynadka, *Nat. Struct. Mol. Biol.*, 2002, **9**, 870–876.
69 P. E. Reynolds, *Eur. J. Clin. Microbiol. Infect. Dis.*, 1989, **8**, 943–950.
70 A. Müller, A. Klöckner and T. Schneider, *Nat. Prod. Rep.*, 2017, **34**, 909–932.
71 Y. Cetinkaya, P. Falk and C. G. Mayhall, *Clin. Microbiol. Rev.*, 2000, **13**, 686–707.
72 P. Courvalin, *Clin. Infect. Dis.*, 2006, **42**, 25–34.
73 R. D. Suessmuth and A. Mainz, *Angew. Chem., Int. Ed.*, 2017, **56**, 3770–3821.
74 R. H. Baltz, *Curr. Opin. Pharmacol.*, 2008, **8**, 557–563.
75 H. Hamamoto, M. Urai, K. Ishii, J. Yasukawa, A. Paudel, M. Murai, T. Kaji, T. Kuranaga, K. Hamase, T. Katsu, J. Su, T. Adachi, R. Uchida, H. Tomoda, M. Yamada, M. Souma, H. Kurihara, M. Inoue and K. Sekimizu, *Nat. Chem. Biol.*, 2015, **11**, 127–133.
76 L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schaberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen and K. Lewis, *Nature*, 2015, **517**, 455–459.
77 B. Hover, S. Kim, M. Katz, Z. Charlop-Powers, J. Owen, M. Ternei, J. Maniko, A. Estrela, H. Molina, S. Park, D. Perlin and S. Brady, *Nat. Microbiol.*, 2018, **3**, 415–422.
78 M. S. Rappé and S. J. Giovannoni, *Annu. Rev. Microbiol.*, 2003, **57**, 369–394.
79 K. Lewis, *Nat. Rev. Drug Discovery*, 2013, **12**, 371–387.
80 J. W. Blunt, B. R. Copp, W.-P. Hu, M. Munro, P. T. Northcote and M. R. Prinsep, *Nat. Prod. Rep.*, 2009, **26**, 170–244.
81 P. G. Stansly, R. G. Shepherd and H. J. White, *Bull. Johns Hopkins Hosp.*, 1947, **81**, 43–54.
82 M. H. Mccormick, J. M. Mcguire, G. E. Pittenger, R. C. Pittenger and W. M. Stark, *Antibiot. Annu.*, 1955, **3**, 606–611.
83 F. Ehlert and H. C. Neu, *Eur. J. Clin. Microbiol.*, 1987, **6**, 84–90.
84 A. Müller, M. Wenzel, H. Strahl, F. Grein, T. N. Saaki, B. Kohl, T. Siersma, J. E. Bandow, H. G. Sahl and T. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 7077–7086.
85 L. Boeck, H. Papiska, R. Wetzel, J. Mynderse, D. Fukuda, F. Mertz and D. Berry, *J. Antibiot.*, 1990, **43**, 587–593.
86 W. Aretz, J. Meiwes, G. Seibert, G. Vobis and J. Wink, *J. Antibiot.*, 2000, **53**, 807–815.
87 T. Barsby, M. T. Kelly, S. M. Gagné and R. J. Andersen, *Org. Lett.*, 2001, **3**, 437–440.
88 C. Bassarello, S. Lazzaroni, G. Bifulco, P. Lo Cantore, N. S. Iacobellis, R. Riccio, L. Gomez-Paloma and A. Evidente, *J. Nat. Prod.*, 2004, **67**, 811–816.
89 H. He, *Appl. Microbiol. Biotechnol.*, 2005, **67**, 444–452.
90 T. Barsby, K. Warabi, D. Sørensen, W. T. Zimmerman, M. T. Kelly and R. J. Andersen, *J. Org. Chem.*, 2006, **71**, 6031–6037.
91 K. Desjardine, A. Pereira, H. Wright, T. Matainaho, M. Kelly and R. J. Andersen, *J. Nat. Prod.*, 2007, **70**, 1850–1853.
92 Y. Xie, R. Chen, S. Si, C. Sun and H. Xu, *J. Antibiot.*, 2007, **38**, 158–161.
93 M. Gualtieri, A. Aumelas and J. O. Thaler, *J. Antibiot.*, 2009, **62**, 295–302.
94 V. G. Isabelle, N. Alexey, A. Luis, L. Peter, B. Gérard, P. T. Maria, C. Pierre, K. Christoph, C. Martine and L. Bruno, *Appl. Environ. Microbiol.*, 2010, **76**, 910–921.
95 T. Janek, M. Łukaszewicz, T. Rezanka and A. Krasowska, *Bioresour. Technol.*, 2010, **101**, 6118–6123.
96 C.-D. Qian, X.-C. Wu, Y. Teng, W.-P. Zhao, O. Li, S.-G. Fang, Z.-H. Huang and H.-C. Gao, *Antimicrob. Agents Chemother.*, 2012, **56**, 1458–1465.
97 Y. Guo, E. Huang, C. Yuan, L. Zhang and A. E. Yousef, *Appl. Environ. Microbiol.*, 2012, **78**, 3156–3165.
98 M. N. Thaker, W. Wang, P. Spanogiannopoulos, N. Waglechner, A. M. King, R. Medina and G. D. Wright, *Nat. Biotechnol.*, 2013, **31**, 922–927.
99 K. Yamanaka, K. A. Reynolds, R. D. Kersten, K. S. Ryan, D. J. Gonzalez, V. Nizet, P. C. Dorrestein and B. S. Moore, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 1957–1962.

100 S. Müller, E. Garcia-Gonzalez, A. Mainz, G. Hertlein, N. C. Heid, E. Mösker, H. van den Elst, H. S. Overkleeft, E. Genersch and R. D. Süssmuth, *Angew. Chem., Int. Ed.*, 2014, **53**, 10821–10825.
101 E. Garcia-Gonzalez, S. Muller, P. Ensle, R. D. Sussmuth and E. Genersch, *Environ. Microbiol.*, 2014, **16**, 1297–1309.
102 F. S. Tareq, M. A. Lee, H. S. Lee, Y. J. Lee, J. S. Lee, C. M. Hasan, M. T. Islam and H. J. Shin, *Org. Lett.*, 2014, **16**, 928–931.
103 L. Jiang, L. Wang, J. Zhang, H. Liu, B. Hong, H. Tan and G. Niu, *Sci. Rep.*, 2015, **5**, 14111.
104 S. Cociancich, A. Pesic, D. Petras, S. Uhlmann, J. Kretz, V. Schubert, L. Vieweg, S. Duplan, M. Marguerettaz, J. Noell, I. Pieretti, M. Hugelland, S. Kemper, A. Mainz, P. Rott, M. Royer and R. D. Sussmuth, *Nat. Chem. Biol.*, 2015, **11**, 195–197.
105 A. Kling, P. Lukat, D. Almeida, A. Bauer, E. Fontaine, S. Sordello, N. Zaburannyi, J. Herrmann, S. Wenzel, C. König, N. Ammerman, M. Barrio, K. Borchers, F. Bordon-Pallier, M. Brönstrup, G. Courtemanche, M. Gerlitz, M. Geslin, P. Hammann, D. Heinz, H. Hoffmann, S. Klieber, M. Kohlmann, M. Kurz, C. Lair, H. Matter, E. Nuermberger, S. Tyagi, L. Fraisse, J. Grosset, S. Lagrange and R. Müller, *Science*, 2015, **348**, 1106–1112.
106 S. A. Cochrane, B. Findlay, A. Bakhtiary, J. Z. Acedo, E. M. Rodriguez-Lopez, P. Mercier and J. C. Vederas, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, 11561–11566.
107 J. Chu, X. Vila-Farres, D. Inoyama, M. Ternei, L. J. Cohen, E. A. Gordon, B. V. Reddy, Z. Charlop-Powers, H. A. Zebroski, R. Gallardo-Macias, M. Jaskowski, S. Satish, S. Park, D. S. Perlin, J. S. Freundlich and S. F. Brady, *Nat. Chem. Biol.*, 2016, **12**, 1004–1006.
108 C. W. Johnston, M. A. Skinnider, C. A. Dejong, P. N. Rees, G. M. Chen, C. G. Walker, S. French, E. D. Brown, J. Berdy, D. Y. Liu and N. A. Magarvey, *Nat. Chem. Biol.*, 2016, **12**, 233–239.
109 A. Zipperer, M. C. Konnerth, C. Laux, A. Berscheid, D. Janek, C. Weidenmaier, M. Burian, N. A. Schilling, C. Slavetinsky, M. Marschal, M. Willmann, H. Kalbacher, B. Schittek, H. Brotz-Oesterhelt, S. Grond, A. Peschel and B. Krismer, *Nature*, 2016, **535**, 511–516.
110 E. Huang, X. Yang, L. Zhang, S. H. Moon and A. E. Yousef, *FEMS Microbiol. Lett.*, 2017, **364**, fnx049.
111 Y. Liu, S. Ding, R. Dietrich, E. Märtlbauer and K. Zhu, *Angew. Chem., Int. Ed.*, 2017, **56**, 1486–1490.
112 J. Ma, H. Huang, Y. Xie, Z. Liu, J. Zhao, C. Zhang, Y. Jia, Y. Zhang, H. Zhang and T. Zhang, *Nat. Commun.*, 2017, **8**, 391.
113 S. Son, Y. S. Hong, M. Jang, K. T. Heo, B. Lee, J. P. Jang, J. W. Kim, I. J. Ryoo, W. G. Kim and S. K. Ko, *J. Nat. Prod.*, 2017, **80**, 3025–3031.
114 T. Velkov, A. Gallardo-Godoy, J. Swarbrick, M. Blaskovich, A. Elliott, M. Han, P. Thompson, K. Roberts, J. Huang, B. Becker, M. Butler, L. Lash, S. Henriques, R. Nation, S. Sivanesan, M. Sani, F. Separovic, H. Mertens, D. Bulach, T. Seemann, J. Owen, J. Li and M. Cooper, *Cell Chem. Biol.*, 2018, **25**, 380–391.
115 L. Pantel, T. Florin, M. Dobosz-Bartoszek, E. Racine, M. Sarciaux, M. Serri, J. Houard, J. M. Campagne, R. M. de Figueiredo, C. Midrier, S. Gaudriault, A. Givaudan, A. Lanois, S. Forst, A. Aumelas, C. Cotteaux-Lautard, J. M. Bolla, C. Vingsbo Lundberg, D. L. Huseby, D. Hughes, P. Villain-Guillot, A. S. Mankin, Y. S. Polikanov and M. Gualtieri, *Mol. Cell*, 2018, **70**, 83–94.
116 S. Müller, E. Garcia-Gonzalez, E. Genersch and R. D. Süssmuth, *Nat. Prod. Rep.*, 2015, **32**, 765–778.
117 W. K. Mousa, B. Athar, N. J. Merwin and N. A. Magarvey, *Nat. Prod. Rep.*, 2017, **34**, 1302–1331.
118 S. Panthee, A. Paudel, H. Hamamoto and K. Sekimizu, *Front. Microbiol.*, 2017, **8**, 373.
119 P. J. Rutledge and G. L. Challis, *Nat. Rev. Microbiol.*, 2015, **13**, 509–523.
120 T. Weber, K. Blin, S. Duddela, D. Krug, H. U. Kim, R. Bruccoleri, S. Y. Lee, M. A. Fischbach, R. Müller and W. Wohlleben, *Nucleic Acids Res.*, 2015, **43**, W237–W243.
121 M. A. Skinnider, C. A. Dejong, P. N. Rees, C. W. Johnston, H. Li, A. L. Webster, M. A. Wyatt and N. A. Magarvey, *Nucleic Acids Res.*, 2015, **43**, 9645–9662.
122 M. Röttig, M. H. Medema, K. Blin, T. Weber, C. Rausch and O. Kohlbacher, *Nucleic Acids Res.*, 2011, **39**, W362.
123 Y. Minowa, M. Araki and M. Kanehisa, *J. Mol. Biol.*, 2007, **368**, 1500–1517.
124 T. Stachelhaus, H. D. Mootz and M. A. Marahiel, *Chem. Biol.*, 1999, **6**, 493–505.
125 Y. Tanaka, M. Izawa, Y. Hiraga, Y. Misaki, T. Watanabe and K. Ochi, *Appl. Microbiol. Biotechnol.*, 2017, **101**, 4417–4431.
126 M. M. Zhang, F. T. Wong, Y. Wang, S. Luo, Y. H. Lim, E. Heng, L. Y. Wan, R. E. Cobb, B. Enghiad and E. L. Ang, *Nat. Chem. Biol.*, 2017, **13**, 607.
127 S. B. Bumpus, B. S. Evans, P. M. Thomas, I. Ntai and N. L. Kelleher, *Nat. Biotechnol.*, 2009, **27**, 951–956.
128 C. Y. Chen, F. R. Chang, Y. C. Shih, T. J. Hsieh, Y. C. Chia, H. Y. Tseng, H. C. Chen, S. J. Chen, M. C. Hsu and Y. C. Wu, *J. Nat. Prod.*, 2000, **63**, 1475–1478.
129 J. L. Meier, S. Niessen, H. S. Hoover, T. L. Foley, B. F. Cravatt and M. D. Burkart, *ACS Chem. Biol.*, 2009, **4**, 948–957.
130 M. M. Schofield and D. H. Sherman, *Curr. Opin. Biotechnol.*, 2013, **24**, 1151–1158.
131 A. T. Tucker, S. P. Leonard, C. D. Dubois, G. A. Knauf, A. L. Cunningham, C. O. Wilke, M. S. Trent and B. W. Davies, *Cell*, 2018, **172**, 618–628.
132 A. R. Awan, B. A. Blount, D. J. Bell, W. M. Shaw, J. C. H. Ho, R. M. Mckiernan and T. Ellis, *Nat. Commun.*, 2017, **8**, 15202.
133 R. A. Oliver, R. Li and C. A. Townsend, *Nat. Chem. Biol.*, 2018, **14**, 5–7.
134 Z. Liu, J. Li, X. Wang, D. Liu, Y. Ke, Y. Wang and J. Shen, *Front. Microbiol.*, 2018, **9**, 248.
135 J. Shoji and H. Hinoo, *J. Antibiot.*, 1975, **28**, 60–63.
136 J. Shoji, H. Hinoo, R. Sakazaki, T. Kato, Y. Wakisaka, M. Mayama, S. Matsuura and H. Miwa, *J. Antibiot.*, 1978, **31**, 652–661.
137 X. Vilafarres, J. Chu, D. Inoyama, M. A. Ternei, C. Lemetre, L. J. Cohen, W. Cho, B. V. Reddy, H. A. Zebroski and J. S. Freundlich, *J. Am. Chem. Soc.*, 2017, **139**, 1404–1407.

138 C. de la Fuente-Nunez, M. D. Torres, F. J. Mojica and T. K. Lu, *Curr. Opin. Microbiol.*, 2017, **37**, 95–102.
139 A. Okano, N. A. Isley and D. L. Boger, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, 5052–5061.
140 A. Markham, *Drugs*, 2014, **74**, 1823–1828.
141 R. A. Leese, Patent application WO2010075416, 2010.
142 J. Quale, N. Shah, P. Kelly, E. Babu, M. Backer, G. Rosas-Garcia, J. Salamera, A. George, S. Bratu and D. Landman, *Microb. Drug Resist.*, 2012, **18**, 132–136.
143 A. Gallardogodoy, C. Muldoon, B. Becker, A. G. Elliott, L. H. Lash, J. X. Huang, M. S. Butler, R. Pelingon, A. M. Kavanagh and S. Ramu, *J. Med. Chem.*, 2016, **59**, 1068–1077.
144 F. Rabanal and Y. Cajal, *Nat. Prod. Rep.*, 2017, **34**, 886–908.
145 B. Terlain and J. P. Thomas, *Bull. Soc. Chim. Fr.*, 1971, **6**, 2363–2365.
146 J. N. Steenbergen, J. Alder, G. M. Thorne and F. P. Tally, *J. Antimicrob. Chemother.*, 2005, **55**, 283–288.
147 Y. Chuang, H. Lin, P. Chen, C. Lin, J. Wang, Y. Chen and S. Chang, *Clin. Infect. Dis.*, 2017, **64**, 1026–1034.
148 M. Strieker and M. A. Marahiel, *ChemBioChem*, 2009, **10**, 607–616.
149 M. A. Farha and E. D. Brown, *Nat. Prod. Rep.*, 2016, **33**, 668–680.
150 S. F. Oppedijk, N. I. Martin and E. Breukink, *Biochim. Biophys. Acta*, 2016, **1858**, 947–957.
151 L. Robbel and M. A. Marahiel, *J. Biol. Chem.*, 2010, **285**, 27501–27508.
152 V. Pader, S. Hakim, K. L. Painter, S. Wigneshweraraj, T. B. Clarke and A. M. Edwards, *Nat. Microbiol.*, 2016, **2**, 16194.
153 C. Sohlenkamp and O. Geiger, *FEMS Microbiol. Rev.*, 2016, **40**, 133–159.
154 C. Fu, L. Keller, A. Bauer, M. Brönstrup, A. Froidbise, P. Hammann, J. Herrmann, G. Mondesert, M. Kurz and M. Schiell, *J. Am. Chem. Soc.*, 2015, **137**, 7692–7705.
155 N. Malanovic and K. Lohner, *Biochim. Biophys. Acta*, 2016, **1858**, 936–946.
156 A. Upadhyay, F. Fontes, M. Gonzalezjuarrero, M. R. Mcneil, D. C. Crans, M. Jackson and D. C. Crick, *ACS Cent. Sci.*, 2015, **1**, 292–302.
157 D. Jonas, I. Engels, D. Hartung, J. Beyersmann, U. Frank and F. D. Daschner, *J. Antimicrob. Chemother.*, 2003, **51**, 275–280.
158 S. H. Moon, X. Zhang, G. Zheng, D. G. Meeker, M. S. Smeltzer and E. Huang, *J. Med. Chem.*, 2017, **60**, 9630–9640.
159 D. M. McGrath, E. M. Barbu, W. H. Driessen, T. M. Lasco, J. J. Tarrand, P. C. Okhuysen, D. P. Kontoyiannis, R. L. Sidman, R. Pasqualini and W. Arap, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 3477–3482.
160 J. V. Olsen, S. E. Ong and M. Mann, *Mol. Cell. Proteomics*, 2004, **3**, 608–614.
161 W. Y. Chen, H. Y. Chang, J. K. Lu, Y. C. Huang, S. G. Harroun, Y. T. Tseng, Y. J. Li, C. C. Huang and H. T. Chang, *Adv. Funct. Mater.*, 2015, **25**, 7189–7199.
162 Y. R. Nobel, L. M. Cox, F. F. Kirigin, N. A. Bokulich, S. Yamanishi, I. Teitler, J. Chung, J. Sohn, C. M. Barber and D. S. Goldfarb, *Nat. Commun.*, 2015, **6**, 7486.
163 C. Reading and M. Cole, *Antimicrob. Agents Chemother.*, 1977, **11**, 852–857.
164 E. Garcia-Fernandez, G. Koch, R. M. Wagner, A. Fekete, S. T. Stengel, J. Schneider, B. Mielich-Suss, S. Geibel, S. M. Markert, C. Stigloher and D. Lopez, *Cell*, 2017, **171**, 1354–1367.
165 W. E. Rose, L. T. Schulz, D. Andes, R. Striker, A. D. Berti, P. R. Hutson and S. K. Shukla, *Antimicrob. Agents Chemother.*, 2012, **56**, 5296–5302.