## Natural Product Reports

## REVIEW

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## Bacterial pathogens: threat or treat (a review on bioactive natural products from bacterial pathogens)<sup>†</sup>

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Threat or treat? While pathogenic bacteria pose significant threats, they also represent a huge reservoir of potential pharmaceuticals to treat various diseases. The alarming antimicrobial resistance crisis and the dwindling clinical pipeline urgently call for the discovery and development of new antibiotics. Pathogenic bacteria have an enormous potential for natural products drug discovery, yet they remained untapped and understudied. Herein, we review the specialised metabolites isolated from entomopathogenic, phytopathogenic, and human pathogenic bacteria with antibacterial and antifungal activities, highlighting those currently in pre-clinical trials or with potential for drug development. Selected unusual biosynthetic pathways, the key roles they play (where known) in various ecological niches are described. We also provide an overview of the mode of action (molecular target), activity, and minimum inhibitory concentration (MIC) towards bacteria and fungi. The exploitation of pathogenic bacteria as a rich source of antimicrobials, combined with the recent advances in genomics and natural products research methodology, could pave the way for a new golden age of antibiotic discovery. This review should serve as a compendium to communities of medicinal chemists, organic chemists, natural product chemists, biochemists, clinical researchers, and many others interested in the subject.

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## 1. Introduction

Antimicrobial resistance (AMR) is amongst the major threats to public health and poses a huge economic burden on global health care. The World Health Organization (WHO) has recently published the priority list of drug-resistant bacteria that pose the greatest danger to human health,<sup>1,2</sup> and among these, a majority of Gram-negative bacteria, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and Enterobacteriaceae. Resistance has emerged to all clinically used antibiotics



Dr Fleurdeliz Maglangit finished her Bachelor of Science in Chemistry at the University of the Philippines in the Visayas Miagao Iloilo. With her continued pursuit for learning, she obtained two Masters degrees, Master in Chemistry and Master of Science in Environmental Studies. She just recently earned her Ph.D. in Chemistry at the University of Aberdeen, Scotland, UK last July 2020. Her PhD

work was mainly focused on natural products discovery and biosynthesis. In her research, she was able to isolate novel compounds with excellent antibacterial bioactivities from Streptomyces bacteria. Currently, she is an Assistant Professor in Chemistry at the University of the Philippines Cebu. She hopes to discover novel bioactive natural products with potential for further drug development using local samples.



Dr Yi Yu obtained his Bachelor's and Master's degrees in Biotechnology (2002) from Huazhong Agricultural University. He received his Ph.D. degree in Genetics (2007) at the Institute of Microbiology, Chinese Academy of Sciences (CAS). He then undertook postdoctoral research with Professor Wen Liu at Shanghai Institute of Organic Chemistry CAS. In 2010, he joined the School of Pharmaceu-

tical Sciences, Wuhan University, and was promoted to professor in 2016. Now, he is the Director of the Institute of Traditional Chinese Medicine and Natural Products. His research interests centre around deciphering the biochemical logic and molecular machinery of bacterial and plant natural product biosynthesis. including those of "last-resort" such as colistin and polymyxin B, and continues to rise at alarming rates.<sup>3,4</sup>

Despite the severity of the situation, the number of new chemical entities in the antibiotic development pipeline is in substantial decline. Nearly all the classes of antibiotics currently in clinical use were discovered during the 'golden era' (1940s–1960s), with several new drugs that are chemically tailored analogues from existing scaffolds.<sup>5</sup> The problem is compounded by the fact that bacteria are evolving resistance at a faster pace than antibiotic development.<sup>6,7</sup> The last new class of antibiotics that target the Gram-negative bacteria are the synthetic fluoroquinolones which were introduced into the clinic about 50 years ago.<sup>8,9</sup> The high rate of the rediscovery of old known molecules in traditional natural product (NP) screening platforms makes this grim situation even worse. Thus, the research community must find new sources of NPs to cope with the looming antibiotic crisis.

Pathogenic bacteria have shown to be rich sources of novel compounds, yet they remained untapped and understudied.<sup>10-13</sup> Virulence factors involved in their pathogenicity have been the subject of extensive study for many decades.<sup>14-23</sup> In recent years, however, it has become apparent that entomopathogenic, phytopathogenic and human and animal pathogenic bacteria are prolific sources of structurally novel and highly bioactive druggable molecules.<sup>11,12</sup>

Threat or treat? While pathogenic bacteria pose a threat to insects, plants, and humans, they also represent gold mines of potential pharmaceuticals to treat various diseases.<sup>11,12,24,25</sup> The opportunistic human pathogen, *Staphylococcus aureus* is a classic example. Despite being a threat, they produce potent bacteriocins (also known as staphylococcins) and several other compounds active against a wide variety of Gram-positive bacteria.<sup>25</sup>



Dr Hai Deng studied chemistry for his Bachelor's and Masters' degrees in China. In 1999, he came to the UK to pursue his Ph.D. in the field of biochemistry and biotransformations at the University of Wales, Swansea. From 2002–2008, he was a postdoctoral researcher of Professor David O'Hagan in University of St Andrews. He was appointed as a lecturer at the Department of Chemistry, University of Aber-

deen in 2008, and promoted to senior lecturer in 2014 and reader in 2018. His current research includes discovery of novel bioactive natural products from various sources, tracing biosynthesis pathways, identifying novel enzyme activities and enzyme mechanism. He became the Fellow of Royal Society of Chemistry in 2017.

studies and Microbial genome-level metabolomic approaches have further revealed the untapped biosynthetic potential of the diverse and underexplored group of pathogenic bacteria. Bacterial genomics has shown that they not only encode for virulence factors but also potential leads for drug development.<sup>11,12</sup> However, it has been estimated that only a very small portion of this gold mine had just been discovered, and that further drug leads or pharmacophores could be mined given the application of suitable and sufficient resources.<sup>11</sup> Thus, this review intends to explore the role that pathogenic bacteria could play in the search for novel compounds and scaffolds. This review should serve as a compendium to communities of medicinal chemists, organic chemists, natural product chemists, biochemists, clinical researchers, and many others interested in the subject.

### 2. Scope of the review

This review surveys the natural products (NPs) isolated from entomopathogenic, phytopathogenic, human, and animal pathogenic bacteria with antibacterial and/or antifungal activity, highlighting those NPs or NP-modified molecules currently in pre-clinical trials or those with potential for future drug development. These include the polyketides (PKSs), nonribosomal peptides (NRPs), peptide–polyketide hybrid metabolites, and ribosomally-synthesised and post-translationally modified peptides (RiPPs). Selected unique and interesting pathways involved in their biosynthesis and the key roles they play in pathogenesis (where known) are also summarized.

Entomopathogenic bacteria such as *Photorhabdus* spp., *Xenorhabdus* spp., and *Serratia marcescens* are the focus of the review. The period from 2017 to the second quarter of 2020 saw a huge rise in the number of bioactive NPs from *Photorhabdus* spp. and *Xenorhabdus* spp. that are not covered in previous synopses,<sup>12,26</sup> and thus they are the emphasis in our review. It is worth noting that the honeybee pathogen, *Paenibacillus larvae* also appears as a rich, yet largely understudied source of novel and structurally diverse NPs. The readers are referred to the review by Müller, *et al.* (2015) which details the metabolites identified from *P. larvae.*<sup>27</sup> Although a rich source, no new metabolite has been identified from this bacterium since 2015.

Phytopathogenic bacteria such as Burkholderia spp., Clostridium puniceum, Dickeya spp., Erwinia amylovora, Pseudomonas syringae, Streptomyces scabies, and Xanthomonas spp. are among the prolific NP producers, and thus they are the topic of this review. The NPs from the diverse genus Burkholderia is summarized in a recent review.28 Another review provided the genomics perspective of NP biosynthesis in phytopathogenic bacteria E. amylovora, Xanthomonas spp., S. scabies, P. syringae, and Dickeya spp.11 Hence, in this review we aim to update and complement previous synopses and cover only those NPs that show the most interesting bioactivities or those that have not been mentioned by Baldeweg, et al. (2019)11 or Kunakom and Eustáquio (2018).28 Furthermore, we included the phytopathogen C. puniceum not mentioned in the above reviews for it produces potent metabolites with antimicrobial activity in nanomolar concentration.

We also explore the human and animal pathogenic bacteria such as Nocardia spp., Staphylococcus spp., Streptococcus mutans, and Yersinia ruckeri as sources of antimicrobials with therapeutic potential. These bacteria have been shown to produce structurally diverse NPs with potent bioactivities.<sup>29-33</sup> The antimicrobials from Nocardia spp. and bacteriocins from Staphylococcus spp. have been summarized in recent reviews,25,33 and thus those NPs with remarkable activities from these bacteria were highlighted. Finally, we provide a thorough compilation of the antimicrobial NPs from bacterial pathogens, Burkholderia spp., C. puniceum, Dickeya spp., E. amylovora, Nocardia spp., Photorhabdus spp., P. larvae, Pseudomonas spp., Staphylococcus spp., S. marcescens, S. mutans, Streptomyces spp., Vibrio spp., Xanthomonas spp., Xenorhabdus spp., and Yersinia ruckeri (see Table S1 in the ESI<sup>†</sup> of this article listed in alphabetical order). We also provide their mode of action (molecular target), activity, and minimum inhibitory concentration (MIC) towards bacteria and fungi (where known), in the pursuit to demonstrate the exceptional biosynthetic ingenuity of the underexplored source of pathogenic bacteria for the production of novel and druggable chemical entities.

# 3. Pathogenic bacteria as novel sources of antimicrobial discovery

Pathogenic bacteria are master engineers of highly diverse and biologically active molecules. To thrive and survive in highly competitive and resource-limited microbial communities, pathogenic bacteria have developed an approach to protect themselves by producing a plethora of structurally diverse metabolites that have been fine-tuned by the producing organism to have potent and selective biological activities.<sup>25,34</sup> It is believed that pathogenic bacteria exploit these molecules to regulate virulence and persistence during infections. Additionally, the vast array of antibacterial armamentarium is thought to fight off predators, compete for nutrients, and protect their host. Other roles have also been suggested such as signalling and quorum sensing, gene expression, stress response, cellular growth and iron acquisition.<sup>12,35</sup>

Pathogenic bacteria represent exceptionally prolific sources of potential therapeutics as indicated in their genomes, yet they have been largely ignored.<sup>11,36</sup> Here, we present an overview of the antimicrobial NPs produced by entomopathogenic, phytopathogenic, and human and animal pathogenic bacteria, and highlight a selection of metabolites with antibiotic activity that show promising potential for future development (Fig. 1).

#### 3.1 Entomopathogenic bacteria

Previously regarded as overlooked and neglected sources, the entomopathogenic bacteria have received considerable interest in the last 15 years owing to the novel druggable chemical entities they generate.<sup>13,34</sup> Those that have been described recently as prolific NP producers include *Photorhabdus* spp. and *Xenorhabdus* spp., *S. marcescens*, and *P. larvae*.

Members of the genera *Photorhabdus* and *Xenorhabdus* (*Enterobacteriaceae*) produce a wide array of NPs to support



Fig. 1 Overview of pathogenic bacteria. Despite a threat to insects, plants, animals, and humans, pathogenic bacteria represent novel sources of potential pharmaceuticals to treat various diseases.

a complex life cycle involving insect pathogenesis and nematode symbiosis with Heterorhabditis spp. and Steinernema spp., respectively.37 The antimicrobial compounds produced by these bacteria are non-toxic to the nematode, but lethal to several insect pathogens and other opportunistic microbes that are direct food competitors.24 This indicates the production of antimicrobials with favourable toxicity, good pharmacokinetics, and are likely druggable and safe to eukaryotic organisms. Serratia marcescens is a Gram-negative, facultatively-anaerobic bacterium (Enterobacteriaceae) often associated with insect infection.38 Several insects are susceptible to Serratia species, including crickets, grasshoppers, locusts, cockroach, termites, beetles, butterflies, moths, fruit fly, wasps,<sup>39</sup> and recently has been discovered as being pathogenic to bees.<sup>40</sup> Some members of S. marcescens also cause opportunistic nosocomial infections of the respiratory tract, urinary tract, brain, meninges, heart, and wounds.<sup>39,41</sup> Despite a threat, S. marcescens has been shown to produce not only the characteristic red pigment prodigiosin but also a huge repertoire of antimicrobial compounds.<sup>41</sup> Paenibacillus larvae is a Gram-positive bacterium that causes fatal intestinal infection of honeybee larvae, called American Foulbrood (AFB). This pathogen spreads very rapidly and poses various threats of different severity leading to massive losses of entire bee colonies. P. larvae secretes a broad spectrum of antibacterial compounds that are critical virulence factors and also, relevant in the quest for new bioactive compounds for drug development. Readers are referred to the recent review by Müller, et al.27

It should be mentioned that several other entomopathogenic bacteria such as *Bacillus thuringiensis* and *Pseudomonas ento-mophila* have the capacity to produce NPs based on their genome sequences but have not been mined further for NP production.<sup>42,43</sup>

#### 3.2 Phytopathogenic bacteria

Plant pathogenic bacteria can have detrimental effects on plant growth, productivity, and yield. They affect a wide range of crops posing a threat to global food production. Hundreds of phytopathogenic bacteria have been identified to date,<sup>44–46</sup> but only a few have been explored for natural product discovery.<sup>11</sup>

Clostridium puniceum, the only known plant pathogenic bacterium from the diverse genus Clostridium to date,44-46 causes potato slimy rot, manifested by the formation of pink pigments by the bacterium.47 All Dickeya species (formerly Erwinia chrysanthemi) cause economically important diseases on different plant hosts worldwide.14,48 D. zeae causes soft rot in a variety of plants (e.g. potato, chicory, maize, banana, rice). Erwinia amylovora is the causative agent of fire blight, a destructive disease of Rosaceae plants such as apple and pear trees<sup>49</sup> that is typically accompanied by the development of black necrosis.<sup>50</sup> Historically, E. amylovora is the first characterised bacterial plant pathogen.<sup>51</sup> Pseudomonas spp. produce a wide spectrum of phytotoxic compounds. P. syringae pathovars are the topmost phytotoxic-producing bacteria among all Pseudomonas, and all phytopathogens identified to date.14,52 Streptomyces species are particularly renowned for their ability to produce numerous bioactive NPs.53-58 Several Streptomyces strains, however, are phytopathogenic and can cause potato common scab diseases such as S. caviscabies, S. acidiscabies, S. turgidiscabies, and S. scabies.21,59 Among the most notable pathogens of the genus Xanthomonas are X. albilineans, the causative agent of leaf scald disease on sugar cane<sup>60</sup> and X. campestris, the causal agent of black rot of crucifers that affects all cultivated brassicas.<sup>14</sup> Members of the genus Burkholderia include strains that can either be beneficial or harmful. Some strains are pathogenic to plants such as B. glumae, which causes rice rot, while others cause opportunistic human infections such as the strains of Burkholderia cepacia complex (Bcc), which

include *B. pseudomallei* and *B. mallei*. For detailed information on the diverse *Burkholderia* genus, refer to the recent review.<sup>28</sup>

Virulence-mechanisms of plant pathogenic bacteria have been the subject of several different reviews.<sup>14,21,28,52,59</sup> Despite being a threat to agriculture, phytopathogens *C. puniceum*, *Dickeya* spp., *E. amylovora*, *Pseudomonas* spp., *Streptomyces* spp., *Xanthomonas* spp., *Burkholderia* spp. – some of which belong to the top 10 most important plant pathogenic bacteria<sup>14</sup> – also serve as huge arsenals for potent drug leads. Genome analyses disclosed that their biosynthetic machinery encodes not only for virulence factors but also for antibiotic-like metabolites with no plant disease-associated function.<sup>11</sup> Furthermore, some phytotoxins were found to exhibit potent antimicrobial properties.<sup>11,28,47</sup>

#### 3.3 Human and animal pathogenic bacteria

While the antimicrobials from non-pathogenic strains are studied in-depth, knowledge of the structural and mechanistic diversity of antibiotics particularly from human and animal pathogenic bacteria is limited. Here, we provide an overview of the potential chemistry to be uncovered from the opportunistic pathogens, *Nocardia* spp., *Staphylococci, S. mutans, Vibrio* spp., and *Y. ruckeri*.

Many different species of *Nocardia* have been identified, and many of these are pathogenic to humans and animals. To date, more than 50 *Nocardia* species are clinically significant.<sup>61</sup> Of these, *N. brasiliensis*, *N. abscessus*, *N. transvalensis*, *N. terpenica*, and *N. pseudobrasiliensis* have been identified to be prolific microbial sources of bioactive novel compounds.<sup>33</sup>

*Staphylococci* represent the normal flora of the skin and mucous membrane of human and animals.<sup>62</sup> There are more than 40 species, but few are important human pathogens such as *S. aureus, S. epidermidis, S. haemolyticus, S. lugdunensis,* and *S. saprophyticus* implicated in various infections, especially in immunocompromised patients.<sup>18</sup> Though they pose a threat, they are also prolific producers of potent bacteriocins (also known as staphylococcins) exhibiting antibacterial activity against closely related species and a wide variety of Grampositive bacteria.<sup>25,63</sup>

*Streptococcus mutans* is the major causative agent of human dental caries (tooth decay).<sup>64</sup> In addition to caries, *S. mutans* is also implicated in infective endocarditis, a lethal infection, and inflammation of heart valves.<sup>65</sup> Bacterial sequence analysis of *S. mutans* discloses a small genome (about 2 Mb) yet surprisingly harbours rich and diverse biosynthetic gene clusters (BGC) for the production of PKS, NRPS, hybrid PKS–NRPS, and RiPP metabolites.<sup>66,67</sup> Several bioactive NPs have recently been isolated from *S. mutans*.<sup>30,68–78</sup>

Vibrionaceae includes several species that cause intestinal (diarrhoea, cholera) and extra-intestinal (septicaemia, skin infection) illnesses in both humans and aquatic animals. Among the opportunistic *Vibrio* pathogens, *V. parahaemolyticus* has been shown to produce metabolites with remarkable bioactivity.<sup>79</sup>

Yersinia ruckeri is the etiological agent of yersiniosis or enteric redmouth (ERM) disease in marine and freshwater fish, particularly salmonids.<sup>17</sup> Infections due to *Y. ruckeri* cause high mortalities in fish, contributing to substantial economic losses in the aquaculture industry.<sup>80</sup> *Y. ruckeri* has also been isolated from human wound infection, however, it remains unclear whether *Y. ruckeri* or another bacterium caused the infection.<sup>81</sup> Interestingly, *Y. ruckeri* has been shown to produce the dithiolopyrrolone natural product, holomycin.<sup>31,32</sup>

# 4. Chemical diversity of antimicrobials produced by pathogens

Pathogenic bacteria produce numerous NPs with highly diverse structures made up of a handful of simple building blocks, usually derived from one or more primary metabolic pathways. These NPs can be classified into five different groups according to their biosynthetic origin: polyketides, nonribosomal peptides, polyketide–nonribosomal peptide hybrid metabolites, ribosomal peptides, and others. Since numerous NPs from pathogenic bacteria are known, only selected compounds with promising therapeutic potential are presented.

#### 4.1 Polyketides

Polyketides, assembled by polyketide synthases (PKS), are among the largest classes of chemically diverse NPs, encompassing molecules such as macrolides, aromatics, and polyenes. The structural diversity exhibited by polyketides is exemplified by the broad spectrum of biological activities they possess, such as antibacterial, antifungal, and anticancer among others (Fig. 2 and Table S1<sup>†</sup>). PKSs occurring in bacteria are classified into three types (type I, II, and III) depending upon their structure and biochemistry. Type I PKSs are large multifunctional enzymes comprised of multiple functional domains as exemplified by borrelidin 1, gladiolin 2, erythromycin 3, and brasilinolide A 4. Type II PKSs are formed by discrete catalytic domains and are responsible for the biosynthesis of bacterial aromatic polyketides such as clostrubins 5-6 and nocardicyclin A 7. Type III PKSs are simpler chalcone synthase-like proteins that catalyse the formation of the product within a single active site. Examples include chalcones, resorcinol, pyrones, and stilbenes (Fig. 2 and Table S1<sup>†</sup>).

Polyketides are biosynthesised from two-carbon acetate units derived from activated acetyl-CoA and malonyl-CoA in successive decarboxylative Claisen condensation reactions, in a manner analogous to fatty acid biosynthesis. Typically, this process involves the core domains comprising of the ketosynthase ( $KS_{\alpha}$  and  $KS_{\beta}$ ), malonyl/acyl transferase (AT), and a phosphopantethienylated acyl carrier protein (ACP) which serves as an anchor for the growing PK chain.<sup>82</sup> A series of post-PKS tailoring enzymes such as ketoreductase (KR), methyltransferase (MT), enoyl reductase (ER), and dehydratase (DH) can variously modify the polyketide backbone, either while the intermediates are still bound to the assembly line or after they are released. Installation of different polyketide starter and extender units also represents a significant route to add unusual moieties such as nitrile functionality, carboxylates, and



Fig. 2 Examples of antimicrobial polyketide natural products with unusual chemical motifs highlighted in red, isolated from pathogenic bacteria.

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branched-alkyl chains into polyketide scaffolds to generate mature final products with a high degree of chemical complexity and activity. The mechanistic enzymology of diverse polyketide assembly lines has been the subject of comprehensive reviews.<sup>82,83</sup> This section covers some representatives of interesting polyketide antimicrobials containing unusual chemical functionalities from pathogenic bacteria such as PKSI borrelidin **1**, PKSII clostrubins **5** and **6**, and stilbene-containing PKSIII metabolites **8–11**.

**4.1.1 Borrelidin.** Borrelidin **1** was first isolated from *Streptomyces rochei* in 1949 as an antibiotic exhibiting anti-*Borrelia* activity,<sup>84</sup> and then more recently as a product of the potato pathogen *Streptomyces* GK18<sup>85</sup> and other *Streptomyces* species<sup>86–90</sup> as well as marine-derived microorganisms (Fig. 2 and Table S1†).<sup>91–94</sup> Borrelidin **1** features an 18-membered macrolide with a nitrile functionality.<sup>95,96</sup> To date, numerous analogues have been discovered including borrelidins B-O,<sup>87,90,91,93,94</sup> acetyl-borrelidin<sup>89</sup> as well as amide containing congeners, borrelidin CR1 and CR2.<sup>92,93,97</sup>

More than 30 nitrile-containing pharmaceuticals are currently marketed for a wide range of medical indications, including vildagliptin for diabetes and anastrozole for breast cancer treatment.<sup>98</sup> The nitrile functionality renders the molecule more water-soluble and less susceptible to oxidative metabolism in the liver.<sup>98</sup> Furthermore, nitrile moiety is rare in natural products, hence the biosynthetic mechanism of borrelidin, particularly the nitrile group has attracted significant interest. The biosynthesis of borrelidin proceeds through the typical pathway known for type 1 PKS to form the macrolide ring except for the unique *trans*-cyclopentane-(1*R*-2*R*)-dicarboxylic acid (CDPA) starter unit (Fig. 3). CDPA is likely derived from

tyrosine or 4-hydroxyphenyl acetic acid (4-HPA) catabolism.86 The nitrile formation in 1 may start from oxidation of the pendant methyl group in pre-borrelidin 1c to an aldehyde 1e catalysed by cytochrome P450, BorI, and alcohol dehydrogenase, BorK. This is followed by the conversion of the aldehyde to aminomethyl group (borrelidin B) 1b catalysed by the putative aminotransferase, BorJ.94 BorJ is related to CynN1 and CyaN1 aminotransferases in nitrile-containing cyanosporasides that typically act upon carbonyl groups, catalysing conversion to amines.99 The aminomethyl intermediate 1b is finally converted to the nitrile catalysed by the putative BorI and BorK enzymes via a series of oxidation and dehydration reactions. Mutants obtained by inactivation of either BorI or BorJ failed to generate any borrelidin but led to the production of pre-borrelidin 1c, suggesting that BorI/I are responsible for nitrile biosynthesis.<sup>86,94</sup> Furthermore, the isolation of borrelidin B 1b from a marine-derived Streptomyces strain supports the plausible mechanism of nitrile formation.94

Borrelidin is a potent threonyl-tRNA synthetase inhibitor.<sup>100</sup> Borrelidin **1** is active against a wide range of bacteria, including *Enterococcus faecalis, Micrococcus luteus, Enterococcus faecium, Proteus hauseri*, and *Klebsiella pneumoniae* (MIC = 0.5–65  $\mu$ M).<sup>90,91,93</sup> Additionally, borrelidin exhibits 3× potent activity against *Salmonella enterica* (MIC = 0.51  $\mu$ M), the causative agent of foodborne salmonellosis than the antibiotic ampicillin (MIC = 1.4  $\mu$ M).<sup>91</sup> This remarkable activity has received considerable clinical interest in the search for privileged scaffolds that selectively target *S. enterica*. On the other hand, borrelidin C and D analogues with an additional hydroxy moiety in the cyclopentane ring are inactive against the tested bacteria and show reduced activity in *S. enterica* (MIC = 16–63  $\mu$ M). SAR investigation of the borrelidin scaffold has



Fig. 3 Proposed nitrile formation in borrelidin biosynthesis.



Fig. 4 Noncanonical polyketide cyclisation folding in clostrubin biosynthesis.

indicated that the vinylic nitrile and the carboxylic acid moieties are essential for the activity.<sup>87,90,93,94,101</sup>

**4.1.2 Clostrubins.** Clostrubin A **5** was first isolated as a deep purple pigment from the strictly anaerobic bacterium, *Clostridium beijerinckii* (HKI0724) in 2014 (Fig. 2 and Table S1<sup>†</sup>).<sup>102</sup> A year later, clostrubin A **5** and its related compound clostrubin B **6** were identified from the potato cultures of *C. puniceum.*<sup>47</sup> Both compounds feature a highly unusual pentacyclic polyphenol with an exceptional benzo[*a*]tetraphene scaffold that is rare in anaerobes, and clostrubin B **6** differs from A **5** in the presence of an extra sugar-like linear side chain.<sup>47</sup>

The biosynthesis of clostrubins in the anaerobic C. puniceum is proposed to originate from type II PKS (clr) with high homology to the pentacyclic resistomycin (rem) PKS in aerobic bacteria, Streptomyces resistomycificus (Fig. 4).<sup>103</sup> Type II PKSs are very common in actinomycetes; only two examples of type II polyketides have been identified in non-actinomycete bacteria so far. Stable-isotope labelling experiments indicated that the striking perifused ring feature of clostrubin is formed from a noncanonical polyketide folding which delineates from the conserved cyclization patterns of typical angucylic decaketides from aerobic bacteria. Numerous tailoring enzymes catalyse diverse post-modification reactions, such as cyclodehydration steps and decarboxylation leading to a loss of one C1 carbon to afford 5. Furthermore, labelling experiments suggest that the polycyclic core undergoes acetylation at ring A, and that ring E could be formed by condensation with an activated aceto-acetyl building block.<sup>102</sup> The benzo[a] tetraphene scaffold has also recently been identified in borolithochromes from the specimens of the Jurassic putative macroalgae Solenopora jurassica that has been preserved for over 150 million years, illustrating the evolutionary significance of clostrubin-type polyketides.<sup>104</sup>

Clostrubin A 5 displayed nanomolar potency against *Bacillus* subtilis (MIC = 75 nm) and superior antibacterial activity against several nosocomial pathogens, methicillin-resistant *S. aureus*, MRSA (MIC = 0.12  $\mu$ M), vancomycin-resistant *Enterococcus*, VRE (MIC = 0.97  $\mu$ M), and *Mycobacterium* including *M. smegmatis*, *M. aurum*, *M. vaccae*, and *M. fortuitum* (MIC = 0.12–0.48  $\mu$ M) than the antibiotic ciprofloxacin.<sup>102</sup> Furthermore, when tested against some common potato disease-causing microbial pathogens like *Clavibacter michiganensis* subsp. sepedonicus (ring rot), *Bacillus pumilus* (soft rot), and *S. scabies* (common scab), clostrubin A 5 displayed nanomolar activity with MIC values of 47 nM, 95 nM, and 95 nM, respectively.

Likewise, clostrubin B 6 displayed activity but weaker than clostrubin A 5 against the potato pathogens (MIC = 0.14–0.27  $\mu M).^{47}$ 

Clostrubins **5–6** are not virulence factors but rather play dual roles beneficial to the anaerobic bacteria.<sup>47,102</sup> First, being potent antibiotics, they act as chemical arsenals to inhibit other microbial competitors in a resource-limited niche.<sup>47,102</sup> Second, clostrubins promote the survival of the anaerobic *C. puniceum* and *C. beijerinckii* in an oxygen-rich plant environment.<sup>47</sup> Taken together, clostrubins represent promising leads for the development of antibacterial agents for use in fighting off potato infections. Furthermore, the total synthesis of clostrubin was achieved,<sup>105</sup> which may provide insight into structure–activity relationships (SAR) to guide the development of novel antibiotics.

4.1.3 Stilbenes. Stilbenes, a class of polyphenols commonly found in plants, are characterised by the presence of 1,2diphenylethylene nucleus known to exhibit diverse biological activities such as antioxidant, anticancer, antihyperglycemic, nematicidal, and antimicrobial activities.<sup>106</sup> Photorhabdus spp. is the only known bacterial producer of stilbenes, with two major products being 3,5-dihydroxy-4-isopropyl-trans-stilbene (also known as tapinarof 8) and its stilbene epoxide (Fig. 2 and Table S1<sup>†</sup>).<sup>107</sup> Tapinarof (benvitimod) 8 is a topical non-steroidal anti-inflammatory drug (NSAID) used for the treatment of psoriasis and atopic dermatitis.<sup>108</sup> Its mode of action (MOA) is mediated by activation of the aryl hydrocarbon receptor (AhR) and nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) signaling pathways.<sup>108</sup> The AhR is a conserved ligand-dependent transcription factor involved in the regulation of the metabolism of drugs, xenobiotics, and endogenous small molecules. Nrf2 is involved in the cellular detoxification and defence against reactive oxygen species (ROS) and electrophilic cell stress.109

Although the carbon framework of stilbene monomers consists only of 1,2-diphenylethylene units, they demonstrate an enormous structural diversity because they are easily polymerized by oxidative coupling to produce diverse oligomers with intricate structures.<sup>110,111</sup> Since stilbenes possess strong antioxidant/radical scavenging properties,<sup>106</sup> their production in *Photorhabdus* spp. can be induced by supplementation of redox stress that generates reactive oxygen species. Feeding of paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) to *P. luminescens* and *P. asymbiotica* cultures under aerobic

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Fig. 5 (A) Annotation of Plu1886, which encodes a cupin enzyme, adjacent to known tapinarof biosynthetic genes in *P. luminescens* TT01 (B) proposed pathway for regioselective oxidative dimerization of tapinarof **8** to duotap-520 **11** and carbocyclinone-534 **12**, and (C) activity of Plu1886 enzyme with plant-derived stilbenes **13–16** in the presence of  $Mn^{2+}$  or  $Cu^{2+}$ .

conditions produced tapinarof **8** and its stilbene epoxide **9**,<sup>107</sup> lumiquinone **10** <sup>112</sup> and two novel tapinarof dimers, duotap-520 **11** and carbocyclinone-534 **12** (Fig. 5B).<sup>113</sup> Duotap-520 **11** contains a resorcinol–benzoquinone C–C bond linkage whereas carbocyclinone-534 **12** features a novel hexacyclic core with a cyclopropane bridge. The complex structure of **12** was elucidated by nuclear magnetic resonance (NMR) experiments, X-ray crystallographic analysis, and electronic circular dichroism (ECD) spectral measurements and characterised as a racemic mixture of (+)-carbocyclinone-534 and (–)-carbocyclinone-534 **12**.

Stilbene monomers such as resveratrol, isorhapontigenin, and piceatannol can undergo spontaneous oxidation and

dimerization into an assortment of oxidized oligomers.<sup>110,111</sup> Likewise, it has been shown that the formation of tapinarofderived products, duotap **11**, and carbocyclinone **12** involved similar oxidation, Diels–Alder cyclization, and dimerization mechanism (Fig. 5B). Under aerobic conditions, duotap **11** was shown to undergo slow spontaneous conversion into **12**. Furthermore, an orphan cupin-type protein, Plu1886 adjacent to tapinarof *bkd* BGC in *P. luminescens* TT01 was identified to enhance the transformation of tapinarof **8** to **11** or **8** to **12** *in vitro* (Fig. 5A).<sup>113</sup> Cupin superfamily of enzymes are widespread in plants and are known to catalyse numerous diverse oxidation reactions, often requiring metal cofactors (*e.g.* Ni<sup>2+</sup>, Ca<sup>2+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Co<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>) for the activity.<sup>114,115</sup> *In vitro* 

enzymatic tapinarof conversion to carbocyclinone-534 **12** is highest in the presence of  $Mn^{2+}$  and to **11** in Cu<sup>2+</sup>. Microaerobic cultures of  $\Delta plu1886$  mutant showed a substantial decrease in carbocylinone **12** production relative to the WT, supporting its role to enhance tapinarof dimerization reactions.<sup>113</sup>

The bacterial Plu1886 enzyme shows substrate promiscuity towards plant-derived stilbenes such as pinosylvin 13, resveratrol 14 (Fig. 5C). The cupin catalysed the robust conversion of pinosylvin 13 to the novel duotap 13a and resveratrol 14 into its new carbocyclinone 14a scaffold in the presence of Mn<sup>2+</sup> or Cu<sup>2+</sup>. The no-enzyme controls only showed a trace amount of dimer 14a and an undetectable level of 13a. The new enzyme

derived products **13a** and **14a** were purified and structurally confirmed by 2D NMR experiments. No duotap production from **14** or carbocyclinone production from **13** was observed and no derivatives corresponding to dimerization of piceatannol **15** or chiricanine **16**.<sup>113</sup>

Stilbenes are prolific sources of lead molecules in the search for new drugs and medicines. Even slight structural modifications of monomeric stilbenes dramatically alter their chemical complexity and improve their overall pharmacokinetic properties.<sup>106</sup> Duotap-520 **11** exhibited much higher potency against MRSA (MIC = 6.5  $\mu$ M) and VRE (MIC = 4.1  $\mu$ M) compared to tapinarof **8** with MIC values of 50.5  $\mu$ M and 27.0  $\mu$ M in MRSA



Fig. 6 Examples of antimicrobial nonribosomal peptides with unusual motifs highlighted in red, isolated from pathogenic bacteria.

and VRE, respectively. Carbocyclinone-534 12 did not show any significant antimicrobial activity but exhibited antimycobacterial activity against M. smegmatis.113 Duotap 11 showed stronger activity than tapinarof 8 in its ability to regulate the Nrf2 antioxidant reporter gene. Furthermore, dimers 11 and 12 showed little to no efficacy in a colitis mouse model, whereas the monomer reduces disease symptoms. Although 8, 11 and 12 were only produced in the pathogenic P-form of Photorhabdus spp., their varying bioactivity data suggest that the bacterium employs a regulatory mechanism to attain its desired functional outcomes required for symbiosis and pathogenesis.113 The much weaker antimicrobial activity of tapinarof relative to duotap-520 is probably a means of cellular detoxification by the bacteria to support its symbiosis with the nematode, whereas the more potent duotap-520 presumably support its pathogenic lifestyle.107,110,113 The promiscuity of Plu1886 biosynthetic enzyme in vitro represents a significant cornerstone towards the development of an efficient system to generate novel stilbene dimers with specific activity.

#### 4.2 Nonribosomal peptides

Non-ribosomal peptide synthetases (NRPSs) are multi-modular enzymes that catalyse the synthesis of numerous peptide and peptide-like natural products that have wide applications in medicine, agriculture, and biotechnology among other fields (Fig. 6 and Table S1†). These mega enzyme complexes are not limited to the 22 proteinogenic amino acids; a large breadth of substrates is now known to be integrated and modified by postsynthesis action. NRPSs can incorporate a wide variety of nonproteinogenic amino acids, such as D-isomers,  $\alpha$ -hydroxy/keto acids, carboxylic acids, and *N*-methylated residues, as well as several other building blocks such heterocyclic rings and fatty acids. Other common post-synthetic modifications associated with the NRPS machinery include glycosylation and oxidative cross-linking giving rise to diverse molecules with precise functionality for a particular molecular target.<sup>116</sup>

Typical NRPS modules feature an adenylation (A) domain that selects and activates an amino acid monomer (and sometimes other carboxylic acids) as an adenylate followed by acyl transfer to a peptidyl carrier protein (PCP; also known as thiolation domain, T). This thiolation domain loads the activated amino acid on a 4'-phosphopantetheine (4'-Ppant) arm and covalently tethers it to form a peptide bond with an amino acid on the succeeding module, a reaction catalysed by the condensation (C) domain. Together, these three core domains (C, A, T) comprise a minimal NRPS module. In addition to these essential domains, each module may contain an epimerase (E) for the conversion of an L to D-configuration of amino acid, methyltransferase (MT) for N-methylation of the amide nitrogen, oxidase (Ox) for the conversion of a thiazoline to a thiazole or for  $\alpha$ -hydroxylation of the incorporated amino acid, and reductase (R) for reductive release of an aldehyde product. The C domain replaced by the cyclization (Cy) domain catalyses both condensation and the intramolecular heterocyclisation of Ser, Cys, or Thr to afford thiazoline or oxazaline heterocycles. The release of the final peptide product from the NRPS is catalysed by a C-terminal reductase (R), thioesterase (TE), or a cyclizing C domain to yield linear, cyclic, or branched peptide chain topologies. The structural biology and enzymology of NRPSs have been the subject of several reviews.83,117,118 This section covers some of the interesting linear and cyclic

 $\begin{array}{c} \begin{pmatrix} \mathsf{NH}_2 \\ \mathsf{H}_2\mathsf{N} \\ \mathsf{H}_2 \\ \mathsf{H}_2\mathsf{N} \\ \mathsf{H}_2 \\ \mathsf{H}_2\mathsf{N} \\ \mathsf{H}_2 \\ \mathsf{H}_2 \\ \mathsf{NH}_2 \\ \mathsf{N$ 

Fig. 7 Chemical structures of synthetic analogues NOSO-95179 24 and NOSO-502 25

nonribosomal peptide antimicrobials from pathogenic bacteria such as odilorhabdins **17a–c**, nematophin **18**, photoditritide **19**, serrawettins **20**, stephensiolides **21**, lugdunin **22**, and holomycin **23** (Fig. 6 and Table S1†).

**4.2.1 Odilorhabdins.** Odilorhabdins (ODLs) are a new class of ribosome-targeting antibiotics produced by the NRPS gene cluster in *Xenorhabdus nematophila* strain K102 (CNCM I-4530) (Fig. 6 and Table S1†).<sup>119</sup> Three ODLs were isolated, NOSO-95A **17a** (1296 Da), NOSO-95B **17b** (1280 Da), and NOSO-95C **17c** (1264 Da). Compounds **17a–c** are 10-mer linear peptides containing four types of non-proteinogenic amino-acid residues:  $\alpha,\gamma$ -diamino- $\beta$ -hydroxybutyric acid (Dab( $\beta$ OH)) at positions 2 and 3,  $\delta$ -hydroxylysine (Dhl) at positions 8 and 10,  $\alpha,\beta$ -dehydro arginine at position 9, and a putrescine moiety at the C-terminal position.<sup>119</sup>

Lead optimization strategies identified a synthetic analogue, NOSO-95179 24 (Fig. 7)<sup>120</sup> with improved antibacterial properties over the natural compound NOSO-95C 17c.120,121 NOSO-95179 24 differs from NOSO-95C 17c by the replacement of  $Dab(\beta OH)_3$  by alanine and the removal of the lateral lysine10 and putrescine at the C-terminus. Further structural modification at Ala3 and His7 positions of 24 led to the selection of NOSO-502 25 as the first odilorhabdin clinical candidate (Fig. 7).9,122,123 NOSO-502 25 exhibits potent activity to all classes (Ambler A, B, C, and D classification) of carbapenem-resistant Enterobacteriaceae (CRE) strains (MIC =  $0.5-4 \ \mu g \ mL^{-1}$ ). Furthermore, 25 shows excellent in vivo efficacy in several CRE murine infection models, exhibits good in vitro safety profile, and has a low potential for resistance development.119,120,122,123 Notably, 25 exhibits good stability in plasma, microsomes, and hepatocytes.<sup>123</sup> Taken together, NOSO-502 25 represents a promising drug candidate.

Antimicrobial peptides that interfere with bacterial ribosomes are rare.<sup>124,125</sup> Nine classes of ribosome-targeting antibiotics are known, five of which, including odilorhabdins target the 30S subunit.<sup>125</sup> However, the specific binding site of ODLs on the ribosome and its bactericidal mechanism is distinct from the other four classes.<sup>119</sup> ODLs bind to the decoding centre of the 30S small ribosomal subunit<sup>119</sup> that has never been exploited by any other known ribosome targeting antibiotics such as negamycin, tetracycline, streptomycin and paromomycin.<sup>125-128</sup> ODLs display concentration-dependent bactericidal activity similar to the mechanism described for aminoglycosides and negamycin antibiotics.<sup>126,127,129</sup> At lower concentrations, ODLs induce miscoding of the genetic code, likely by increasing the affinity of aminoacyl-tRNAs to the ribosome,<sup>128</sup> whereas at higher concentrations they inhibit translocation.<sup>119</sup>

**4.2.2** Nematophin. Nematophin 18a, first described in 1997,<sup>130</sup> is produced by all strains of *X. nematophila* (Fig. 6 and Table S1<sup>†</sup>). Chemically 18a, 3-indole-ethyl-(3'-methyl-2'-oxo)-pentanamide, contains an N-terminal  $\alpha$ -keto group and a C-terminal tryptamine residue, showing structural resemblance to the Rhabdopeptide–Xenortide Peptides (RXPs).<sup>130</sup> Recently, new nematophin analogues 18b–d and nematophins with valine building blocks, nevaltophins 26a–f were identified in *Xenorhabdus* strains (Fig. 8).<sup>131</sup>

The biosynthesis of nematophin is proposed to originate from the monomodular NRPS, RdpD, which is closely related to the RXP-producing NRPS, RdpABC but differs in the incorporation of  $\alpha$ -keto carboxylic acid as the starting unit.<sup>131</sup> Heterologous expression of the *rdpD* gene from *X. nematophila* ATCC 19601 strain in *Escherichia coli* fed with either phenylethylamine (PEA) or tryptamine (TRA), resulted in the production of new nematophin congeners, **18b–d** (Fig. 8A). In contrast, the wild type (WT) *X. nematophila* strain only produced nematophin **18a** even when fed with PEA or TRA and the presence of the amine compounds did not enhance its production level.<sup>131</sup>

Very few non-ribosomal peptides containing α-keto acid building blocks have been described to date.  $^{\scriptscriptstyle 131,132}$  The  $\alpha\text{-keto}$ acid precursors in nonribosomal cereulide from Bacillus cereus and valinomycin from Streptomyces spp. occur via deamination of α-amino acids such as valine, isoleucine or alanine.<sup>132-134</sup> A similar deamination mechanism to the corresponding acids is proposed in RdpD biosynthesis which is activated by the A domain and subsequently loaded onto the adjacent T domain. Nucleophilic attack by the free amine *via* the C<sub>term</sub> generates nematophin 18a and analogues (18b-d). The Cterm domains in RXP-NRPS and RdpD-NRPS indicate that various amines such as TRA and PEA commonly found in Xenorhabdus strains can be used as substrates to access the production of TRA- (18b) and PEA-containing nematophin derivatives (18c-d). The PEA analogues are produced in minor amounts, implying that the substrate preference of the Cterm domain in RdpD is likely tryptamine over phenylethylamine.131

A similar BGC was identified in Xenorhabdus PB62.4 containing two monomodular NRPS, Pb62A resembling RdpD with a broken Cstarter domain, and Pb62B like the RXP RdpC terminal module with a complete C domain. Heterologous expression of the pb62 gene cluster in E. coli fed with either PEA or TRA has permitted to unlock the production of new elongated nematophin derivatives containing an additional valine motif in the structure which was assigned the name nevaltophins 26a-f. The structures of 26a-f suggest a biosynthetic pathway very similar to that of 18a-d but with the incorporation of a valine subunit with α-keto acid building blocks (Fig. 8B).<sup>131</sup> The production of 26a-f was abolished in the Ser<sub>1303</sub>Ala mutation on the conserved Ser of the PCP domain in PB62A and led to the accumulation of 26g, further supporting the proposed biosynthesis (Fig. 8C). Furthermore, when Pb62A was used as a starting module in XndB involved in xenortide biosynthesis,135 nevaltophins with phenylalanine motif 26h-i were produced.131 The results provided a platform for engineered biosynthesis further expanding the nematophin chemical space.

While the crude extracts containing nematophins displayed zone of inhibition against the Gram-positive bacteria *M. luteus*, the nevaltophins containing-extracts did not exhibit activity.<sup>131</sup> The authors, however, only tested the antibacterial activity of nevaltophins against *M. luteus*;<sup>131</sup> and the results may not provide conclusive evidence that the valine unit incorporation in the nematophin core structure may enhance or decrease its bioactivity. In stark contrast, another study indicated that nematophin **18a** has no activity against *M. luteus* at the highest concentration tested (100  $\mu$ g mL<sup>-1</sup>). Nematophin, however,



Fig. 8 (A) Nematophin 18a and analogues 18b-d from heterologous expression of the *rdpD* gene in *E. coli* and proposed biosynthesis, (B) nevaltophin and analogues 26a-f from heterologous expression of the *pb62* gene cluster in *E. coli*, and (C) chemical structures of 26g-i.

showed potent activity against other Gram-positive bacteria such as S. aureus (MIC = 0.125  $\mu g~mL^{-1}$ ),  $^{130,136}$  MRSA (MIC = 1.5  $\mu g \text{ mL}^{-1}$ ) and fungal pathogen, *Botrytis cinerea* (MIC = 12  $\mu g$ mL<sup>-1</sup>).<sup>130</sup> Furthermore, the  $\delta$ -keto amide functionality in nematophin is essential for its anti-staphylococcal activity;136 and the activity is substantially enhanced by N-substitution of the indole ring with an alkyl or a phenyl group.131,136,137 The synthetic N-methyl substituted nematophin analogue displayed nanomolar activity towards several strains of S. aureus (15 ng mL<sup>-1</sup>), Staphylococcus hyicus (60 ng mL<sup>-1</sup>), and Staphy*lococcus intermedius* 9503 (50 ng mL<sup>-1</sup>)<sup>136</sup> including MRSA ATCC 43300 (31 ng mL $^{-1}$ ) and methicillin-susceptible *S. aureus*, MSSA ATCC 29213 (125 ng mL<sup>-1</sup>).<sup>137</sup> Conversely, incorporation of azaindole moieties in the nematophin scaffold significantly reduced the antibiotic activity (MIC =  $16-128 \ \mu g \ mL^{-1}$ ).<sup>137</sup> Nematophin 18a and nevaltophin 26a showed weak activity against parasites, Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani, and Plasmodium falciparum.131 Phenylethylamide-containing compounds such as nematophin were found to specifically inhibit an insect serotonin receptor facilitating its role in insect pathogenesis.138

**4.2.3 Nonribosomal peptides** *via* **promoter exchange.** Several known and cryptic nonribosomal peptides were identified in *Photorhabdus* and *Xenorhabdus via* the promoter exchange strategy, including GameXPeptides, xenoamicins,

mevalagmapeptides, xenorhabdin, indigoidine,139,140 and the pentadecapeptide, kolossin.<sup>141</sup> Recently, photoditritide **19** was identified after the photoditritide synthetase (pdtS) gene was activated in Photorhabdus temperata Meg1 via substitution of the native promoter with a transcriptionally active arabinoseinducible promoter, P<sub>BAD</sub> (Fig. 6 and Table S1<sup>†</sup>).<sup>142</sup> Overexpression of the pdtS gene was achieved with arabinose (induced strain), resulting in the production of a hexapeptide that is not previously detected in the wild type (WT) strain. Photoditritide 19 consists of two homoarginines (Har), two tyrosines (Tyr), and two tryptophans (Trp).139 Although nonproteinogenic amino acid Har-containing peptides have been reported in various marine organisms such as a sponge (cupolamide A),143 cyanobacteria (nodularin-Har)144,145 and marinederived actinomycetes (lucentamycins A-D),146 photoditritide is the first peptide from entomopathogenic bacteria that contains the rare homoarginine residue.142 Photoditritide 19 displayed antimicrobial activity against M. luteus (MIC = 3.0  $\mu$ M) and *E. coli* (MIC = 24  $\mu$ M) and weak antiprotozoal activity against T. cruzi (IC<sub>50</sub> = 71  $\mu$ M), P. falciparum (IC<sub>50</sub> = 27  $\mu$ M) and *T. brucei rhodesiense* ( $IC_{50} = 13 \mu M$ ). No cytotoxic activity against mammalian L6 cells was observed.142 Nonribosomal peptides 27–31 were produced via promoter exchange in  $\Delta hfq$  mutants of Photorhabdus and Xenorhabdus strains (Fig. 9 and Table S1<sup>†</sup>). The global post-transcriptional regulator, Hfq, is widespread in

8



Fig. 9 Structures of nonribosomal peptides identified from  $\Delta hfq$  mutants of X. szentirmaii (szentirazine 27, lipopeptides 28a–c), Photorhabdus PB45.5 (silathride 29, flesusides A and B 30a–b), Xenorhabdus KJ12.1 (cuidadopeptide 31) via promoter exchange.

bacteria and performs diverse functions, one of which is the modulation of BGC expression through mediating interactions between the small regulatory RNAs (sRNAs) and their target mRNAs.<sup>147-149</sup> An *hfq* deletion mutant in *P. luminescens* abolished the production of all known NPs.<sup>150</sup> Exchanging the native promoter of a BGC of interest with a constitutively strong active  $P_{BAD}$  promoter in  $\Delta hfq$  mutants resulted in the production of numerous known and new compounds **27–31**. While promoter

exchange in the wild type strains produces several background peaks,<sup>139-142</sup> promoter substitution in  $\Delta h f q$  mutants leads to culture supernatants containing only the compounds of interest, thereby enabling direct bioactivity testing, requiring no laborious and time-consuming isolation and purification steps.<sup>151</sup>

The promoter exchange strategy resulted in overproducing mutants with significantly higher production titres relative to

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the WT strains.<sup>151</sup> In X. szentirmaii- $\Delta hfq$ , two silent BGCs were activated that encode for the known depsipeptides, xenobactin<sup>152</sup> and szentiamide.<sup>153</sup> Additionally, a new oxidized diketopiperazine (DKP), szentirazine 27, and three new shortened PAX-peptides (28a-c) were produced. The new compounds 27–28 were exclusively produced by the induced  $\Delta hfg$  mutant. The structures of the lipopeptides (28a-c) were elucidated by detailed MS-MS analysis while szentirazine 27 was isolated from a large-scale culture, and its structure was characterized by NMR spectroscopy.<sup>151</sup> Furthermore, new peptides silathride 29 and flesusides A 30a and B 30b were identified from Photo*rhabdus* PB45.5- $\Delta hfq$  and the new lipopeptide cuidadopeptide 28 from Xenorhabdus KJ12.1- $\Delta hfq$  via a similar approach. The structures of 29-31 were elucidated by detailed MS/MS fragmentation analysis, labeling experiments and by comparison with synthetic compounds.<sup>151</sup> All new NPs 27-31 showed weak to moderate antimicrobial activity against several Gram-positive and Gram-negative bacteria, and fungi.151

**4.2.4 Serrawettins.** Serrawettins are non-ionic biosurfactants produced exclusively by the genus *Serratia*. They exhibit diverse activities such as emulsification, surface, antifouling, antitumor, and antimicrobial.<sup>154</sup> Three molecular species have been reported from *S. marcescens*, serrawettin W1 (also known as serratamolide A **20a**), serrawettin W2 **32a**, and serrawettin W3. Serratamolide A, a symmetrical dilactone molecule, was discovered by Wasserman, *et al.* in 1961 (Fig. 10 and Table S1<sup>†</sup>).<sup>155,156</sup> It is composed of two L-serine amino acids linked to two  $\beta$ -hydroxy serratamic acids (D-3-hydroxydecanoyl-L-serine).<sup>157</sup> It differs from depsipeptides valinomycin,<sup>158</sup> cereulide,<sup>158</sup> and enniatins<sup>159</sup> in the presence of  $\beta$ -hydroxy acids, rather than  $\alpha$ -hydroxy acid residues. Several serrawettin W1 **20a** congeners have also been identified from *Serratia* sp., serratamolides B–G (**20b–g**), which varies in the length of the fatty acid chain and the presence of a methoxy unit (**20g**) and a double bond in the alkyl chain (Fig. 10).<sup>160,161</sup>

The general chemical structure of serrawettin W2 consists of five amino acid residues (D-Leu–L-Ser–L-Thr–D-Phe–L-Ile) attached to a  $\beta$ -hydroxy fatty acid moiety (Fig. 10 and Table S1†).<sup>162,163</sup> Four analogues of serrawettin W2 **32b–d** were recently isolated from *Serratia* sp. which differs based on the amino acids present (Ile or Val, Phe or Tyr) or the length of the fatty acid chain (C5 or C7).<sup>162</sup> Further putative analogues (W7–W8)



Fig. 10 Analogues of serratamolide (serrawettin W1) A-G (20a-g) and serrawettin W2 32a-d identified in Serratia sp.

were tentatively identified in *Serratia surfactantfaciens* sp. nov. YD25 by MS/MS fragmentation analysis.<sup>164</sup> The structure of serrawettin W3 described in 1986 is still yet to be determined.<sup>165</sup> It is partially characterised and is composed of five amino acid residues (Thr, Ser, Val, Leu, Ile) and one dodecanoic fatty acid.<sup>163</sup>

The dilactone serrawettin W1 is believed to be formed solely by the action of the monomodular NRPS, SwrW encoding for aminolipid synthetase (Fig. 10). Initially, the biosynthesis of 20a was thought to occur via condensation of two serratamic acid molecules. However, mutational studies indicate the absence of the presumed precursors, suggesting the involvement of NRPS machinery in 16a production. Consequently, the presence of SwrW was identified in S. marcescens 274 by transposon mutagenesis. SwrW exhibits a C-A-T-TE domain architecture specific for only L-serine, and is presumed to be the simplest enzyme in the NRPS family. This simple NRPS system features an unusual dimerization, most likely via two following transesterification steps to assemble the symmetric and cyclic product, serrawettin W1 with no peptide bonds.<sup>166</sup> Biosynthesis of serrawettin W1 presumably starts with the adenylation of the L-serine, after which the activated L-serine binds as a thioester to the thiolation domain which has been phosphopantetheinylated through the action of the PPTase, PswP.<sup>167</sup> The amino group of the L-serine bound to the thiolation domain forms a bond with the 3-D-hydroxydecanoyl fatty acid which is speculated to come from a yet unknown ACP domain to form the first serratamic acid intermediate, and then subsequently transferred to the TE active site.<sup>166</sup> Thereafter, biosynthesis of the second serratamic acid occurs and follows similar dimerization and cyclization processes to the ones catalysed by the multi-modular synthetase in the biosynthesis of the symmetric decapeptide gramicidin S from *Brevibacillus brevis*.<sup>168</sup>

Biosynthesis of serrawettin W2 in S. surfactantfaciens sp. YD25<sup>T</sup> is proposed to be catalysed by the NRPS peptide synthetase, SwrA consisting of five modules (Fig. 10). The unusual feature of SwrA (like SwrW) stems from the assembly of the starter unit. Typical NRPS contains A domains at the initiation site, but the SwrA NRPS harbours a C domain at its Nterminus suggesting that the initiation of peptide synthesis may form from the condensation of a fatty acid rather than an amino acid. It is presumed that a fatty acid adenylate, acyl-ACP, or acyl-CoA is likely the substrate for this C domain, catalysing the N-acylation of leucine. The fatty acid precursor in serrawettin W2 is speculated to be synthesised by the putative PKS SwrEFG gene cluster and other unknown enzymes. Chain elongation then occurs via the action of the other domains by successive incorporation of serine, threonine, phenylalanine, and isoleucine. Finally, cyclisation and chain release of the oligopeptide is catalysed by the TE domain to yield serrawettin W2.164



sphA (stephensiolide)

Fig. 11 Structures of stephensiolides A-K (21a-k) from Serratia sp.

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Serrawettin W1 **20a** exhibits antimycobacterial activity against *M. tuberculosis*, *M. diernhoferi*, and *M. avium* (MIC = 25  $\mu$ g mL<sup>-1</sup>),<sup>155,161</sup> and antibacterial and antifungal activities towards *S. aureus*, *B. subtilis*, *M. luteus*, *Trichophyton* spp., and MRSA (MIC = 6.25–50  $\mu$ g mL<sup>-1</sup>).<sup>155,169,170</sup> Likewise, serrawettin W2 **32a** is active against Gram-positive (*e.g. S. aureus*, *Rhodococcus* sp. and *Micrococcus* spp.) and Gram-negative bacteria (*e.g. Pseudomonas* spp., *Shigella* spp.) including drug-resistant *S. aureus* clinical isolates.<sup>164</sup> Serrawettin W2 **32a** is a potent biofilm inhibitor of *Candida albicans* (IC<sub>50</sub> = 7.7  $\mu$ M), while the W2 analogues **32b-f** are moderately active (IC<sub>50</sub> = 13.4–60.0  $\mu$ M).<sup>162</sup> Furthermore, **32a** is cytotoxic towards Hela (IC<sub>50</sub> = 20.9  $\mu$ M) and Caco2 (IC<sub>50</sub> = 54.1  $\mu$ M) cell lines.

The cyclic lipodepsipeptides, stephensiolides A-K 21a-k were produced by a Serratia strain that was isolated from the midgut and salivary glands of Anopheles stephensi mosquitoes (Fig. 11).<sup>171</sup> Stephensiolides were also isolated from the fungal endophyte, Lecanicillium sp. (Hypocreales) obtained from the latex of Sandwithia guyanensis plant.<sup>172</sup> Stephensiolides 21a-k mimic the core structure of serrawettin W2 32a as both are cyclic pentapeptides162,163 but differ in the sequence of the amino acid constituents.<sup>171</sup> The peptide sequence in stephensiolides is Thr-Ser-Ser-Val/Ile-Ile/Val while serrawettin W2 is Leu-Ser-Thr-Phe-Ile. Furthermore, the lactone in stephensiolides is cyclized through the hydroxy group of the threonine, whereas serrawettin W2 is cyclized via a 3-hydroxy group of the fatty acid chain. Stephensiolide congeners (A to K) 21a-k vary in the length of the alkyl chain, amino acid residues (Ile or Val) or the presence of a double bond in the lipid side chain.171

Like serrawettin W1 **20a** and serrawettin W2 **32a**, stephensiolides are biosynthesised by a similar NRPS machinery (Fig. 11). Bioinformatics analysis identified the penta-modular NRPS, *sphA* which is presumed to be responsible for the incorporation of five amino acids, threonine, serine, serine, valine/isoleucine, and isoleucine/valine.<sup>171</sup> SphA contains a unique initial C domain that is homologous to the lipopeptide-loading C module of EndA in the enduracidin biosynthesis,<sup>173</sup> which is probably responsible for the incorporation of the fatty acid in **21a-k** from an ACP.<sup>171</sup>

Antimicrobial testing of the stephensiolide mixture (A to K) revealed activity against *B. subtilis* 3610 ( $IC_{50} = 15 \ \mu g \ mL^{-1}$ ), *P. falciparum* Dd2 ( $IC_{50} = 14 \ \mu g \ mL^{-1}$ ), and the human hepatocytes, HepG2 ( $IC_{50} = 21 \ \mu g \ mL^{-1}$ ).<sup>171</sup> Stephensiolides also demonstrated antibacterial activity against MRSA with stephensiolide I **21i** as the most active (MIC = 4  $\mu g \ mL^{-1}$ ).<sup>172</sup> Like serrawettins, stephensiolides facilitate bacterial surface motility within mosquitoes is not fully understood, however, it is speculated that an enhanced swarming ability enables the bacteria to colonize and migrate in the different tissues within the insect host. A close relative to *S. marcescens, Serratia* strain AS1 colonizes diverse anopheline species and infect multiple different tissues within mosquitoes, including the midgut, female ovaries, and male accessory glands.<sup>174</sup>

**4.2.5 Lugdunin.** Lugdunin **22** is a macrocyclic peptide antibiotic isolated from the nasal and skin commensal

bacterium, *S. lugdunensis* (Fig. 12 and Table S1†). Structural features of lugdunin comprise an unusual thiazolidine heterocycle and five amino acids (Val, Trp, Leu, Val, and Val) in alternating D- and L-configuration.<sup>29</sup> This five-membered thiazolidine resembles a clasp that "adorns" the peptide backbone, hence the term fibupeptides was coined for this new class of compounds (Latin *fibula*, meaning clasp).<sup>175</sup>

The biosynthetic mechanism for lugdunin production features several unusual aspects of the domains and their overall organization (Fig. 12). Four NRPS genes, lugA, B, C, and D, are proposed to direct the biosynthesis of lugdunin. Interestingly for a heptapeptide, the gene cluster encodes adenylation domains for only five amino acids. Biosynthesis presumably starts at the characteristic initiation module of LugD specific for L-cysteine, followed by sequential addition of D-valine and L-tryptophan by LugA, and D-leucine by LugB. The modules encoded in LugC exhibit a very peculiar organization, featuring a single valine-incorporating A domain but two downstream condensation and three PCP domains for peptide bond formation and amino acid transfer, respectively.29 This suggests an iterative biosynthetic logic similar to that of koranimine176 and yersiniabactin,177 where the single LugC adenylation domain activates three successive valine residues for subsequent installation in alternating L- and D-configurations. Chain release of the thioester-bound heptapeptide is catalysed by the terminal reductase of LugC, followed by subsequent cyclisation. Finally, the nucleophilic attack of the cysteine thiol group at either the re or si face of the imine yields two thiazolidine-containing structural diastereomers (depicted with wavy bond). The thiazolidine heterocycle is present in some linear NRPS compounds, such as watasemycins<sup>178</sup> and yersiniabactin,177 but is yet unreported in macrocyclic peptides. Lugdunin is the first thiazolidine-containing macrocyclic peptide. Interestingly, production of lugdunin in ample amounts for chemical characterisation and biological profiling was only obtained via substitution of the native tetR-like regulatory gene, lugR, with a xylose-based expression approach.29

Lugdunin 22 exhibits potent bactericidal activities against a wide range of Gram-positive bacteria, including B. subtilis, Listeria monocytogenes, S. aureus, Streptococcus pneumoniae, and opportunistic pathogens MRSA, VRE, and glycopeptideintermediate resistant S. aureus (GISA) (MIC =  $1.5-12 \ \mu g$  $mL^{-1}$ ).<sup>29</sup> In contrast to rifampicin, S. aureus did not show any resistance to lugdunin even under prolonged exposure to suboptimal doses of the compound for over 30 days. Furthermore, it shows no toxicity in primary human erythrocytes, neutrophils, or human monocytic cell line HL60, and demonstrates good in vivo efficacy in the mouse model of S. aureus skin infection. In vivo tests show significant reduction and even total eradication of viable S. aureus on the skin surface and in the mouse tissue indicating that the compound can penetrate the deeper layers of the skin.<sup>29</sup> This inhibitory mechanism is achieved by the bactericidal activity of lugdunin as well as by the increased innate defence of epithelial cells resulting in efficient protection against S. aureus skin colonization. Lugdunin offers the host three layers of protection. Firstly, it can directly inhibit and kill S. aureus. Secondly, it can work synergistically with the

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Lugdunin

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antimicrobial peptides produced naturally by the host as part of the immune response (for example, hCAP18/LL-37 and the dermcidin-derived peptides DCD-1L), enhancing their ability to kill *S. aureus*. Finally, it can induce an immune response within the skin, thus enabling it to recruit phagocytic immune cells to aid with the clearing of the competing pathogen. Other factors derived from the skin commensal *S. epidermidis* may serve to amplify this response, increasing efficacy.<sup>179</sup>

SAR studies indicate that the cyclic structure of the peptide, the N-unsubstituted thiazolidine "clasp", two amino acids tryptophan and leucine, and an alternating D- and L-amino acid backbone are integral to the activity.<sup>175</sup> The nonpolar tryptophan and leucine residues interact with the hydrophobic regions of the bacterial cell membranes similar to the activity of poly-(Trp-Leu)-octapeptides.<sup>180</sup> Fibupeptides like lugdunin carry electronically charged particles across the membrane and consequently disintegrate the membrane potential, thereby killing the bacteria. Incorporation of an additional tryptophan motif in the peptide backbone intensifies this membrane interaction and further strengthens the antibacterial effect, exhibiting two-fold increased activity over the parent compound.175 Lugdunin or analogues thereof are promising candidates for the treatment of multi-drug resistant Grampositive infections. However, it may be challenging to develop these into systemic therapeutics considering that they are membrane-targeting antibiotics. Such compounds also tend to perturb mammalian plasma membranes.181

**4.2.6 Holomycin**. Holomycin **23** was first discovered in *Streptomyces griseus* in 1959<sup>182</sup> and later was reported to be produced by several other *Streptomyces* species<sup>183–188</sup> and other bacteria, including the marine Gram-negative bacterium *Photobacterium halotolerans*<sup>79</sup> and the fish pathogen *Y. ruckeri* (Fig. 6 and Table S1<sup>†</sup>).<sup>31,32</sup> Structurally, holomycin belongs to a class of dithiolopyrrolone (DTP) natural products<sup>189</sup> which contains a unique heterobicyclic core with a disulfide bridge and a variety of *N*-alkyl and *N*-acyl substituents.<sup>31,79,182–184</sup>

Dithiolopyrrolones possess broad-spectrum inhibitory activity against bacteria, fungi, and cancer cell lines.<sup>189-191</sup> Holomycin 23 is potent against several Gram-positive and Gram-

negative bacteria including *E. coli* (MIC =  $0.2-2 \ \mu g \ mL^{-1}$ ), *S.* aureus (MIC =  $2-4 \ \mu g \ mL^{-1}$ ), S. epidermidis (MIC =  $1 \ \mu g \ mL^{-1}$ ), S. pneumoniae (MIC = 0.1–0.3  $\mu$ g mL<sup>-1</sup>), Haemophilus influenzae (MIC = 0.3  $\mu$ g mL<sup>-1</sup>), and *Moraxella catarrhalis* (MIC = 0.1–0.3  $\mu g m L^{-1}$ ,<sup>192</sup> as well as rifampicin-resistant S. aureus (RRSA) mutants containing modified RNA polymerase β-subunit (MIC = 4-8  $\mu$ g mL<sup>-1</sup>).<sup>193</sup> Despite this attractive biological activity, holomycin is toxic, so it may need to be modified for possible future antibiotic use. Chemical synthesis of DTP analogues with modifications at the N-positions has attracted significant interest by several groups.194-199 N-Aryl DTP analogues have been shown antitumor activity198 and antileukopenia activity.194,197 N-Aryl DTP with 2,4-dimethoxyphenyl moiety displayed potent antibacterial activity against clinical isolates of MRSA, RRSA, vancomycin-resistant S. aureus (VRSA), and moderately penicillin-resistant S. pneumoniae (MPRSP) with MIC values in the range of 0.125–2  $\mu g m L^{-1}$  comparable to the antibiotic rifampin.<sup>195</sup> Previous works also showed that the biosynthetic pathway of DTPs is susceptible to be manipulated by feeding different organic acids or fatty acids to the cultures to modify the lateral acyl chain.200-202 Another approach involved the generation of hybrid-type antimicrobials by incorporating the holomycin antibiotic into the myxopyronin core.203 The holomycin nucleus has also been more recently identified in the marine hybrid antibiotic thiomarinol 35, in which it is joined to a pseudomonic acid motif, an analogue of the FDA-approved topical antibiotic mupirocin (Bactroban®) (Fig. 13).190,204 The biosynthetic hybridity of thiomarinol may have advantageous effects; when one antibiotic fragment is modified by inactivating enzymes, the other constituent might remain functionally active.<sup>205,206</sup> Attempts to stimulate holomycin production have also received considerable interest. Holomycin-high producing variants of S. clavuligerus were obtained via competition-based adaptive evolution against MRSA N315 (ref. 207) as well as manipulation of the regulatory gene, argR which regulates the expression of arginine biosynthesis.208

Owing to the promising antimicrobial activity of DTPs, several studies into their mode of action (MOA) have been conducted using some of the more well-studied group



Fig. 13 Structures of thiolutin 33, thiomarinols 35, and synthetic holomycin analogue 31 that exhibits notable bacterial RNA polymerase inhibition.

members. Two opposing plausible mechanisms of action have been proposed. The first one identifies DTPs as inhibitors of bacterial RNA polymerase (RNAP). Thiolutin 33, a holomycin variant, has been shown to reversibly inhibit RNA and protein synthesis of Saccharomyces cerevisiae at a concentration of 2-4 µg mL<sup>-1</sup> in the whole-cell and spheroplasts assays and inactivates yeast RNA transcription in vitro.191,209-211 However, subsequent studies of holomycin or thiolutin in E. coli RNA synthesis inhibition have indicated that although both exhibit activity in vivo, they show weak (or no activity) in vitro. Furthermore, it was also not clear which step of RNA synthesis thiolutin inhibits. Induction of β-galactosidase in E. coli has suggested both RNA transcription initiation and chain elongation as possible targets of thiolutin. These opposing results cast doubt as to whether RNAP is the main target of the antibiotic in E. coli.186,192,212-214 To uncover the intriguing aspects of DTP mechanisms, Tan and coworkers synthesised various N-aryl DTP analogues and investigated their in vitro inhibitory against E. coli RNAP. Among all the tested compounds, synthetic 34 inhibited the most potent RNAP activity in vitro and is also the least cytotoxic. Additionally, molecular docking studies (Fig. 13) of 34 revealed interaction and high binding affinity with the amino acid residues in the switch region of the E. coli RNAP in the same manner as myxopyronin A, indicating that DTP and analogues are bacterial RNA inhibitors.195,199

The second alternative mechanism is proposed by Li and coworkers in which holomycin **23** is considered as an intracellular metal-chelating antibiotic that sequesters free metal ions and selectively targets *E. coli* metalloenzymes, and not RNA polymerase *in vitro*.<sup>215</sup> The proposed model suggested holomycin acts as a prodrug<sup>192,216</sup> whose activation involves the conversion of the ene-disulfide in the cytoplasm to the active ene-dithiol, reduced holomycin (red-holomycin) with high affinity for zinc ions.<sup>215,216</sup> The mechanism by which the cyclic disulfide **23** is reduced in the cells is as yet unknown. After entering the cells, the red-holomycin **23a** is proposed to exert its metallophoric activity *via* two different routes (Fig. 14): (1) red-holomycin **23a** sequesters essential metals, especially zinc, thereby limiting

zinc availability in the bacterial cell, and (2) red-holomycin 23a removes zinc from a subset of zinc-dependent metalloproteins (i.e. E. coli class II fructose bisphosphate aldolase, FbaA), thereby disrupting the cell's metal homeostasis and potentially interfering the essential metabolic processes such as glucose utilization, RNA synthesis, and respiration. Although both routes contribute to the inhibitory effect of holomycin, route two may play a more prominent role in the MOA, consistent with the findings that an increased zinc concentration renders no enhanced effect on the E. coli growth inhibition. Disruption of the zinc import machinery involved in the maintenance of metal homeostasis, such as ZnuABC restricts zinc uptake and further sensitizes *E. coli* to holomycin.<sup>215</sup> This MOA is unique amongst antibiotics and may be further explored to understand the specificity of holomycin and other DTPs against metalloenzymes for the development of novel potent chelators.

# 4.3 Hybrid polyketide-nonribosomal peptide natural products

Owing to the structural and catalytic resemblances between PKS and NRPS, they have evolved the ability to communicate with each other and combine modules to form hybrid assembly lines. During the transfer of the growing peptide or polyketide intermediate across NRPS/PKS interfaces, ketosynthase (KS) and condensation (C) domains facilitate chain elongation by accepting upstream PCP-bound peptidyl thioesters and ACPbound polyketide thioesters, respectively, thereby switching efficiently between C-C bond and C-N bond formation. Together, the biosynthetic versatility of PKS machinery and the substrate flexibility of NRPS modules that can incorporate almost 500 different proteinogenic and nonproteinogenic amino acids coalesce to yield hybrid natural products with astounding structural and biological diversity (Fig. 15 and Table S1<sup>†</sup>). This biosynthetic machinery has been described extensively elsewhere.83,217 Examples of antimicrobial hybrid polyketide-peptide metabolites produced by pathogenic bacteria include the red-pigment prodigiosin, the broad-spectrum antibiotic althiomycin, the DNA-gyrase inhibitor albicidin, and the



**Fig. 14** A model for the mechanism of action of holomycin in which it acts as a prodrug which undergoes intracellular reduction to the active red-holomycin that sequester free metal ions, particularly zinc (route 1) or removes zinc from metalloproteins (route 2).





antibacterial metabolite associated with dental caries reutericyclin.

**4.3.1 Prodigiosin.** Prodigiosin **36** was first characterized in *S. marcescens*<sup>218,219</sup> and was later identified in several other bacterial genera including *Streptomyces*,<sup>220</sup> *Vibrio*,<sup>221</sup> *Zooshikella*,<sup>219</sup> *Hahella*,<sup>222</sup> and *Pseudoalteromonas* (Fig. 15 and Table **S1**†).<sup>219</sup> Chemically, prodigiosin **36** is 2-methyl-3-pentyl-6-methoxyprodiginine consisting of three pyrroles in the structure. Prodigiosin production yields are greatly influenced by

various nutritional and environmental factors, such as carbon, phosphate, and nitrogen sources, inorganic salts, media composition, oxygen availability, temperature, pH, and incubation time.<sup>41,223,224</sup> The biosynthesis of **36** in the genus *Serratia* is dependent on the *pig* gene cluster consisting of *pigA–N* or *pigA–O*.<sup>225,226</sup> The regulation and biosynthesis of prodiginines in *Serratia* spp., *Streptomyces* spp. and *Pseudoalteromonas* spp. have been reviewed recently.<sup>227,228</sup>

Prodigiosin 36 has numerous potential beneficial properties such as antibacterial,229 antifungal,223 antimalarial,230 antiprotozoal,231 anticancer,232 immunosuppressant,233 and as natural colourants for the dyeing of silk and wool.<sup>227,234</sup> It is active against a wide range of Gram-positive bacteria including S. aureus and B. subtilis,<sup>221</sup> and Gram-negative E. coli, Erwinia carotovora, S. enterica, as well as drug-resistant strains such as MRSA and oxacillin-resistant S. aureus (ORSA).223 Prodigiosin targets the bacterial plasma membrane and causes disruption and loss of vital intracellular substances (K<sup>+</sup> ions, sugars, amino acids, proteins) via a chaotropicity-mediated mode-of-action.235 Bacterial prodigiosin and related analogues exhibit in vitro antiproliferative activity against over 60 human cancer cell lines with an average inhibitory concentration of 2 µM. Furthermore, they are also potent inhibitors of T lymphocyte proliferation.<sup>223</sup> Findings associated with anticancer and immunosuppressive properties of prodiginines and their possible modes of action have been subject to several reviews.236-238 Prodigiosin has also been used as inspiration to develop potent analogues such as obatoclax mesylate (GX15-070) which is currently in clinical trials for the treatment of various types of cancer including lymphoma, myelofibrosis, leukaemia, and mastocytosis.236,239-241

The physiological and ecological function of prodigiosin remains elusive. Its ubiquitous nature suggests that it may be ecologically beneficial to the producer organism. However, the precise role of the pigment remains elusive due to the diversity of prodiginine producers.<sup>228</sup> In S. marcescens, prodigiosin 36 is not an essential virulence factor.<sup>218</sup> Some reports have suggested potential roles of the pigment which is likely a mode of defence against microbial competitors in a continuously dynamic environment or as a response to natural stressors.<sup>223,228</sup> Apart from its protective function against predators, prodigiosin may also serve as a metabolic sink (energy overflow) through the consumption of the excess NAD(P)H or proline from primary metabolism.<sup>242</sup> S. marcescens colonizes and propagates in the environment via swarming, swimming, and air dispersal. It is speculated that prodigiosin contributes to Serratia's cell surface hydrophobicity and consequently its improved motility facilitates bacterial dispersion through the air.223,243

**4.3.2 Althiomycin.** The broad spectrum-antibiotic, althiomycin 37 ( $C_{18}H_{17}N_5O_6S_2$ , 439 Da) was first isolated from *Streptomyces althioticus* in 1957 (Fig. 15 and Table S1<sup>†</sup>).<sup>244</sup> Its structure consisting of two glycines, two cysteines, and one serine was elucidated in 1974 by X-ray crystallography.<sup>245,246</sup> Althiomycin 37, which is also produced by myxobacteria of the genera *Cystobacter*<sup>247</sup> and *Myxococcus*<sup>248</sup> and other *Streptomyces* species,<sup>246</sup> was only identified from the entomopathogen *S. marcescens* Db10 in 2012.<sup>249</sup> The biosynthesis of althiomycin in *S. marcescens* is proposed to involve six genes (*alb1–6*) that encode a hybrid of NRPS and PKS systems closely related to *Myxococcus xanthus* DK897.<sup>248</sup>

Althiomycin **36** displays wide spectrum antibiotic activity against several Gram-positive bacteria including strains of *S. aureus* (MIC = 16–25  $\mu$ g mL<sup>-1</sup>),<sup>250,251</sup> *E. faecalis* (MIC = 16  $\mu$ g mL<sup>-1</sup>),<sup>251</sup> *Corynebacterium diphtheriae* (MIC = 0.8  $\mu$ g mL<sup>-1</sup>)<sup>250</sup> and Gram-negative bacteria including *E. coli* (MIC = 1  $\mu$ g mL<sup>-1</sup>),<sup>251</sup> *K. pneumoniae* (MIC = 6.3  $\mu$ g mL<sup>-1</sup>)<sup>250</sup> and *Shigella* 

*flexneri* (MIC = 25  $\mu$ g mL<sup>-1</sup>)<sup>250</sup> but exhibits no such effects in mammalian cells.<sup>252</sup> Althiomycin 36 blocks the action of the peptidyl transferase by binding to the 50S ribosomal subunit, thus inhibiting prokaryotic protein synthesis.244,252 Althiomycin and derivatives have been chemically synthesised (albeit with low efficiency).<sup>251,253</sup> The synthetic de(hydroxymethyl) althiomycin analogue showed comparable antibiotic activity to that of the parent compound. SAR studies indicated that the 4methoxy-3-pyrrolin-2-one moiety, and the configuration of the oxime group and thiazoline ring are relevant to its bioactivity.250 This methoxypyrrolinone pharmacophoric feature in althiomycin is also present in other bioactive natural products such as malyngamide A,<sup>254</sup> sintokamide A,<sup>255</sup> and mirabimide E.<sup>256</sup> To date, the difficulties encountered in chemical synthesis have hampered further investigations into the potential of althiomycin-based compounds as antibacterial drugs.249

**4.3.3 Albicidin.** The antibiotic albicidin **40** was first characterized in 1985 from the chlorosis-inducing cultures of *X. albilineans* isolated from diseased sugarcane (*Saccharum officinarum* L.) (Fig. 15 and Table S1<sup>†</sup>).<sup>257–259</sup> It took 30 years before the structure of **40** was fully elucidated owing to its extremely low yields in *X. albilineans* cultures.<sup>257,260</sup> The development of a viable heterologous expression system in a fast-growing bacterium, *Xanthomonas axonopodis* pv. vesicatoria optimized albicidin production<sup>261</sup> (albeit with low efficiency ~1 mg per 100 L cell culture), which enabled unambiguous structural elucidation of **40**.<sup>260</sup> Albicidin **40** is a rather extraordinary linear polyaromatic oligopeptide composed of a cinnamoyl residue at the N-terminus, an unusual β-cyano-L-alanine (Cya), two *para*-aminobenzoic acids and a dipeptidic moiety at the C-terminus (4-amino-2-hydroxy-3-methoxybenzoic acids).<sup>260</sup>

The structure determination of 40 paved the way for chemical synthesis providing multigram quantities of albicidin and enabling SAR studies of the albicidin scaffold.262 Albicidin 40 targets the GyrA subunit of the DNA gyrase (topoisomerase II),<sup>263</sup> an essential enzyme that catalyses and modulates the extent of supercoiling of double-stranded DNA.264 Albicidin inhibits this supercoiling activity of E. coli DNA gyrase with half-maximal inhibitory concentrations (~40 nM) lower than those of most coumarins and quinolones.263 Albicidin is bactericidal against a wide range of Gram-positive and Gram-negative bacteria with nanomolar potency particularly against fluoroquinolone-resistant strains of *E. coli* (MIC =  $0.031-0.5 \ \mu g \ mL^{-1}$ ), Salmonella enter*itidis* (MIC = 0.5  $\mu$ g mL<sup>-1</sup>), and *P. aeruginosa* DSM 117 (MIC = 1.0  $\mu g m L^{-1}$ ).<sup>265</sup> Structural modifications of 40 such as the substitution of the central amino acid  $\beta$ -cyanoalanine with polar threonine residue266 or azahistidine leads to analogues with increased bioactivity over the natural albicidin.267 Replacement of the Nterminal methylcoumaric acid moiety with benzoyl or acyl residues leads to inactivity towards the E. coli gyrase<sup>268,269</sup> whereas carbamoylation of the N-terminus motif, which is most likely a post-NRPS reaction gives rise to a more potent bacterial gyrase inhibitor (IC<sub>50</sub>  $\sim$  8 nM).<sup>270</sup> Synthetic azahistidine–albicidin variants with ethoxy group substitution on the C-terminal dipeptide motif exhibits increased potency against Gram-positive B. subtilis, Mycobacterium phlei and ciprofloxacin-sensitive (MIC =  $0.031 \ \mu g$ mL<sup>-1</sup>) and -resistant S. aureus (MIC = 0.063 µg mL<sup>-1</sup>).<sup>267</sup> Variation

in the molecule's stereocenter has minimal effect on the activity as indicated by *ent*-albicidin containing the D-Cya exhibiting comparable gyrase activity (IC<sub>50</sub> ~ 40 nM) with the natural product albicidin.<sup>265</sup> Furthermore, replacing the central amide bond with a triazole moiety leads to a novel albicidin analogue that can overcome the serine endopeptidase AlbD resistance while preserving biological activity.<sup>267,271</sup>

4.3.4 Reutericyclin. Reutericyclin 38a, N-acyl tetramic acid, was initially isolated from the cultures of lactic acid bacteria Lactobacillus reuteri LTH2584 originating from an industrial sourdough isolate (Fig. 15 and Table S1<sup>†</sup>).<sup>71,272,273</sup> Its chemical structure was confirmed by chemical synthesis.272,274 More recently, reutericyclin (renamed reutericyclin A 38a) and analogues reutericyclin B 38b and C 38c including the unacylated tetramic acid mutanocylin 39 were produced from the muc gene cluster in S. mutans B04Sm5 isolated from the mouth of a child with severe dental caries.<sup>30</sup> Mutanocyclin 39 was also reported to be produced after the unidentified BGC1 in S. mutans 35 was activated via a new heterologous expression system.275 The chemical structures of reutericylins A-B 38ab differ from C–D 38c–d in the presence of the N-substituted  $\alpha$ , $\beta$ -unsaturated fatty acid whereas the latter have saturated acyl chains.30 In solution, tetramic acids undergo keto-enol tautomerism, and the preferred tautomeric form of reutericyclin is the pyrrolidine-2,4-dione which differs from all other naturally-occurring 3-acyl-tetramic acids.272,276

The reutericyclin BGC in *S. mutans* comprises 9 genes (*mucA–I*) that encode a hybrid modular PKS–NRPS assembly line, as well as enzymes involved in transport and regulation. Reutericyclin **38a–c** are proposed to be assembled from C10 or C11 fatty acids as starter units through elongation with leucine, which is subsequently extended *via* a malonyl-CoA unit (Fig. 16). The reutericyclin genomic island does not code for enzymes related to fatty acid metabolism,<sup>277</sup> thus the C10 or C11 lipid

chain in **38a-c** may come from the general metabolism through the action of hydroxyacyl-ACP dehydratases to generate *trans*-2decenoyl-ACP, decanoyl-ACP, and *trans*-2-dodecenoyl-ACP.<sup>30</sup>

Another interesting feature of the *muc* assembly line is the lack of an epimerase (E) domain or dual functioning C/E domains required in the conversion of L-to D-leucine residue. The A domain in MucD appears to incorporate the p-leucine building block in 38a-c.30 Most Gram-positive bacteria have the ability to synthesise p-alanine and p-glutamic acid as components of the peptidoglycan cell wall, however, the synthesis of other D-amino acids is less common.<sup>278</sup> Feeding of [<sup>13</sup>C<sub>1</sub>] L- and p-leucine to fermentation cultures of S. mutans and L. reuteri revealed incorporation of only [<sup>13</sup>C<sub>1</sub>] L-leucine.<sup>30</sup> Conversely, an isoleucine 2-epimerase with leucine epimerase activity has been characterised in lactobacilli,279 and L. reuteri strains have been reported to produce D-leucine.277 Presumably, S. mutans may also contain isoleucine 2-epimerase homologues responsible for p-leucine synthesis. Additionally, the muc TE domain may also act as epimerase as exemplified by the NocTE domain in nocardicin biosynthesis.280 However, MucTE exhibits very low homology to the dual functioning NocTE domain.30 It is currently unclear which enzyme is responsible for the epimerization reaction in reutericyclin biosynthesis. The first three genes, mucABC are homologous to the phloroglucinol biosynthetic proteins PhlABC, and are believed to catalyse the acetylation of the pyrrolidine ring in 38a-c. Expression of the MucA-E in E. coli BAP1 strain resulted in the production of 38a-c and a new analogue reutericyclin D 38d containing an N-dodecanoyl substituent, indicating that genes mucA-E indeed compose the minimal BGC for 38a-c production. Furthermore, heterologous expression and deletion experiments characterised MucF as a new deacylase responsible for converting reutericyclin 38a-c to the tetramic acid 38d lacking the lipid chain.



Fig. 16 Proposed reutericyclin and mutanocyclin biosynthesis.

Reutericyclin exhibits potent activity against a broad range of Gram-positive bacteria, including B. cereus, B. subtilis, E. faecalis, S. aureus, Lactobacillus spp., Weissella confusa and clinical isolates of *E. faecium* (MIC =  $0.06-6.5 \ \mu g \ mL^{-1}$ )<sup>71</sup> as well as pathogens associated with topical infections such as mupirocin-resistant MRSA (MIC =  $0.8-3.12 \ \mu g \ mL^{-1}$ ),<sup>72</sup> macrolide-resistant *Streptococcus pyogenes* (MIC =  $0.012-0.4 \mu g$  $mL^{-1}$ )<sup>72</sup> and *Clostridium difficile* (MIC = 0.09-0.38 µg mL<sup>-1</sup>).<sup>71,281</sup> Gram-negative bacteria, yeast, and fungi are resistant to reutericyclin.<sup>71</sup> The natural reutericyclin exhibits slightly higher antibacterial activity compared to the synthetic reutericyclin racemate, indicating that the stereochemistry is vital to the compound's bioactivity.282 Reutericyclin is an amphiphilic molecule consisting of a hydrophilic negatively charged group and two hydrophobic side chains. Thus, it acts as a proton ionophore and targets the cytoplasmic membrane causing dissipation of the transmembrane proton potential ( $\Delta pH$ ) in sensitive cells.72,73,283 SAR revealed that substitution of these hydrophobic groups with polar or charged substituents diminishes the antibacterial activity. The loss of activity in polarsubstituted reutericyclins is probably due to the decreased interaction with the hydrophobic regions of the bacterial membrane.283 Although the in vitro profile of reutericyclin 38a is comparable to the antibiotic mupirocin, it's in vivo activity in S. aureus murine infection model is 5-fold weaker compared to the antibiotic. The primary factor that may decrease the efficacy of 38a in vivo is likely the slow partitioning of the aqueous dermis by the highly lipophilic reutericyclin molecules.72 Reutericyclin is cytotoxic towards Vero epithelial cells and causes hemolysis in mammalian cells.283 Conversely, modifications of the substituents in the N-substituted position has shown to modulate the cytopathic effects of this class of compounds.<sup>284</sup> Mutanocyclin 39 consisting mainly of the tetramic acid core lacks antibacterial activity, demonstrating that the presence of the appropriate ring moieties plays a critical role in the bioactivity.30,275,276,284 Taken together, reutericyclins appear to be potent candidates for controlling recalcitrant skin infections caused by Gram-positive pathogens. Further medicinal chemistry optimization efforts are necessary to discover reutericyclinbased chemotypes with reduced toxicity whilst retaining or increasing antibacterial activity.

The production of reutericyclin **38a** in sourdough is thought to inhibit other competing Gram-positive competitor *L. sanfranciscensis* while enabling the stable persistence of the producing organism *L. reuteri*. A wide variety of food-related spoilage pathogens is inhibited by reutericyclin. Hence, reutericyclin-producing strains may find application in food preservation and fermentations.<sup>68</sup>

In *S. mutans*, the tetramic acids reutericyclins **38a–d** and mutanocyclin **39** are found to inhibit the growth of healthy oral microbes, suggesting that the pathogen likely use these molecules to remove the bacteria that block its growth to further cause severe dental caries.<sup>30</sup> The findings lay a foundation for the continued exploration of antibiotic-producing strains within the complex competing microbial niche of the human microbiota.

# 4.4 Ribosomally synthesized and post-translationally modified peptides (RiPPs)

Ribosomally synthesised and post-translationally modified peptides (RiPPs) are a large class of structurally diverse natural products (Fig. 17 and Table S1<sup>†</sup>). RiPPs are produced from a short precursor peptide comprising of a leader peptide and a core peptide. Biosynthesis begins with the synthesis of a precursor peptide by the ribosome. Then, the core peptide is subject to post-translational modifications (PTMs) beyond the 20 canonical amino acids; many of which are guided by leader peptides and recognition sequences to install a wide variety of unusual structural features onto the peptide backbone. Such PTMs can often render significant advantages over unmodified linear peptides, including enhanced target affinity and stability, as well as resistance to proteolytic degradation. Following modifications, the leader peptide and recognition sequences are cleaved by proteolysis, sometimes concomitant with cyclisation of the polypeptide chain, to produce the mature active product. In some cases, additional post-translational modifications occur after cleavage of the flanking sequences. For further information and perspectives on RiPP biosynthesis, we direct the readers to several recent reviews.285,286 Numerous ribosomally-synthesised bacteriocins have been isolated from pathogenic bacteria, and they have been the subject of several different reviews.<sup>25,287,288</sup> In this section, we highlight those interesting antibiotic RiPPs with unusual PTMs from bacterial pathogens such as darobactin 45, bottromycin 46, and nocardithiocin 47 (Fig. 17 and Table S1<sup>†</sup>).

4.4.1 Darobactin. Darobactin 45 is the first member of a new class of antibiotics that selectively kills Gram-negative bacteria produced by Photorhabdus khanii (Fig. 17 and Table S1<sup>†</sup>).<sup>289</sup> Darobactin 45 is a 7-mer modified peptide with an amino acid sequence of Trp1-Asn2-Trp3-Ser4-Lys5-Ser6-Phe7. The unprecedented chemical architecture of darobactin features a novel scaffold with two fused macrocycles, an aromatic-aliphatic ether linkage between two tryptophans and a unique tryptophan-lysine bond between two inactivated carbons.<sup>289</sup> This unusual lysine-tryptophan crosslink was also previously identified in the peptide pheromone, streptide from Streptococcus thermophilus. Genetic and biochemical studies implicated a radical S-adenosylmethionine (rSAM) enzyme, StrB containing two [4Fe-4S] clusters likely responsible for the installation of the unique lysine-to-tryptophan crosslink in streptide.290 Enzymes of the rSAM class catalyse free radical based reactions to incorporate a wide variety of unique and difficult modifications during RiPP biosynthesis, including α-,  $\beta$ -, and  $\gamma$ -thioether bridge, tyramine excision, epimerization, methylation, aliphatic-ether crosslink, and carbon-carbon bond formation.291,292

The putative BGC involved in darobactin **45** biosynthesis consists of a propeptide DarA, transporters DarB and DarD, membrane fusion protein DarC, and a RaS enzyme DarE. Deletion of the *dar* operon in *P. khanii* DSM3369 by double crossover abrogated production of **45**. Notably, heterologous expression of the *dar* BGC into *E. coli* produced the peptide suggesting that the *dar* is sufficient for darobactin production.



MIC < 0.0078 µg/mL against Corynebacterium xerosis



DarE showed little homology to StrB, nonetheless, DarE harbours the rSAM and SPASM/Twitch domains that are characteristic of this diverse protein superfamily. It is speculated that DarE catalyses the versatile formation of Lys-to-Trp macrocyclic crosslink in darobactin. Recently, a novel rSAM enzyme TqqB has been shown to install a C-O-C Thr-Gln ether cross-link.<sup>291</sup> The dar operon does not encode a separate putative enzyme that incorporates the C-O-C Trp-Trp ether bond. It was speculated that DarE may not only catalyse the linkage of the Trp-Lys C-C bond but also the formation of the aromaticaliphatic ether linkage in darobactin. RiPP operons often encode a protease that cleaves out the mature peptide; however, this is not present in the dar operon. Generic proteolysis is presumed to be involved in the maturation of the propeptide.<sup>289</sup> It is anticipated that structural and biochemical investigations of the novel darobactin enzymatic system will further expand the repertoire of rSAM enzymes and will aid future engineering efforts of RiPP natural products.

Darobactin 45 is effective against multiple Gram-negative bacteria in vitro, including drug-resistant human pathogens such as polymyxin-resistant P. aeruginosa and extendedspectrum β-lactam-resistant K. pneumoniae and E. coli and carbapenem-resistant clinical isolates (MIC =  $2-64 \ \mu g \ mL^{-1}$ ). It exhibits better efficacy in several mouse septicaemia infection

models than the antibiotic gentamicin. Darobactin, however, showed little to no activity on Gram-positive bacteria, gut commensals including Bacteroides and human cell lines (HepG2, FaDu, HEK293) up to 128 µg mL<sup>-1</sup> concentration.<sup>289</sup>

Gram-negative bacteria are difficult to treat due to their double-membrane cell wall, which forms a protective barrier from antibiotics.<sup>293</sup> The outer membrane contains a layer of negatively charged lipopolysaccharides in addition to proteins and phospholipids that blocks the entry of large and hydrophobic molecules.9 The cut-off size for compounds that can penetrate the membrane is about 600 Da. Given the size of darobactin (966 Da), it cannot breach this permeability barrier but instead acts on the surface of the cell. Darobactin binds to the β-barrel assembly machine (BAM) A protein and induces the closed-gate conformation, thereby preventing the normal protein folding and membrane insertion necessary for bacterial survival.<sup>289</sup> The discovery of darobactin 45 offers a promising lead in the dwindling pipeline of antibiotics that selectively target the Gram-negatives. Currently, darobactin 45 is in preclinical stage.9

The production of darobactin 45 in large amounts remains a challenge. Heterologous expression in different Photorhabdus species yielded highest in P. khanii DSM 3369 strain  $(3 \text{ mg L}^{-1})$ ,<sup>289</sup> yet the production titre is still low. The complexity

of the structure and the stereochemistry make it difficult to be obtained by chemical synthesis. The poor yield complicates both drug development and further biosynthetic studies of the molecule. Nonetheless, bacterial genome sequences identified further tentative analogues, darobactins B–E from *Yersinia*, and *Photorhabdus* species.<sup>289</sup> Expression of these putative darobactins may provide insight into the structure–activity relationships (SAR) and determine the pharmacophoric regions of the molecule. The identification of the biosynthetic route of **45** should facilitate the generation of a library of darobactin-like antibiotics that selectively targets the Gram-negatives.

**4.4.2 Bottromycin.** Bottromycin **46** was first isolated from the fermentation cultures of *Streptomyces bottropensis* in 1957 (ref. 294) and was later characterised in several other *Streptomyces* species<sup>295</sup> including the plant pathogen *S. scabies* (Fig. 17 and Table S1†).<sup>296,297</sup> The structure elucidation process involved several repeated revisions since its first isolation<sup>298–301</sup> which ultimately led to the assignment of bottromycin **46** and was later confirmed by chemical synthesis.<sup>302</sup> The structure features an unprecedented macrolactamidine ring, rare β-methylated amino acid residues, and a terminal methyl ester and a thiazole heterocycle.<sup>294,296,298</sup> Through untargeted metabolomics and mass spectral networking analysis, the biosynthetic pathway of bottromycins in *S. scabies* was determined which involves

a series of complex and unprecedented modifications from the precursor peptide (BtmD) catalysed by the enzymes encoded in the *btm* gene cluster (Fig. 18).<sup>303</sup> The regulation of bottromycin biosynthesis in *S. scabies* was recently elucidated. The only regulatory gene *btmL* encoded in the cluster was identified to be a positive modulator of *btmD* and not a master regulator that controls bottromycin expression.<sup>304</sup> The mechanism by which BtmL modulates BtmD transcription in *S. scabies* remains elusive. An understanding of the regulation of bottromycin biosynthesis may shed light into further engineering and overproduction of medicinally promising bottromycin-based compounds as well as expression of other RiPP pathways.

Total synthesis of bottromycins and analogues enabled evaluation of their antibacterial activity.<sup>302,305-307</sup> Bottromycin inhibits the growth of a wide range of microorganisms by blocking the binding of aminoacyl tRNAs to the A-site on the 50S ribosome, ultimately leading to inhibition of bacterial protein synthesis.<sup>308,309</sup> The bottromycins, particularly bottromycin A2 **46**, display potent antibacterial activity against Gram-positive bacteria including clinically-isolated MRSA and VRE strains (MIC = 0.5–2.0  $\mu$ g mL<sup>-1</sup>)<sup>305,306,310</sup> and mycoplasma.<sup>306</sup> A natural de-methyl analogue of **46**, bottromycin B2, exhibits slightly reduced antibacterial activity (MIC = 4  $\mu$ g mL<sup>-1</sup>).<sup>305</sup> The three-dimensional structure of bottromycin is



Fig. 18 Biosynthesis of bottromycin A2 in S. scabies.

essential for the antibacterial activity while the thiazole and methyl ester moieties are not required.<sup>305</sup>

The development of bottromycin as an antibacterial drug is impeded by the reduced in vivo efficacy in MRSA-infected mice. This reduced efficacy is mainly due to the instability of the terminal methyl ester moiety, which undergoes rapid hydrolysis to carboxylic acid in blood plasma rendering it inactive. Notably, the substitution of this ester with a ketone functionality resulted in potent and stable analogues with improved pharmacological properties and superior in vivo efficacy in the mouse infection model than 46.306 Therefore, further structural optimization of bottromycin-based compounds via engineering of the biosynthetic pathway or chemical synthesis offers promising leads for the development of new bottromycin-based anti-infectives. Recently, yeast-mediated pathway engineering of the bottromycin BGC through an inducible, theophyllinecontrolled riboswitch system led to an overall 120-fold increase in pathway productivity in a heterologous Streptomycete host.311 Another approach involved promoter exchange that resulted in 5–50 fold higher productivity of a suite of new bottromycin-related compounds compared to the wild type strain.<sup>312</sup> Application of these strategies to turn-up or upregulate biosynthetic pathways that are involved in controlling metabolic yields will undoubtedly facilitate the discovery of known NPs and new bioactive NPs in Actinobacteria.

**4.4.3 Nocardithiocin.** Nocardithiocin **4**7 is a thiocillin-like thiopeptide produced by the opportunistic pathogen *N. pseudobrasiliensis* strain IFM 0757 obtained from a clinical sample (Fig. 17 and Table S1†).<sup>313</sup> Thiopeptides (or thiazolyl peptides) are a family of highly modified sulfur-rich peptides, characterised by a macrocycle bearing a nitrogen-containing sixmembered ring core, numerous thiazole rings, and several dehydrated amino acid residues.<sup>314</sup> Nocardithiocin contains the characteristic 2,3,6-trisubstituted pyridine core, and hence it is classified into series d.<sup>313</sup>

Biosynthesis of nocardithiocin 47 is proposed to be directed by a 12 gene-cluster (*notA-L*), with NotG as the precursor peptide (Fig. 19).<sup>315</sup> The characteristic pyridine core in 47 is likely



Nocardithiocin 47

Fig. 19 Proposed biosynthetic pathway of nocardithiocin 47.

formed by macrocyclization of the precursor peptide at sites Ser1 and Ser10. The structure of 47 features isoleucine (Ile8) bearing two hydroxy moieties resembling PTMs observed in thiostrepton that is probably catalysed by a cytochrome P450.316 A second P450 is predicted to hydroxylate a dehydroalanine (Dha4), similar to those observed in berninamycin compounds,<sup>317</sup> which is subsequently methylated by a putative methyltransferase (NotC or NotE). Another methyltransferase (NotC or NotE) likely installs a methyl group at the Cterminus.315

Nocardithiocin 47 exhibits potent bacteriostatic activity against a variety of bacteria including Corynebacterium xerosis (MIC < 0.0078  $\mu$ g mL<sup>-1</sup>), *M. smegmatis* (MIC = 0.062  $\mu$ g mL<sup>-1</sup>), Nocardia asteroides (MIC = 0.062  $\mu g m L^{-1}$ ), and Gordonia bronchialis (MIC = 0.03  $\mu g \text{ mL}^{-1}$ ). It is also highly active

against rifampicin-resistant bacteria as well as -sensitive M. tuberculosis strains, and most of the resistant strains were inhibited at concentrations ranging from 0.025 to 1.56 µg mL<sup>-1</sup>.<sup>313</sup> Despite the impressive antibiotic activity of 47, its clinical use is hampered by poor aqueous solubility and light instability.<sup>313,314</sup> The identification of the nocardithiocin BGC expands the possibility for further structural modifications to generate stable analogues with improved pharmacokinetic properties. Genetic modification of the nocardithiocin scaffold via substitution of Val6 of the core peptide by ten mostly hydrophobic amino acids yielded nocardithiocin analogues, two of which showed improved MIC against a panel of Grampositive bacteria. Furthermore, nocardithiocin and all analogues were stable to light. However, they remained poorly water-soluble.<sup>318</sup> Introduction of polar groups at the



Fig. 20 Other categories of metabolites discovered from pathogens.

tail end of the nocathiacin thiopeptide enhanced its watersolubility while retaining its potent *in vitro* and *in vivo* antibacterial activity.<sup>319</sup> Similar tail modifications could also improve the solubility of nocardithiocin without diminishing antibacterial potency.

### 4.5 Other categories of metabolites from pathogens

Most of the PKS and peptidyl compounds discussed in this review can be classified based on their biosynthetic class. Other antimicrobial metabolites, including nucleosides, indoles, guanine,  $\beta$ -lactams, and carbapenems will be covered in this section, highlighting those with remarkable activity.

**4.5.1 Carbapenems.** *Serratia* strains generate antibiotics of the carbapenem group (Fig. 20 and Table S1<sup>†</sup>).<sup>320</sup> Carbapenems belong to a diverse group of  $\beta$ -lactam antibiotics, which are now the most widely used class of clinical antibiotics to date.<sup>321</sup> Their biosynthesis involves a biochemical route unique from the other four known classes of  $\beta$ -lactams, such as penicillins,<sup>322</sup> cephalosporins,<sup>322-324</sup> monobactams,<sup>325</sup> and clavams.<sup>326</sup> The biosynthesis and regulation of carbapenems have been extensively studied in *Serratia* and involve a nine-gene cluster, *carRABCDEFGH*.<sup>327,328</sup>

Carbapenems have broad-spectrum activity against important Gram-positive and Gram-negative pathogens, particularly nosocomial multidrug-resistant bacteria.<sup>321,328</sup> Furthermore, they exhibit potent antibacterial and  $\beta$ -lactamase-inhibitory activity.<sup>328,329</sup> *S. marcescens* makes the simplest known  $\beta$ -lactam antibiotic containing only the bicyclic nucleus, 1-carbapen-2-em-3-carboxylic acid (SQ 27860) **48** and two saturated diastereomers, (3*R*,5*R*)- and (3*S*,5*R*)-carbapenam-3-carboxylic acids **49–50** (Fig. 20 and Table S1<sup>†</sup>).<sup>320,330–332</sup> Gram-negative enteric *Erwinia* strains also produce carbapenem **48** and carbapenams **49–50**, and later **48** was identified as a metabolite of the entomopathogen, *P. luminescens*.<sup>330–332</sup> Unlike **48**, these carbapenams **49–50** lack antibacterial activity but are resistant to β-lactamases I and II from *B. cereus*.<sup>332</sup> Carbapenem **48** is a potent antibacterial, but it is highly unstable and requires initial derivatization to the *p*-nitrobenzyl ester for isolation. Carbapenem **48** is active against several strains of *S. aureus*, *E. coli*, and *Enterobacter cloacae*.<sup>331</sup>

**4.5.2 Thioguanine.** Thioguanine **51** (also known as tioguanine or 6-thioguanine, 6-TG or 2-aminopurine-6-thiol), introduced in the early 1950s for antimetabolite therapy,<sup>333</sup> is now in clinical use for the treatment of various diseases including psoriasis, inflammatory bowel disease and acute and chronic myelogenous leukaemia (Fig. 20 and Table S1†).<sup>334-337</sup> Thioguanine **51** is a sulfur-containing guanine analogue that works by disrupting DNA and RNA. Originally known as a synthetic compound,<sup>333</sup> thioguanine was first isolated from the cultures of *Pseudomonas* sp. GH<sup>338</sup> and later identified as a critical virulence factor of the plant pathogen, *E. amylovora*.<sup>50,339</sup>

The biosynthesis of thioguanine **51** has been recently elucidated in *E. amylovora* and is encoded by the *ycf* gene cluster (Fig. 21A). The rare thioamide moiety in thioguanine is likely derived from the action of two key enzymes YcfA and YcfC which constitutes a bipartite enzyme system, unique from those previously described thionation in RNA systems (Fig. 21B).<sup>337,339</sup> The ATP-dependent YcfA enzyme catalyses the transfer of sulfur onto the guanine backbone<sup>339</sup> and uses a pyridoxal phosphate



Fig. 21 (A) Organization of the *ycf* gene cluster in *Erwinia amylovora*, and (B) model for the bipartite enzyme system, YcfA and YcfC for the oxygen-to-sulfur substitution in thioamide formation.

(PLP)-dependent specialised sulfur shuttle enzyme, YcfC that functions independently from the general sulfur mobilization pathways.<sup>337</sup> While the sulfur source in universal RNA-systems often originates from L-cysteine through the action of cysteine desulfurases (IscS),<sup>340</sup> the cysteine-derived sulfur nucleophile in thioguanine biosynthesis is provided by YcfC and then, transferred and bound onto one of the cysteine residues (Cys113) of the YcfA active site.<sup>337,339</sup> Meanwhile, no thionated products were detected using the IscS homologue (Ea-IscS)-catalysed reaction in *E. amylovora*.<sup>337</sup> YcfA initially activates the guanine backbone by adenylating the carbonyl oxygen prior to thionation.<sup>339</sup> Subsequent YcfA-mediated sulfur transfer to the activated substrate generates the thioamides with concomitant release of adenosine monophosphate (AMP).<sup>337,339</sup>

In addition to its anticancer properties,<sup>334</sup> thioguanine **51** is bacteriostatic towards *E. coli*<sup>338</sup> and strains of *Salmonella typhimurium* and *Pantoea agglomerans*,<sup>341</sup> with the resumption of cell growth after prolonged incubation. At 0.25  $\mu$ M concentration, thioguanine completely inhibited the growth of *E. coli* strains B and K12.<sup>341</sup> This inhibition did not occur when either adenine or guanine was present in the assay medium.<sup>338</sup> Growth inhibition was also reported for *B. subtilis*,<sup>342,343</sup> and the abolishment of flagella formation for *B. cereus*.<sup>344</sup> Other organisms like *Streptococcusfaecium* strain, *S. cerevisiae*, *Rahnella aquatilis*, and *Gibbsiella* sp. (strain BK1) were insensitive to thioguanine.<sup>50,341,345</sup>

**4.5.3 Caryoynencin.** Caryoynencin A, B, and C **52a–c** were isolated from the cultures of the plant carnation pathogen *Burkholderia caryophylli* through bioactivity guided screening. The structure features an exceedingly rare polyacetylene functionality, and remarkably, caryoynencin is the only known bacterial polyyne with four conjugated triple bonds. However, caryoynencin is extremely unstable. Isolation of **52** from the active extract was carried out by applying argon at 0 °C to obtain a concentrated mixture of caryoynencins A, B, and C. By contrast, concentration on a rotary evaporator (20 °C) or storage at -20 °C led to a complete loss of bioactivity. Furthermore, the individual component was not achievable since they equilibrate

rapidly.<sup>346</sup> Recently, a derivatization strategy that selectively targets terminal alkynes was used to isolate and characterise these extremely unstable compounds directly from the culture extracts (Fig. 22).<sup>347</sup> The so-called CuAAC (copper-catalysed azide–alkyne cycloaddition) "click reaction"<sup>348</sup> was carried out by treatment of the *B. caryophilli* extracts with benzyl azide and copper(1) catalyst to obtain the triazoles **60a–b** and **61b**, the structures of which were fully elucidated by NMR.<sup>347</sup>

Transposon mutagenesis and genome sequencing of B. carvophilli (DSM50341) have provided the first insight into the unusual polyyne biosynthesis in bacteria which involves novel desaturases and a cytochrome P450 monooxygenase (Fig. 23A). Disruption of the transposon site points to a  $\Delta^9$  desaturase-like gene, orthologs of which were identified in the genomes of the plant pathogens Burkholderia gladioli BSR3 and B. gladioli pv. cocovenenans. Comparative genomics analyses further revealed that the carvoynencin (cay) locus is conserved in several Burkholderia strains, and homologous gene clusters were also identified in various other bacteria.347 Metabolomic analyses also revealed that strain BSR3 and B. gladioli Lv-StA are capable of caryoynencin production.<sup>347,349</sup> The characterisation of the cay BGC will thus facilitate the discovery of numerous polyynebearing NPs from bacteria and lead to the expansion of the polyyne biosynthetic machinery capable of producing polyacetylenes.

Caryoynencin is likely derived from fatty acid-ACP, followed by desaturation to yield the alkyne motifs, **62** (Fig. 23B). Three putative desaturase genes (*cayB*, *cayC*, *cayE*) were implicated to be responsible for the incorporation of the triple bonds. Phylogenetic analyses have revealed that the CayBCE desaturases are unique and have probably evolved independently from those found in fungi, plants, and insects.<sup>347</sup> Notably, CayB and CayC form a separate clade with the closest desaturase homologue JamB,<sup>350</sup> which has been suggested to introduce the terminal alkyne functionality in the jamaicamide pathway of *Moorea producens*. Subsequent hydroxylation and elimination reactions of **62** catalysed by the putative cytochrome P450



Fig. 22 Chemical trapping of the tetraynes by an *in situ* copper(*i*)-catalysed azide–alkyne cycloaddition (CuAAC) click reaction. (A) Structures of triazoles 60a-b produced from *B. caryophylli* wild type after click reaction, and (B) structures of 61a from *B. caryophylli*  $\Delta$ cayG and 61b after click reaction, and (B) structures of 61a from *B. caryophylli*  $\Delta$ cayG and 61b after click reaction, and 61c shunt product.

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Fig. 23 (A) Biosynthetic gene cluster of caryoynencin (cay) in B. caryophilli, and (B) Proposed biosynthetic pathway of caryoynencin.

(CayG) generates the allylic alcohol moiety in caryoynencin **52**. Formation of the triazole **61b** lacking a hydroxyl group in  $\Delta cayG$  mutant after an *in situ* click reaction supports the plausible function of CayG. Compound **61c** with an alcohol moiety instead of a triple bond is likely a shunt product of terminal alkyne formation.<sup>347</sup>

Caryoynencin has been shown to possess outstanding activity than the antibiotic kanamycin A. It is active against a wide range of bacteria and fungi including *E. coli* (MIC = 0.63  $\mu$ g mL<sup>-1</sup>), *K. pneumoniae* (MIC = 0.04  $\mu$ g mL<sup>-1</sup>), *S. aureus* (MIC = 0.02  $\mu$ g mL<sup>-1</sup>), *B. subtilis* (MIC = <0.02  $\mu$ g mL<sup>-1</sup>), *C. albicans* (MIC = 0.05  $\mu$ g mL<sup>-1</sup>), and several *Trichophyton* species (MIC = 0.02–0.05  $\mu$ g mL<sup>-1</sup>).<sup>346,351</sup> Owing to their high instability, synthetic approaches have been developed to gain more insight

into their structure and function.<sup>352,353</sup> The terminal alkyne and the hydroxy group were crucial for the antibacterial activity while the diene motif and the butanoic acid were not essential.<sup>351,353</sup> The triazole **60b** is active against *B. subtilis* (MIC = 3.12  $\mu$ g mL<sup>-1</sup>) and MRSA (12.5  $\mu$ g mL<sup>-1</sup>),<sup>346</sup> consistent with earlier studies that the tetrayne molecules were more potent than the corresponding triyne or diyne analogues.<sup>351</sup> Remarkably, the introduction of a trimethylsilyl motif generates stable polyacetylene derivatives with potent antibacterial activity. Hydrophilicity also plays an important role in the bioactivity, and thus conjugates of polyynes and sugars, amino acids, and nucleic acids are attractive molecules.<sup>353</sup>

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#### Conclusions and future 5. perspective

Pathogenic bacteria have an enormous yet unexploited potential for natural product drug discovery. Entomopathogenic, phytopathogenic, and human and animal pathogenic bacteria produce a repertoire of novel potential therapeutics, with an assortment of unprecedented structures, activities, and modes of action. Some of them are in pre-clinical trials (darobactin, NOSO-95C analogue) or have huge potential for drug development. Darobactin, odilorhabdin, and albicidin are promising candidates in the dwindling pipeline of antibiotics that selectively target the Gram-negatives.

Although darobactin is a potent drug lead for Gram-negative bacteria, the greater bottleneck is to produce it in large amounts for pre-clinical and clinical development. Moreover, the complexity and stereochemistry of darobactin make it difficult to be obtained by chemical synthesis, and thus SAR studies to determine the key bioactive moieties remain a challenge. Nevertheless, modifications of the complex vancomycin antibiotic have been achieved by several groups to produce potent analogues with less propensity to antibiotic resistance.354 Lead optimisation of the odilorhabdin scaffold was also achieved and identified NOSO-502 as a clinical candidate for carbapenemresistant Enterobacteriaceae.128 Several other metabolites that possess potent in vitro activity can be chemically-modified to increase in vivo efficacy and further enhance pharmacokinetic properties without diminishing activity such as bottromycin, althiomycin, caryoynencin, nocardithiocin, lugdunin, nematophin, holomycin, and reutericyclin. The NPs covered in this review could be clinical leads or could provide structural templates for further medicinal chemistry optimisation efforts.

The ecological functions of currently known NPs in pathogenic bacteria remain to be deciphered. Understanding this role might be key to determining their potential use. For example, clostrubins which serve dual functions - kills potential microbial competitors and permits survival of the pathogenic anaerobe in an oxygen-rich potato niche - represent promising leads for the design and development of antibacterial therapeutics and plant protection agents.47 Impairing clostrubin production in C. puniceum could also help prevent potatoes from "soft rot".

Given that some pathogenic bacteria are a threat to humans, how can one prioritize natural products discovery from these huge untapped resources? Developments in culture-independent meta-omics approaches have provided greater access to underinvestigated taxa that contain unique metabolic profiles that probably encode novel chemistry. The exploitation of these metagenomic data has proven to be beneficial in the characterisation and isolation of cryptic metabolites from the complex human microbiota.355-357 For example, the thiopeptide antibiotic lactocillin was discovered via a sequence-based metagenomic analysis.358 Such techniques can be exploited to identify potential genetic markers of disease in NP producing bacterial pathogens, thereby circumventing the threat of opportunistic infections caused by pathogenic bacteria. Metagenomic approaches also provide a means to access novel bioactive molecules with diverse structures. Integrating other emerging techniques in these NP discovery efforts, such as elicitation of cryptic biosynthetic pathways and refactoring of silent BGCs should help illuminate the chemical "dark matter" in bacterial pathogens. The substitution of native promoters with strong constitutive promoters in cryptic gene clusters has been one such productive strategy in activating biosynthesis and improving antibiotic expression. For example, promoter exchange in Photorhabdus spp. and Xenorhabdus spp. led to the expression of several cryptic nonribosomal peptides. Additionally, promoter exchange in  $\Delta hfq$  mutants resulted in the production of desired metabolites for further bioactivity testing.<sup>151</sup> The development of high-throughput next-generation sequencing methods, together with the development of new bioinformatics tools that can assemble nearly complete genomes, will continue to revolutionize microbial "dark matter" exploration. Furthermore, improvements in analytical platforms (mass spectrometry, NMR) coupled with recent advancements in metabolomics enable the detection and identification of compounds in minute quantities from complex biological samples.359-361 Application of recent machine learning tools for structure recognition, bioactivity prediction, drug-target interactions<sup>362</sup> such as the NMR-based Small Molecule Accurate Recognition Technology (SMART 2.0)<sup>363</sup> further accelerates the drug discovery process.

Taken together, access to the immense repertoire of novel cryptic metabolites encoded in pathogenic bacteria is only achievable through improvements in, and integration of, various approaches and available methods from multiple disciplines. Further exploitation of the untapped chemical diversity of pathogenic bacteria will undoubtedly yield many more novel bioactive molecules and might reboot the antibiotic pipeline. Soon, we can predict a second "Golden Era of Antibiotics" discovery.

#### Conflicts of interest 6.

The authors declare no conflicts of interest.

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## 8. References

- 1 Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics, World Health Organization, Geneva Switz., 2017, pp. 1–7.
- 2 C. Willyard, Nature, 2017, 543, 15.
- 3 A. P. Zavascki, L. Z. Goldani, J. Li and R. L. Nation, J. Antimicrob. Chemother., 2007, 60, 1206–1215.
- 4 Y. Y. Liu, Y. Wang, T. R. Walsh, L. X. Yi, R. Zhang, J. Spencer,
  Y. Doi, G. Tian, B. Dong, X. Huang, L. F. Yu, D. Gu, H. Ren,
  X. Chen, L. Lv, D. He, H. Zhou, Z. Liang, J. H. Liu and
  J. Shen, *Lancet Infect. Dis.*, 2016, 16, 161–168.
- 5 A. R. Coates, G. Halls and Y. Hu, *Br. J. Pharmacol.*, 2011, **163**, 184–194.
- 6 J. L. Martínez and F. Baquero, *Upsala J. Med. Sci.*, 2014, **119**, 68–77.
- 7 R. A. Bonomo, *Cold Spring Harbor Perspect. Med.*, 2017, 7, 1–15.
- 8 A. Luther, M. Urfer, M. Zahn, M. Müller, S. Y. Wang, M. Mondal, A. Vitale, J. B. Hartmann, T. Sharpe, F. Lo Monte, H. Kocherla, E. Cline, G. Pessi, P. Rath, S. M. Modaresi, P. Chiquet, S. Stiegeler, C. Verbree, T. Remus, M. Schmitt, C. Kolopp, M. A. Westwood, N. Desjonquères, E. Brabet, S. Hell, K. LePoupon, A. Vermeulen, R. Jaisson, V. Rithié, G. Upert, A. Lederer, P. Zbinden, A. Wach, K. Moehle, K. Zerbe, H. H. Locher, F. Bernardini, G. E. Dale, L. Eberl, B. Wollscheid, S. Hiller, J. A. Robinson and D. Obrecht, *Nature*, 2019, 576, 452–458.
  9 K. Lewis, *Cell*, 2020, 181, 29–45.
- 10 E. M. Molloy and C. Hertweck, *Curr. Opin. Microbiol.*, 2017, **39**, 121–127.
- 11 F. Baldeweg, D. Hoffmeister and M. Nett, *Nat. Prod. Rep.*, 2019, **36**, 307–325.
- 12 Y. M. Shi and H. B. Bode, Nat. Prod. Rep., 2018, 35, 309-335.
- 13 S. J. Pidot, S. Coyne, F. Kloss and C. Hertweck, *Int. J. Med. Microbiol.*, 2014, **304**, 14–22.
- 14 J. Mansfield, S. Genin, S. Magori, V. Citovsky, M. Sriariyanum, P. Ronald, M. A. X. Dow, V. Verdier, S. V Beer, M. A. Machado, I. A. N. Toth, G. Salmond, G. D. Foster, I. P. Lipm and F.-C. Tolosan, *Mol. Plant Pathol.*, 2012, 13, 614–629.
- 15 Y. Ichinose, F. Taguchi and T. Mukaihara, J. Gen. Plant Pathol., 2013, **79**, 285–296.
- 16 J. A. Banas, Front. Biosci., 2004, 9, 1267-1277.
- 17 A. Wrobel, J. C. Leo and D. Linke, Genes, 2019, 10, 1-20.
- 18 K. Becker, C. Heilmann and G. Peters, *Clin. Microbiol. Rev.*, 2014, 27, 870–926.
- 19 K. R. Soumya, S. Philip, S. Sugathan, J. Mathew and E. K. Radhakrishnan, *3 Biotech*, 2017, 7, 1–10.
- 20 S. J. Hinchliffe, M. C. Hares, A. J. Dowling and R. H. Ffrench-Constant, *Open Toxicol. J.*, 2010, **3**, 83–100.
- 21 D. R. D. Bignell, J. K. Fyans and Z. Cheng, J. Appl. Microbiol., 2014, 116, 223–235.
- 22 A. Rodou, D. O. Ankrah and C. Stathopoulos, *Toxins*, 2010, 2, 1250–1264.

- 23 J. W. Wilson, M. J. Schurr, C. L. LeBlanc, R. Ramamurthy, K. L. Buchanan and C. A. Nickerson, *Postgrad. Med. J.*, 2002, 78, 216–224.
- 24 N. J. Tobias, Y. M. Shi and H. B. Bode, *Trends Microbiol.*, 2018, **26**, 833–840.
- 25 L. L. Newstead, K. Varjonen, T. Nuttall and G. K. Paterson, *Antibiotics*, 2020, **9**, 1–19.
- 26 J. Dreyer, A. P. Malan and L. M. T. Dicks, *Front. Microbiol.*, 2018, 9, 1–14.
- 27 S. Müller, E. Garcia-Gonzalez, E. Genersch and R. D. Süssmuth, *Nat. Prod. Rep.*, 2015, **32**, 765–778.
- 28 S. Kunakom and A. S. Eustáquio, J. Nat. Prod., 2019, 82, 2018–2037.
- 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. Brötz-Oesterhelt, S. Grond, A. Peschel and B. Krismer, *Nature*, 2016, 535, 511–516.
- 30 X. Tang, Y. Kudo, J. L. Baker, S. Labonte, P. A. Jordan, S. M. K. McKinnie, J. Guo, T. Huan, B. S. Moore and A. Edlund, ACS Infect. Dis., 2020, 6, 563–571.
- 31 Z. Qin, A. T. Baker, A. Raab, S. Huang, T. Wang, Y. Yu, M. Jaspars, C. J. Secombes and H. Deng, *J. Biol. Chem.*, 2013, 288, 14688–14697.
- 32 H. Deng and C. J. Secombes, Virulence, 2014, 5, 9-11.
- 33 D. Dhakal, V. Rayamajhi, R. Mishra and J. K. Sohng, J. Ind. Microbiol. Biotechnol., 2019, 46, 385–407.
- 34 V. L. Challinor and H. B. Bode, Ann. N. Y. Acad. Sci., 2015, 1354, 82–97.
- 35 Z. Hu and W. Zhang, ACS Infect. Dis., 2020, 6, 25-33.
- 36 H. B. Bode, Curr. Opin. Chem. Biol., 2009, 13, 224-230.
- 37 S. Forst, B. Dowds, N. Boemare and E. Stackebrandt, *Annu. Rev. Microbiol.*, 1997, **51**, 47–72.
- 38 M. L. Pineda-Castellanos, Z. Rodríguez-Segura, F. J. Villalobos, L. Hernández, L. Lina and M. Eugenia Nuñez-Valdez, *Pathogens*, 2015, 4, 210–228.
- 39 P. A. D. Grimont and F. Grimont, Annu. Rev. Microbiol., 1978, 32, 221–248.
- 40 A. Fünfhaus, J. Ebeling and E. Genersch, *Curr. Opin. Insect. Sci.*, 2018, 26, 89–96.
- 41 A. Soenens and J. Imperial, *Phytochem. Rev.*, 2020, **19**, 577–587.
- 42 N. Vodovar, D. Vallenet, S. Cruveiller, Z. Rouy, V. Barbe, C. Acosta, L. Cattolico, C. Jubin, A. Lajus, B. Segurens, B. Vacherie, P. Wincker, J. Weissenbach, B. Lemaitre, C. Médigue and F. Boccard, *Nat. Biotechnol.*, 2006, 24, 673–679.
- J. F. Challacombe, M. R. Altherr, G. Xie, S. S. Bhotika, N. Brown, D. Bruce, C. S. Campbell, M. L. Campbell, J. Chen, O. Chertkov, C. Cleland, M. Dimitrijevic, N. A. Doggett, J. J. Fawcett, T. Glavina, L. A. Goodwin, L. D. Green, C. S. Han, K. K. Hill, P. Hitchcock, P. J. Jackson, P. Keim, A. R. Kewalramani, J. Longmire, S. Lucas, S. Malfatti, D. Martinez, K. McMurry, L. J. Meincke, M. Misra, B. L. Moseman, M. Mundt, A. C. Munk, R. T. Okinaka, B. Parson-Quintana, L. P. Reilly, P. Richardson, D. L. Robinson, E. Saunders,

R. Tapia, J. G. Tesmer, N. Thayer, L. S. Thompson, H. Tice, L. O. Ticknor, P. L. Wills, P. Gilna and T. S. Brettin, *J. Bacteriol.*, 2007, **189**, 3680–3681.

- 44 C. T. Bull, S. H. De Boer, T. P. Denny, G. Firrao, M. F. Saux, G. S. Saddler, M. Scortichini, D. E. Stead, Y. Takikawa, T. P. Denny, G. Firrao, M. F. Saux, G. S. Saddler, M. Scortichini, D. E. Stead and Y. Takikawa, *J. Plant Pathol.*, 2010, 92, 551–592.
- 45 C. T. Bull, S. H. De Boer, T. P. Denny, G. Firrao, M. F. Saux, G. S. Saddler, M. Scortichini, D. E. Stead and Y. Takikawa, *J. Plant Pathol.*, 2012, **94**, 21–27.
- 46 C. T. Bull, T. A. Coutinho, T. P. Denny, G. Firrao, M. F. Saux,
  X. Li, G. S. Saddler, M. Scortichini, D. E. Stead and
  Y. Takikawa, *J. Plant Pathol.*, 2014, 96, 223–226.
- 47 G. Shabuer, K. Ishida, S. J. Pidot, M. Roth, H.-M. Dahse and C. Hertweck, *Plant Sci.*, 2015, **350**, 670–675.
- 48 I. K. Toth, J. M. van der Wolf, G. Saddler, E. Lojkowska,
  V. Hélias, M. Pirhonen, L. Tsror (Lahkim) and
  J. G. Elphinstone, *Plant Pathol.*, 2011, 60, 385–399.
- 49 M. Malnoy, S. Martens, J. L. Norelli, M.-A. Barny, G. W. Sundin, T. H. M. Smits and B. Duffy, *Annu. Rev. Phytopathol.*, 2012, 50, 475–494.
- 50 G. Feistner and C. M. Staub, *Curr. Microbiol.*, 1986, **13**, 95–101.
- 51 C. S. Oh and S. V. Beer, *FEMS Microbiol. Lett.*, 2005, 253, 185–192.
- 52 X. F. Xin, B. Kvitko and S. Y. He, *Nat. Rev. Microbiol.*, 2018, **16**, 316–328.
- 53 Y. Mast and E. Stegmann, Antibiotics, 2019, 8, 10-13.
- 54 F. Maglangit, Q. Fang, V. Leman, S. Soldatou, R. Ebel and H. Deng, *Molecules*, 2019, **24**, 3384.
- 55 R. E. de Lima Procópio, I. R. da Silva, M. K. Martins, J. L. de Azevedo and J. M. de Araújo, *Braz. J. Infect. Dis.*, 2012, 16, 466–471.
- 56 F. Maglangit, Q. Fang, K. Kyeremeh, J. M. Sternberg, R. Ebel and H. Deng, *Molecules*, 2020, 25, 256.
- 57 Q. Fang, F. Maglangit, L. Wu, R. Ebel, K. Kyeremeh, J. H. Andersen, F. Annang, G. Pérez-Moreno, F. Reyes and H. Deng, *Molecules*, 2020, 25, 460.
- 58 Q. Fang, F. Maglangit, M. Mugat, C. Urwald, K. Kyeremeh and H. Deng, *Molecules*, 2020, 25, 1108.
- 59 Y. Li, J. Liu, G. Díaz-Cruz, Z. Cheng and D. R. D. Bignell, *Microbiology*, 2019, 165, 1025–1040.
- 60 I. Pieretti, A. Pesic, D. Petras, M. Royer, R. D. Süssmuth and S. Cociancich, *Front. Plant Sci.*, 2015, 6, 1–7.
- 61 P. S. Conville, B. A. Brown-Elliott, T. Smith and A. M. Zelazny, *J. Clin. Microbiol.*, 2018, **56**, 1–10.
- 62 J. N. O'Sullivan, M. C. Rea, P. M. O'Connor, C. Hill and R. P. Ross, *FEMS Microbiol. Ecol.*, 2019, **95**, 1–10.
- 63 A. F. d. S. Duarte, H. Ceotto, M. L. V. Coelho, M. A. V. d. P. Brito and M. d. C. d. F. Bastos, *Food Control*, 2013, 32, 313–321.
- 64 J. A. Lemos, S. R. Palmer, L. Zeng, Z. T. Wen, J. K. Kajfasz, I. A. Freires, J. Abranches and L. J. Brady, *Microbiol. Spectrum*, 2019, 7, 1–18.
- 65 K. Nakano, R. Nomura and T. Ooshima, *Jpn. Dent. Sci. Rev.*, 2008, 44, 29–37.

- 66 L. Liu, T. Hao, Z. Xie, G. P. Horsman and Y. Chen, *Sci. Rep.*, 2016, **6**, 1–10.
- 67 S. S. Momeni, S. M. Beno, J. L. Baker, A. Edlund, T. Ghazal, N. K. Childers and H. Wu, *J. Dent. Res.*, 2020, **99**, 969–976.
- 68 M. G. Gänzle, Appl. Microbiol. Biotechnol., 2004, 64, 326–332.
- 69 X. Wang, L. Du, J. You, J. B. King and R. H. Cichewicz, Org. Biomol. Chem., 2012, 10, 2044–2050.
- 70 F. Qi, P. Chen and P. W. Caufield, *Appl. Environ. Microbiol.*, 2001, 67, 15–21.
- 71 M. G. Ganzle, A. Holtzel, J. Walter, G. Jung and W. P. Hammes, *Appl. Environ. Microbiol.*, 2000, 66, 4325– 4333.
- 72 J. G. Hurdle, R. Yendapally, D. Sun and R. E. Lee, *Antimicrob. Agents Chemother.*, 2009, **53**, 4028–4031.
- 73 M. G. Gänzle and R. F. Vogel, *Appl. Environ. Microbiol.*, 2003, 69, 1305–1307.
- 74 D. Dufour, A. Barbour, Y. Chan, M. Cheng, T. Rahman, M. Thorburn, C. Stewart, Y. Finer, S. G. Gong and C. M. Lévesque, *J. Bacteriol.*, 2020, 202, 1–14.
- 75 G. Nicolas and M. Lavoie, *Genome and Genomics*, 2007, 1, 193–208.
- 76 M. Mota-Meira, G. LaPointe, C. Lacroix and M. C. Lavoie, Antimicrob. Agents Chemother., 2000, 44, 24–29.
- 77 M. Mota-Meira, H. Morency and M. C. Lavoie, *J. Antimicrob. Chemother.*, 2005, **56**, 869–871.
- 78 P. M. Joyner, J. Liu, Z. Zhang, J. Merritt, F. Qi and R. H. Cichewicz, Org. Biomol. Chem., 2010, 8, 5486–5489.
- 79 M. Wietz, M. Mansson, C. H. Gotfredsen, T. O. Larsen and L. Gram, *Mar. Drugs*, 2010, 8, 2946–2960.
- 80 G. Kumar, S. Menanteau-Ledouble, M. Saleh and M. El-Matbouli, *Vet. Res.*, 2015, 46, 1–10.
- 81 S. de Keukeleire, A. de Bel, Y. Jansen, M. Janssens,
  G. Wauters and D. Piérard, New Microbes New Infect., 2014, 2, 134–135.
- 82 C. Hertweck, Angew. Chem., Int. Ed., 2009, 48, 4688-4716.
- 83 M. A. Fischbach and C. T. Walsh, *Chem. Rev.*, 2006, 106, 3468-3496.
- 84 J. Berger, L. M. Jampolsky and M. W. Goldberg, Arch. Biochem., 1949, 22, 476–478.
- 85 Z. Cao, G. Khodakaramian, K. Arakawa and H. Kinashi, *Biosci., Biotechnol., Biochem.*, 2012, **76**, 353–357.
- 86 C. Olano, B. Wilkinson, C. Sanchez, S. J. Moss, R. Sheridan, V. Math, A. J. Weston, A. F. Brana, C. J. Martin, M. Oliynyk, C. Mendez, P. F. Leadlay and J. A. Salas, *Chem. Biol.*, 2004, 11, 87–97.
- 87 J. Sun, J. Shao, C. Sun, Y. Song, Q. Li, L. Lu, Y. Hu, C. Gui, H. Zhang and J. Ju, *Bioorg. Med. Chem.*, 2018, 26, 1488–1494.
- 88 X. Gao, Y. Jiang, L. Han, X. Chen, C. Hu, H. Su, Y. Mu,
   P. Guan and X. Huang, *RSC Adv.*, 2017, 7, 44401–44409.
- 89 A. Hamed, A. S. Abdel-Razek, M. Frese, D. Wibberg, A. F. El-Haddad, T. M. A. Ibrahim, J. Kalinowski, N. Sewald and M. Shaaban, *Z. Naturforsch., C: J. Biosci.*, 2018, 73, 49–57.
- 90 L. Zhang, J. Shi, C. L. Liu, L. Xiang, S. Y. Ma, W. Li, R. H. Jiao, R. X. Tan and H. M. Ge, *Tetrahedron Lett.*, 2018, **59**, 4517–4520.

- 91 J. Kim, D. Shin, S. H. Kim, W. Park, Y. Shin, W. K. Kim, S. K. Lee, K. B. Oh, J. Shin and D. C. Oh, *Mar. Drugs*, 2017, **15**, 1–11.
- 92 A. Sidhu, J. R. Miller, A. Tripathi, D. M. Garshott,
  A. L. Brownell, D. J. Chiego, C. Arevang, Q. Zeng,
  L. C. Jackson, S. A. Bechler, M. U. Callaghan, G. H. Yoo,
  S. Sethi, H. S. Lin, J. H. Callaghan, G. Tamayo-Castillo,
  D. H. Sherman, R. J. Kaufman and A. M. Fribley, ACS Med. Chem. Lett., 2015, 6, 1122–1127.
- 93 Z. Zhou, Q. Wu, Q. Xie, C. Ling, H. Zhang, C. Sun and J. Ju, *Chem. Biodiversity*, 2020, **17**, e1900560.
- 94 C. J. Schulze, W. M. Bray, F. Loganzo, M. Lam, T. Szal, A. Villalobos, F. E. Koehn and R. G. Linington, *J. Nat. Prod.*, 2014, 77, 2570–2574.
- 95 M. Kuo, D. A. Yurek and D. A. Kloosterman, *J. Antibiot.*, 1989, **42**, 1006–1007.
- 96 B. F. Anderson, A. J. Herit, R. W. Rickards and G. B. Robertson, *Aust. J. Chem.*, 1989, 42, 717–730.
- 97 E. M. Novoa, N. Camacho, A. Tor, B. Wilkinson, S. Moss, P. Marín-García, I. G. Azcárate, J. M. Bautista, A. C. Mirando, C. S. Francklyn, S. Varon, M. Royo, A. Cortés and L. R. De Pouplana, *Proc. Natl. Acad. Sci. U.* S. A., 2014, **111**, E5508–E5517.
- 98 F. F. Fleming, L. Yao, P. C. Ravikumar, L. Funk and B. C. Shook, *J. Med. Chem.*, 2010, 53, 7902–7917.
- 99 A. L. Lane, S. J. Nam, T. Fukuda, K. Yamanaka, C. A. Kauffman, P. R. Jensen, W. Fenical and B. S. Moore, *J. Am. Chem. Soc.*, 2013, **135**, 4171–4174.
- 100 D. Habibi, N. Ogloff, R. B. Jalili, A. Yost, A. P. Weng, A. Ghahary and C. J. Ong, *Invest. New Drugs*, 2012, 30, 1361–1370.
- 101 C. Hu, H. Su, J. Luo, L. Han, Q. Liu, W. Wu, Y. Mu, P. Guan, T. Sun and X. Huang, *Bioorg. Med. Chem.*, 2018, 26, 6035– 6049.
- 102 S. Pidot, K. Ishida, M. Cyrulies and C. Hertweck, Angew. Chem., Int. Ed., 2014, 53, 7856–7859.
- 103 K. Jakobi and C. Hertweck, J. Am. Chem. Soc., 2004, 126, 2298–2299.
- 104 K. Wolkenstein, H. Sun, H. Falk and C. Griesinger, J. Am. Chem. Soc., 2015, 137, 13460–13463.
- 105 M. Yang, J. Li and A. Li, Nat. Commun., 2015, 6, 6-11.
- 106 T. Shen, X. N. Wang and H. X. Lou, *Nat. Prod. Rep.*, 2009, **26**, 916–935.
- 107 H. B. Park, P. Sampathkumar, C. E. Perez, J. H. Lee, J. Tran, J. B. Bonanno, E. A. Hallem, S. C. Almo and J. M. Crawford, *J. Biol. Chem.*, 2017, **292**, 6680–6694.
- 108 S. H. Smith, C. Jayawickreme, D. J. Rickard, E. Nicodeme, T. Bui, C. Simmons, C. M. Coquery, J. Neil, W. M. Pryor, D. Mayhew, D. K. Rajpal, K. Creech, S. Furst, J. Lee, D. Wu, F. Rastinejad, T. M. Willson, F. Viviani, D. C. Morris, J. T. Moore and J. Cote-Sierra, *J. Invest. Dermatol.*, 2017, 137, 2110–2119.
- 109 Q. Ma, Annu. Rev. Pharmacol. Toxicol., 2013, 53, 401-426.
- 110 R. H. Cichewicz, S. A. Kouzi and M. T. Hamann, *J. Nat. Prod.*, 2000, **63**, 29–33.
- 111 X. Wan, X. B. Wang, M. H. Yang, J. S. Wang and L. Y. Kong, *Bioorg. Med. Chem.*, 2011, **19**, 5085–5092.

- 112 H. B. Park and J. M. Crawford, *J. Nat. Prod.*, 2015, **78**, 1437–1441.
- 113 H. B. Park, T. N. Goddard, J. Oh, J. Patel, Z. Wei, C. E. Perez, B. Q. Mercado, R. Wang, T. P. Wyche, G. Piizzi, R. A. Flavell and J. M. Crawford, *Angew. Chem., Int. Ed.*, 2020, **59**, 7871– 7880.
- 114 J. M. Dunwell, A. Purvis and S. Khuri, *Phytochemistry*, 2004, 65, 7–17.
- 115 S. Khuri, F. T. Bakker and J. M. Dunwell, *Mol. Biol. Evol.*, 2001, **18**, 593–605.
- 116 C. T. Walsh, ACS Chem. Biol., 2014, 9, 1653-1661.
- 117 M. A. Martínez-Núñez and V. E. L. y. López, Sustainable Chem. Processes, 2016, 4, 1-8.
- 118 M. Winn, J. K. Fyans, Y. Zhuo and J. Micklefield, *Nat. Prod. Rep.*, 2016, **33**, 317–347.
- 119 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.
- 120 M. Sarciaux, L. Pantel, C. Midrier, M. Serri, C. Gerber, R. Marcia De Figueiredo, J. M. Campagne, P. Villain-Guillot, M. Gualtieri and E. Racine, *J. Med. Chem.*, 2018, 61, 7814–7826.
- 121 S. C. Blanchard, Mol. Cell, 2018, 70, 3-5.
- 122 M. Zhao, A. J. Lepak, K. Marchillo, J. Vanhecker and D. R. Andes, *Antimicrob. Agents Chemother.*, 2018, **62**, 1–9.
- 123 E. Racine, P. Nordmann, L. Pantel, M. Sarciaux, M. Serri,
  J. Houard and P. Villain-guillot, *Antimicrob. Agents Chemother.*, 2018, 62, 1–16.
- 124 D. N. Wilson, G. Guichard and C. Axel Innis, *Oncotarget*, 2015, **6**, 16826–16827.
- 125 Y. S. Polikanov, N. A. Aleksashin, B. Beckert and D. N. Wilson, *Front. Mol. Biosci.*, 2018, **5**, 1–21.
- 126 N. B. Olivier, R. B. Altman, J. Noeske, G. S. Basarab, E. Code,
  A. D. Ferguson, N. Gao, J. Huang, M. F. Juette, S. Livchak,
  M. D. Miller, D. B. Prince, J. H. D. Cate, E. T. Buurman
  and S. C. Blanchard, *Proc. Natl. Acad. Sci. U. S. A.*, 2014,
  111, 16274–16279.
- 127 Y. S. Polikanov, T. Szal, F. Jiang, P. Gupta, R. Matsuda, M. Shiozuka, T. A. Steitz, N. Vázquez-laslop and A. S. Mankin, *Mol. Cell*, 2014, 56, 541–550.
- 128 E. Racine and M. Gualtieri, Front. Microbiol., 2019, 10, 1–10.
- 129 L. Wang, A. Pulk, M. R. Wasserman, M. B. Feldman, R. B. Altman, J. H. Doudna Cate and S. C. Blanchard, *Nat. Struct. Mol. Biol.*, 2012, **19**, 957–963.
- 130 J. Li, G. Chen and J. M. Webster, *Can. J. Microbiol.*, 1997, **43**, 770–773.
- 131 X. Cai, V. L. Challinor, L. Zhao, D. Reimer, H. Adihou,
   P. Grün, M. Kaiser and H. B. Bode, *Org. Lett.*, 2017, 19, 806–809.
- 132 A. Stanišić and H. Kries, *ChemBioChem*, 2019, **20**, 1347–1356.
- 133 N. A. Magarvey, M. Ehling-Schulz and C. T. Walsh, J. Am. Chem. Soc., 2006, **128**, 10698–10699.

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- 134 J. Jaitzig, J. Li, R. D. Süssmuth and P. Neubauer, *ACS Synth. Biol.*, 2014, **3**, 432–438.
- 135 D. Reimer, F. I. Nollmann, K. Schultz, M. Kaiser and H. B. Bode, *J. Nat. Prod.*, 2014, 77, 1976–1980.
- 136 T. Himmler, F. Pirro and N. Schmeer, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2045–2050.
- 137 F. Wesche, H. Adihou, T. A. Wichelhaus and H. B. Bode, *Beilstein J. Org. Chem.*, 2019, **15**, 535–541.
- 138 A. Hasan, H. S. Yeom, J. Ryu, H. B. Bode and Y. Kim, *Sci. Rep.*, 2019, **9**, 1–18.
- 139 E. Bode, A. O. Brachmann, C. Kegler, R. Simsek, C. Dauth, Q. Zhou, M. Kaiser, P. Klemmt and H. B. Bode, *ChemBioChem*, 2015, 16, 1115–1119.
- 140 A. O. Brachmann, F. Kirchner, C. Kegler, S. C. Kinski, I. Schmitt and H. B. Bode, *J. Biotechnol.*, 2012, **157**, 96–99.
- 141 H. B. Bode, A. O. Brachmann, K. B. Jadhav, L. Seyfarth, C. Dauth, S. W. Fuchs, M. Kaiser, N. R. Waterfield, H. Sack, S. H. Heinemann and H. D. Arndt, *Angew. Chem.*, *Int. Ed.*, 2015, 54, 10352–10355.
- 142 L. Zhao, R. M. Awori, M. Kaiser, J. Groß, T. Opatz and H. B. Bode, *J. Nat. Prod.*, 2019, **82**, 3499–3503.
- 143 L. S. Bonnington, J. Tanaka, T. Higa, J. Kimura, Y. Yoshimura, Y. Nakao, W. Y. Yoshida and P. J. Scheuer, J. Org. Chem., 1997, 62, 7765–7767.
- 144 H. Ishii, M. Nishijima and T. Abe, *Water Res.*, 2004, 38, 2667–2676.
- 145 K. Saito, A. Konno, H. Ishii, H. Saito, F. Nishida, T. Abe and C. Chen, *J. Nat. Prod.*, 2001, **64**, 139–141.
- 146 J. W. Cha, J. S. Park, T. Sim, S. J. Nam, H. C. Kwon, J. R. Del Valle and W. Fenical, *J. Nat. Prod.*, 2012, 75, 1648–1651.
- 147 N. De Lay, D. J. Schu and S. Gottesman, J. Biol. Chem., 2013, 288, 7996–8003.
- 148 J. Vogel and B. F. Luisi, *Nat. Rev. Microbiol.*, 2015, **9**, 578–589.
- 149 M. Dietrich, R. Munke, M. Gottschald, E. Ziska, J. P. Boettcher, H. Mollenkopf and A. Friedrich, *FEBS J.*, 2009, **276**, 5507–5520.
- 150 N. J. Tobias, A. K. Heinrich, H. Eresmann, P. R. Wright, N. Neubacher, R. Backofen and H. B. Bode, *Environ. Microbiol.*, 2017, **19**, 119–129.
- 151 E. Bode, A. K. Heinrich, M. Hirschmann, D. Abebew, Y. Shi, T. D. Vo, F. Wesche, Y. Shi, P. Grün, S. Simonyi, N. Keller, Y. Engel, S. Wenski, R. Bennet, S. Beyer, I. Bischoff, A. Buaya, S. Brandt, I. Cakmak, H. Çimen, S. Eckstein, D. Frank, R. Fürst, M. Gand, G. Geisslinger, S. Hazir, M. Henke, R. Heermann, V. Lecaudey, W. Schäfer, S. Schiffmann, A. Schüffler, R. Schwenk, M. Skaljac, E. Thines, M. Thines, T. Ulshöfer, A. Vilcinskas, T. A. Wichelhaus and H. B. Bode, *Angew. Chem., Int. Ed.*, 2019, 131, 19133–19139.
- 152 F. Grundmann, M. Kaiser, M. Kurz, M. Schiell, A. Batzer and H. B. Bode, *RSC Adv.*, 2013, **3**, 22072–22077.
- 153 B. Ohlendorf, S. Simon, J. Wiese and J. F. Imhoff, *Nat. Prod. Commun.*, 2011, **6**, 1247–1250.
- 154 T. Clements, T. Ndlovu and W. Khan, *Microbiol. Res.*, 2019, **229**, 126329.

- 155 H. H. Wasserman, J. J. Keggi and J. E. McKeon, *J. Am. Chem. Soc.*, 1962, **84**, 2978–2982.
- 156 H. H. Wasserman, J. J. Keggi and J. E. McKeon, *J. Am. Chem. Soc.*, 1961, **83**, 4107–4108.
- 157 T. Matsuyama, M. Fujita and I. Yano, *FEMS Microbiol. Lett.*, 1985, **28**, 125–129.
- 158 M. A. Kroteń, M. Bartoszewicz and I. Święcicka, *Pol. J. Microbiol.*, 2010, **59**, 3–10.
- 159 A. A. Sy-Cordero, C. J. Pearce and N. H. Oberlies, *J. Antibiot.*, 2012, **65**, 541–549.
- 160 L. Zhu, C. Pang, L. Chen and X. Zhu, *Nat. Prod. Chem. Res.*, 2018, **6**, 312.
- 161 D. Dwivedi, R. Jansen, G. Molinari, M. Nimtz, B. N. Johri and V. Wray, *J. Nat. Prod.*, 2008, **71**, 637–641.
- 162 J. L. Motley, B. W. Stamps, C. A. Mitchell, A. T. Thompson, J. Cross, J. You, D. R. Powell, B. S. Stevenson and R. H. Cichewicz, *J. Nat. Prod.*, 2017, **80**, 598–608.
- 163 T. Matsuyama, K. Kaneda, Y. Nakagawa, K. Isa, H. Hara-Hotta and I. Yano, *J. Bacteriol.*, 1992, **174**, 1769–1776.
- 164 C. Su, Z. Xiang, Y. Liu, X. Zhao, Y. Sun, Z. Li, L. Li, F. Chang, T. Chen, X. Wen, Y. Zhou and F. Zhao, *BMC Genomics*, 2016, 17, 1–19.
- 165 T. Matsuyama, T. Murakami and M. Fujita, *J. Gen. Microbiol.*, 1986, 132, 865–875.
- 166 H. Li, T. Tanikawa, Y. Sato, Y. Nakagawa and T. Matsuyama, *Microbiol. Immunol.*, 2005, **49**, 303–310.
- 167 S. Sunaga, H. Li, Y. Sato, Y. Nakagawa and T. Matsuyama, *Microbiol. Immunol.*, 2004, **48**, 723–728.
- 168 J. W. Trauger, R. M. Kohli, H. D. Mootz, M. A. Marahiel and C. T. Walsh, *Nature*, 2000, **407**, 215–218.
- 169 D. E. Kadouri and R. M. Q. Shanks, *Res. Microbiol.*, 2013, 164, 821–826.
- 170 T. Clements, T. Ndlovu, S. Khan and W. Khan, *Appl. Microbiol. Biotechnol.*, 2019, **103**, 589–602.
- 171 J. G. Ganley, G. Carr, T. R. Ioerger, J. C. Sacchettini, J. Clardy and E. R. Derbyshire, *ChemBioChem*, 2018, **19**, 1590–1594.
- 172 P.-Y. Mai, M. Levasseur, D. Buisson, D. Touboul and V. Eparvier, *Plants*, 2020, **9**, 1–11.
- 173 X. Yin and T. M. Zabriskie, *Microbiology*, 2006, **152**, 2969–2983.
- 174 S. Wang, A. L. A. Dos-santos, W. Huang, K. C. Liu, M. A. Oshaghi, G. Wei, P. Agre and M. Jacobs-lorena, *Science*, 2017, 357, 1399–1402.
- 175 N. A. Schilling, A. Berscheid, J. Schumacher, J. S. Saur, M. C. Konnerth, S. N. Wirtz, J. M. Beltrán-Beleña, A. Zipperer, B. Krismer, A. Peschel, H. Kalbacher, H. Brötz-Oesterhelt, C. Steinem and S. Grond, *Angew. Chem., Int. Ed.*, 2019, 58, 9234–9238.
- 176 B. S. Evans, I. Ntai, Y. Chen, S. J. Robinson and N. L. Kelleher, *J. Am. Chem. Soc.*, 2011, **133**, 7316–7319.
- 177 B. A. Pfeifer, C. C. C. Wang, C. T. Walsh and C. Khosla, *Appl. Environ. Microbiol.*, 2003, **69**, 6698–6702.
- 178 Y. Inahashi, S. Zhou, M. J. Bibb, L. Song, M. M. Al-Bassam, M. J. Bibb and G. L. Challis, *Chem. Sci.*, 2017, 8, 2823–2831.
- 179 K. Bitschar, B. Sauer, J. Focken, H. Dehmer, S. Moos, M. Konnerth, N. A. Schilling, S. Grond, H. Kalbacher,

F. C. Kurschus, F. Götz, B. Krismer, A. Peschel and B. Schittek, *Nat. Commun.*, 2019, **10**, 2730.

- 180 M. R. Ghadiri, J. R. Granja and L. K. Buehler, *Nature*, 1994, 369, 301–304.
- 181 J. G. Hurdle, A. J. O'Neill, I. Chopra and R. E. Lee, *Nat. Rev. Microbiol.*, 2011, **9**, 62–75.
- 182 L. Ettlinger, E. Gäumann, R. Hütter, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog and H. Zähner, *Helv. Chim. Acta*, 1959, 42, 563–569.
- 183 K. Okamura, K. Soga, Y. Shimauchi, T. Ishikura and J. Lein, *J. Antibiot.*, 1977, **30**, 334–336.
- 184 M. Kenig and C. Reading, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 498–501.
- 185 S. Huang, Y. Zhao, Z. Qin, X. Wang, M. Onega, L. Chen, J. He, Y. Yu and H. Deng, *Process Biochem.*, 2011, 46, 811– 816.
- 186 P. Liras, Appl. Microbiol. Biotechnol., 2014, 98, 1023-1030.
- 187 Z. Wei, C. Xu, J. Wang, F. Lu, X. Bie and Z. Lu, *PeerJ*, 2017, 5, 1–19.
- 188 H. Ding, J. N. Wang, D. S. Zhang and Z. J. Ma, *Chem. Biodiversity*, 2017, 14, 2–9.
- 189 Z. Qin, S. Huang, Y. Yu and H. Deng, *Mar. Drugs*, 2013, **11**, 3970–3997.
- 190 B. Li, W. J. Wever, C. T. Walsh and A. A. Bowers, *Nat. Prod. Rep.*, 2014, **31**, 905–923.
- 191 S. Huang, M. H. Tong, Z. Qin, Z. Deng, H. Deng and Y. Yu, *Anticancer Agents Med. Chem.*, 2015, **15**, 277–284.
- 192 B. Oliva, A. O'Neill, J. M. Wilson, P. J. O'Hanlon and I. Chopra, *Antimicrob. Agents Chemother.*, 2001, 45, 532–539.
- 193 A. O'Neill, B. Oliva, C. Storey, A. Hoyle, C. Fishwick and I. Chopra, Antimicrob. Agents Chemother., 2000, 44, 3163– 3166.
- 194 X. Tan, C. Li, Z. Yu, P. Wang, S. Nian, Y. Deng, W. Wu and G. Wang, *Chem. Pharm. Bull.*, 2013, **61**, 351–357.
- 195 J. Meng, B. Kong, J. Wang, X. Yang, Y. Lv, L. Lyu, Z. Jiang and X. Tan, *Med. Chem. Res.*, 2020, **29**, 1376–1386.
- 196 J. E. Ellis, J. H. Fried, I. T. Harrison, E. Rapp and C. H. Ross, J. Org. Chem., 1977, 42, 2891–2893.
- 197 C. Li, Y. Sun, G. Wang and X. Tan, Bull. Korean Chem. Soc., 2014, 35, 3489–3494.
- 198 B. Li, M. P. A. Lyle, G. Chen, J. Li, K. Hu, L. Tang, M. A. Alaoui-Jamali and J. Webster, *Bioorg. Med. Chem.*, 2007, 15, 4601–4608.
- 199 X. Tan, M. Huang, S. Nian, Y. Peng, J. Qin, B. Kong and X. Duan, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127146.
- 200 N. Bouras, R. Merrouche, L. Lamari, F. Mathieu, N. Sabaou and A. Lebrihi, *Process Biochem.*, 2008, **43**, 1244–1252.
- 201 N. Bouras, F. Mathieu, N. Sabaou and A. Lebrihi, *Process Biochem.*, 2007, 42, 925–933.
- 202 A. C. Chorin, L. Bijeire, M. C. Monje, G. Baziard, A. Lebrihi and F. Mathieu, *J. Appl. Microbiol.*, 2009, **107**, 1751–1762.
- 203 F. Yakushiji, Y. Miyamoto, Y. Kunoh, R. Okamoto, H. Nakaminami, Y. Yamazaki, N. Noguchi and Y. Hayashi, ACS Med. Chem. Lett., 2013, 4, 220–224.
- 204 A. C. Murphy, S. S. Gao, L. C. Han, S. Carobene, D. Fukuda, Z. Song, J. Hothersall, R. J. Cox, J. Crosby, M. P. Crump,

C. M. Thomas, C. L. Willis and T. J. Simpson, *Chem. Sci.*, 2014, **5**, 397–402.

- 205 T. A. Wencewicz, J. Mol. Biol., 2019, 431, 3370-3399.
- 206 Z. D. Dunn, W. J. Wever, D. N. J. Economou and P. B. Li, Angew. Chem., Int. Ed., 2015, 54, 5137–5141.
- 207 P. Charusanti, N. L. Fong, H. Nagarajan, A. R. Pereira, H. J. Li, E. A. Abate, Y. Su, W. H. Gerwick and B. O. Palsson, *PLoS One*, 2012, 7, e33727.
- 208 H. Yin, S. Xiang, J. Zheng, K. Fan, T. Yu, X. Yang, Y. Peng,
   H. Wang, D. Feng, Y. Luo, H. Bai and K. Yang, *Appl. Environ. Microbiol.*, 2012, 78, 3431–3441.
- 209 A. Jimenez, D. J. Tipper and J. Davies, *Antimicrob. Agents Chemother.*, 1973, **3**, 729–738.
- 210 G. G. Khachatourians and D. J. Tipper, *Antimicrob. Agents Chemother.*, 1974, **6**, 304–310.
- 211 D. J. Tipper, J. Bacteriol., 1973, 116, 245-256.
- 212 N. Sivasubramanian and R. Jayaraman, *Mol. Genet. Genomics*, 1976, **145**, 89–96.
- 213 G. G. Khachatourians and D. J. Tipper, *J. Bacteriol.*, 1974, **119**, 795–804.
- 214 L. Lauinger, J. Li, A. Shostak, I. A. Cemel, N. Ha, Y. Zhang,
  P. E. Merkl, S. Obermeyer, N. Stankovic-Valentin,
  T. Schafmeier, W. J. Wever, A. A. Bowers, K. P. Carter,
  A. E. Palmer, H. Tschochner, F. Melchior, R. J. Deshaies,
  M. Brunner and A. Diernfellner, *Nat. Chem. Biol.*, 2017,
  13, 709–714.
- 215 A. N. Chan, A. L. Shiver, W. J. Wever, S. Z. A. Razvi, M. F. Traxler and B. Li, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, 114, 2717–2722.
- 216 B. Li, F. Ry R., B. Albert A., S. Frank C. and W. Christopher T., *ChemBioChem*, 2013, **13**, 2521–2526.
- 217 C. T. Walsh, R. V. O'Brien and C. Khosla, *Angew. Chem., Int. Ed.*, 2013, **52**, 7098–7124.
- 218 W. Zhou, J. H. Li, J. Chen, X. Y. Liu, T. T. Xiang, L. Zhang and Y. J. Wan, *J. Invertebr. Pathol.*, 2016, **136**, 92–94.
- 219 J. S. Lee, Y. S. Kim, S. Park, J. Kim, S. J. Kang, M. H. Lee, S. Ryu, J. M. Choi, T. K. Oh and J. H. Yoon, *Appl. Environ. Microbiol.*, 2011, 77, 4967–4973.
- 220 H. N. A. Do and T. H. K. Nguyen, *J. Appl. Pharm. Sci.*, 2014, 4, 21–24.
- 221 T. Danevcic, M. B. Vezjak, M. Tabor, M. Zorec and D. Stopar, *Front. Microbiol.*, 2016, 7, 1–10.
- 222 H. Zhang, H. Wang, W. Zheng, Z. Yao, Y. Peng and S. Zhang, *Front. Microbiol.*, 2017, **8**, 1–12.
- 223 N. Stankovic, L. Senerovic, T. Ilic-Tomic, B. Vasiljevic and J. Nikodinovic-Runic, *Appl. Microbiol. Biotechnol.*, 2014, 98, 3841–3858.
- 224 S. Shahitha and K. Poornima, *J. Appl. Pharm. Sci.*, 2012, **2**, 138–140.
- 225 R. Sakuraoka, T. Suzuki and T. Morohoshi, *Genome Biol. Evol.*, 2019, **11**, 931–936.
- 226 N. R. Williamson, H. T. Simonsen, R. A. A. Ahmed, G. Goldet, H. Slater, L. Woodley, F. J. Leeper and G. P. C. Salmond, *Mol. Microbiol.*, 2005, 56, 971–989.
- 227 N. R. Williamson, P. C. Fineran, F. J. Leeper and G. P. C. Salmond, *Nat. Rev. Microbiol.*, 2006, 4, 887–899.

- 228 F. E. Sakai-Kawada, C. G. Ip, K. A. Hagiwara and J. D. Awaya,
- Front. Microbiol., 2019, 10, 1–9.
  229 H. Okamoto, Z. Sato, M. Sato, Y. Koiso, S. Iwasaki and M. Isaka, Jpn. J. Phytopathol., 1998, 64, 294–298.
- 230 H.-S. Kim, M. Hayashi, Y. Shibata, Y. Wataya, T. Mitamura, T. Horii, K. Kawauchi, H. Hirata, S. Tsuboi and Y. Moriyama, *Biol. Pharm. Bull.*, 1999, 22, 532–534.
- 231 P. Azambuja, D. Feder and E. S. Garcia, *Exp. Parasitol.*, 2004, **107**, 89–96.
- 232 D. Li, J. Liu, X. Wang, D. Kong, W. Du, H. Li, C. Y. Hse, T. Shupe, D. Zhou and K. Zhao, *Int. J. Mol. Sci.*, 2018, **19**, 3465.
- 233 K. Kawauchi, K. Tobiume, S. Kaneko, K. Kaneshiro,
  S. Okamoto, E. Ueda, H. Kamata, Y. Moriyama and
  H. Hirata, *Biol. Pharm. Bull.*, 2007, 30, 1792–1795.
- 234 N. Darshan and H. K. Manonmani, J. Food Sci. Technol., 2015, 52, 5393–5407.
- 235 R. K. Suryawanshi, C. D. Patil, S. H. Koli, J. E. Hallsworth and S. V. Patil, *Nat. Prod. Res.*, 2017, **31**, 572–577.
- 236 B. Díaz De Greñu, P. I. Hernández, M. Espona, D. Quiñonero, M. E. Light, T. Torroba, R. Pérez-Tomás and R. Quesada, *Chem.-Eur. J.*, 2011, 17, 14074–14083.
- 237 A. Fürstner and E. J. Grabowski, *ChemBioChem*, 2001, 2, 706–709.
- 238 R. Pérez-Tomás, B. Montaner, E. Llagostera and V. Soto-Cerrato, *Biochem. Pharmacol.*, 2003, **66**, 1447–1452.
- 239 A. Goy, F. J. Hernandez-Ilzaliturri, B. Kahl, P. Ford, E. Protomastro and M. Berger, *Leuk Lymphoma*, 2014, 55, 1–17.
- 240 S. Cournoyer, A. Addioui, A. Belounis, M. Beaunoyer, C. Nyalendo, R. Le Gall, P. Teira, E. Haddad, G. Vassal and H. Sartelet, *BMC Cancer*, 2019, **19**, 1–14.
- 241 C. A. Goard and A. D. Schimmer, *Core Evidence*, 2013, **8**, 15–26.
- 242 D. W. Hood, R. Heidstra, U. K. Swoboda and D. A. Hodgson, *Gene*, 1992, **115**, 5–12.
- 243 S. R. Burger and J. W. Bennett, *Appl. Environ. Microbiol.*, 1985, **50**, 487–490.
- 244 H. Yamaguchi, Y. Nakayama, K. Takeda, K. Tawara, K. Maeda, T. Takeuchi and H. Umezawa, *J. Antibiot.*, 1957, **10**, 195–200.
- 245 H. Sakakibara, H. Naganawa, M. Ohno, K. Maeda and H. Umezawa, *J. Antibiot.*, 1974, XXVII, 897–899.
- 246 H. A. Kirst, E. F. Szymanski, D. E. Dorman, J. L. Occolowitz, N. D. Jones, M. O. Chaney, R. L. Hamill and M. M. Hoehn, *J. Antibiot.*, 1975, XXVIII, 286–291.
- 247 B. Kunze, H. Reichenbach, H. Augustiniak and G. Höfle, J. Antibiot., 1982, 35, 635–636.
- 248 N. S. Cortina, O. Revermann, D. Krug and R. Müller, *ChemBioChem*, 2011, **12**, 1411–1416.
- 249 G. L. Challis, A. J. Gerc, N. R. Stanley-Wall, S. J. Coulthurst and L. Song, *PLoS One*, 2012, 7, e44673.
- 250 K. Inami and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 2185–2189.
- 251 P. Zarantonello, C. P. Leslie, R. Ferritto and W. M. Kazmierski, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 561–565.

- 252 H. Fujimoto, T. Kinoshita, H. Suzuki and H. Umezawa, J. *Antibiot.*, 1970, **23**, 271–275.
- 253 K. Inami and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 352–360.
- 254 J. H. Cardellina, F. J. Marner and R. E. Moore, *J. Am. Chem. Soc.*, 1979, **101**, 240–242.
- 255 C. A. Banuelos, I. Tavakoli, A. H. Tien, D. P. Caley, N. R. Mawji, Z. Li, J. Wang, Y. C. Yang, Y. Imamura, L. Yan, J. G. Wen, R. J. Andersen and M. D. Sadar, *J. Biol. Chem.*, 2016, **291**, 22231–22243.
- 256 S. Paik, J. Cullingham, R. E. Moore, G. M. L. Patterson, M. A. Tius and S. Carmeli, *J. Am. Chem. Soc.*, 1994, **116**, 8116–8125.
- 257 R. G. Birch and S. S. Patil, *J. Gen. Microbiol.*, 1985, **131**, 1069–1075.
- 258 R. G. Birch and S. S. Patil, *Physiol. Mol. Plant Pathol.*, 1987, **30**, 199–206.
- 259 R. G. Birch and S. S. Patil, Antibiotic and Process for the Production Thereof, *US Pat.*, 4,525,354, 1985, 1–3.
- 260 S. Cociancich, A. Pesic, D. Petras, S. Uhlmann, J. Kretz, V. Schubert, L. Vieweg, S. Duplan, M. Marguerettaz, J. Noëll, I. Pieretti, M. Hügelland, S. Kemper, A. Mainz, P. Rott, M. Royer and R. D. Süssmuth, *Nat. Chem. Biol.*, 2015, **11**, 195–197.
- 261 E. Vivien, D. Pitorre, S. Cociancich, I. Pieretti, D. W. Gabriel, P. C. Rott and M. Royer, *Antimicrob. Agents Chemother.*, 2007, **51**, 1549–1552.
- 262 R. D. Süssmuth, D. Kerwat, S. Grätz, I. Behroz, L. von Eckardstein, P. M. Durkin, M. Morkunas and J. Weston, WO2019/015794, 2017, pp. 1–73.
- 263 S. M. Hashimi, M. K. Wall, A. B. Smith, A. Maxwell and R. G. Birch, *Antimicrob. Agents Chemother.*, 2007, 51, 181– 187.
- 264 J. J. Champoux, Annu. Rev. Biochem., 2001, 70, 369-413.
- 265 J. Kretz, D. Kerwat, V. Schubert, S. Grätz, A. Pesic, S. Semsary, S. Cociancich, M. Royer and R. D. Süssmuth, *Angew. Chem., Int. Ed.*, 2015, 54, 1969–1973.
- 266 S. Grätz, D. Kerwat, J. Kretz, L. von Eckardstein, S. Semsary, M. Seidel, M. Kunert, J. B. Weston and R. D. Süssmuth, *ChemMedChem*, 2016, **11**, 1499–1502.
- 267 I. Behroz, P. Durkin, S. Grätz, M. Seidel, L. Rostock,
   M. Spinczyk, J. B. Weston and R. D. Süssmuth, *Chem.-Eur. J.*, 2019, 25, 16538–16543.
- 268 D. Kerwat, S. Grätz, J. Kretz, M. Seidel, M. Kunert, J. B. Weston and R. D. Süssmuth, *ChemMedChem*, 2016, 11, 1899–1903.
- 269 L. von Eckardstein, D. Petras, T. Dang, S. Cociancich, S. Sabri, S. Grätz, D. Kerwat, M. Seidel, A. Pesic, P. C. Dorrestein, M. Royer, J. B. Weston and R. D. Süssmuth, *Chem.-Eur. J.*, 2017, 23, 15316–15321.
- 270 D. Petras, D. Kerwat, A. Pesic, B. F. Hempel, L. Von Eckardstein, S. Semsary, J. Arasté, M. Marguerettaz, M. Royer, S. Cociancich and R. D. Süssmuth, ACS Chem. Biol., 2016, 11, 1198–1204.
- 271 L. Vieweg, J. Kretz, A. Pesic, D. Kerwat, S. Grätz, M. Royer,
   S. Cociancich, A. Mainz and R. D. Süssmuth, *J. Am. Chem. Soc.*, 2015, 137, 7608–7611.

- 272 A. Höltzel, M. G. Gänzle, G. J. Nicholson, W. P. Hammes and G. Jung, *Angew. Chem., Int. Ed.*, 2000, **39**, 2766–2768.
- 273 M. G. Gänzle and R. F. Vogel, *Int. J. Food Microbiol.*, 2003, **80**, 31–45.
- 274 R. Boehme, G. Jung and E. Breitmaier, *Helv. Chim. Acta*, 2005, **88**, 2837–2841.
- 275 T. Hao, Z. Xie, M. Wang, L. Liu, Y. Zhang, W. Wang, Z. Zhang, X. Zhao, P. Li, Z. Guo, S. Gao, C. Lou, G. Zhang, J. Merritt, G. P. Horsman and Y. Chen, *Nat. Commun.*, 2019, 10, 1–13.
- 276 Y. C. Jeong and M. G. Moloney, Synlett, 2009, 15, 2487–2491.
- 277 X. B. Lin, C. T. Lohans, R. Duar, J. Zheng, J. C. Vederas, J. Walter and M. Gänzle, *Appl. Environ. Microbiol.*, 2015, 81, 2032–2041.
- 278 J. T. Park and J. L. Strominger, Science, 1957, 125, 99-101.
- 279 Y. Mutaguchi, T. Ohmori, T. Wakamatsu, K. Doi and T. Ohshima, *J. Bacteriol.*, 2013, **195**, 5207–5215.
- 280 M. Gunsior, S. D. Breazeale, A. J. Lind, J. Ravel, J. W. Janc and C. A. Townsend, *Chem. Biol.*, 2004, **11**, 927–938.
- 281 J. G. Hurdle, A. E. Heathcott, L. Yang, B. Yan and R. E. Lee, *J. Antimicrob. Chemother.*, 2011, **66**, 1773–1776.
- 282 U. Marquardt, D. Schmid and G. Jung, Synlett, 2000, 8, 1131-1132.
- 283 P. T. Cherian, X. Wu, M. M. Maddox, A. P. Singh, R. E. Lee and J. G. Hurdle, *Sci. Rep.*, 2014, 4, 1–9.
- 284 R. Yendapally, J. G. Hurdle, E. I. Carson, R. B. Lee and R. E. Lee, *J. Med. Chem.*, 2008, **51**, 1487–1491.
- 285 G. A. Hudson and D. A. Mitchell, *Curr. Opin. Microbiol.*, 2018, 45, 61–69.
- 286 M. A. Ortega and W. A. Van Der Donk, Cell Chem. Biol., 2016, 23, 31-44.
- 287 I. Holtsmark, V. G. H. Eijsink and M. B. Brurberg, *FEMS Microbiol. Lett.*, 2008, **280**, 1–7.
- 288 E. Meade, M. A. Slattery and M. Garvey, *Antibiotics*, 2020, 9, 1–18.
- 289 Y. Imai, K. J. Meyer, A. Iinishi, Q. Favre-Godal, R. Green, S. Manuse, M. Caboni, M. Mori, S. Niles, M. Ghiglieri, C. Honrao, X. Ma, J. J. Guo, A. Makriyannis, L. Linares-Otoya, N. Böhringer, Z. G. Wuisan, H. Kaur, R. Wu, A. Mateus, A. Typas, M. M. Savitski, J. L. Espinoza, A. O'Rourke, K. E. Nelson, S. Hiller, N. Noinaj, T. F. Schäberle, A. D'Onofrio and K. Lewis, *Nature*, 2019, 576, 459–464.
- 290 K. R. Schramma, L. B. Bushin and M. R. Seyedsayamdost, *Nat. Chem.*, 2015, 7, 431–437.
- 291 K. A. Clark, L. B. Bushin and M. R. Seyedsayamdost, J. Am. Chem. Soc., 2019, 141, 10610–10615.
- 292 A. Benjdia, C. Balty and O. Berteau, *Front. Chem.*, 2017, **5**, 1–13.
- 293 R. M. Epand, C. Walker, R. F. Epand and N. A. Magarvey, Biochim. Biophys. Acta, Biomembr., 2016, 1858, 980–987.
- 294 J. M. Waisvisz, M. G. Van Der Hoeven, J. Van Peppen and W. C. M. Zwennis, J. Am. Chem. Soc., 1957, 79, 4520–4521.
- 295 Y. Hou, M. D. Tianero, J. Kwan, T. P. Wyche, C. R. Michel, G. A. Ellis, E. Varquez-Rivera, D. Braun, W. E. Rose,

E. W. Schmidt and T. S. Bugni, *Org. Lett.*, 2012, **14**, 5050–5053.

- 296 W. J. K. Crone, F. J. Leeper and A. W. Truman, *Chem. Sci.*, 2012, **3**, 3516–3521.
- 297 J. P. Gomez-Escribano, L. Song, M. J. Bibb and G. L. Challis, *Chem. Sci.*, 2012, **3**, 3522–3525.
- 298 Y. Takahashi, H. Naganawa, T. Takita, H. Umezawa and S. Nakamura, *J. Antibiot.*, 1976, **29**, 1120–1123.
- 299 S. Nakamura, T. Yajima, Y. Lin and H. Umezawa, J. Antibiot., 1967, 20, 1–5.
- 300 S. Nakamura, N. Tanaka and H. Umezawa, J. Antibiot., 1966, 19, 10–12.
- 301 D. Schipper, J. Antibiot., 1983, 36, 1076-1077.
- 302 H. Shimamura, H. Gouda, K. Nagai, T. Hirose, M. Ichioka,
  Y. Furuya, Y. Kobayashi, S. Hirono, T. Sunazuka and
  S. Omura, *Angew. Chem., Int. Ed.*, 2009, 48, 914–917.
- 303 W. J. K. Crone, N. M. Vior, J. Santos-Aberturas, L. G. Schmitz, F. J. Leeper and A. W. Truman, *Angew. Chem., Int. Ed.*, 2016, **55**, 9639–9643.
- 304 N. M. Vior, E. Cea-torrescassana, T. H. Eyles, G. Chandra and A. W. Truman, *Front. Microbiol.*, 2020, **11**, 1–16.
- 305 T. Yamada, M. Yagita, Y. Kobayashi, G. Sennari, H. Shimamura, H. Matsui, Y. Horimatsu, H. Hanaki, T. Hirose, S. Omura and T. Sunazuka, *J. Org. Chem.*, 2018, 83, 7135–7149.
- 306 Y. Kobayashi, M. Ichioka, T. Hirose, K. Nagai,
  A. Matsumoto, H. Matsui, H. Hanaki, R. Masuma,
  Y. Takahashi, S. Omura and T. Sunazuka, *Bioorg. Med. Chem. Lett.*, 2010, 20, 6116–6120.
- 307 S. Ackermann, H. G. Lerchen, D. Häbich, A. Ullrich and U. Kazmaier, *Beilstein J. Org. Chem.*, 2012, **8**, 1652–1656.
- 308 T. Otaka and A. Kaji, J. Biol. Chem., 1976, 251, 2299-2306.
- 309 T. Otaka and A. Kaji, FEBS Lett., 1983, 153, 53-59.
- 310 Y. Inouye, M. Hashimoto, M. Sugiyama, Y. Takesue, T. Santo and T. Yokoyama, *Hiroshima J. Med. Sci.*, 1994, 43, 87–92.
- 311 T. H. Eyles, N. M. Vior and A. W. Truman, ACS Synth. Biol., 2018, 7, 1211–1218.
- 312 L. Horbal, F. Marques, S. Nadmid, M. V. Mendes and A. Luzhetskyy, *Metab. Eng.*, 2018, **49**, 299–315.
- 313 A. Mukai, T. Fukai, Y. Hoshino, K. Yazawa, K. I. Harada and Y. Mikami, *J. Antibiot.*, 2009, **62**, 613–619.
- 314 A. A. Vinogradov and H. Suga, Cell Chem. Biol., 2020, 27, 1-20.
- 315 K. Sakai, H. Komaki and T. Gonoi, *PLoS One*, 2015, **10**, e0143264.
- 316 W. L. Kelly, L. Pan and C. Li, *J. Am. Chem. Soc.*, 2009, **131**, 4327–4334.
- 317 S. J. Malcolmson, T. S. Young, J. G. Ruby, P. Skewes-Cox and C. T. Walsh, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 8483–8488.
- 318 K. Sakai, Y. Hara, M. Ishibashi, M. Sakai, S. Kawahara, S. Imanishi, K. Harada, Y. Hoshino, H. Komaki, A. Mukai and T. Gonoi, *Int. J. Pept. Res. Ther.*, 2020, 26, 281–290.
- 319 L. Xu, A. K. Farthing, J. F. Dropinski, P. T. Meinke, C. McCallum, E. Hickey and K. Liu, *Bioorg. Med. Chem. Lett.*, 2013, 23, 366–369.
- 320 R. Li, A. Stapon, J. T. Blanchfield and C. A. Townsend, *J. Am. Chem. Soc.*, 2000, **122**, 9296–9297.

- 321 K. M. Papp-Wallace, A. Endimiani, M. A. Taracila and R. A. Bonomo, *Antimicrob. Agents Chemother.*, 2011, 55, 4943–4960.
- 322 J. F. Martín, J. Casqueiro, K. Kosalková, A. T. Marcos and S. Gutiérrez, *Antonie van Leeuwenhoek*, 1999, 75, 21–31.
- 323 E. K. Schmitt, B. Hoff and U. Kück, *Adv. Biochem. Eng./ Biotechnol.*, 2004, **88**, 1-43.
- 324 J. L. Ott, C. W. Godzeski, D. Pavey, J. D. Farran and D. R. Horton, *Appl. Microbiol.*, 1962, **10**, 515–523.
- 325 J. O'Sullivan, A. M. Gillum, C. A. Aklonis, M. L. Souser and R. B. Sykes, Antimicrob. Agents Chemother., 1982, 21, 558–564.
- 326 S. E. Jensen, J. Ind. Microbiol. Biotechnol., 2012, **39**, 1407–1419.
- 327 P. C. Fineran, H. Slater, L. Everson, K. Hughes and G. P. C. Salmond, *Mol. Microbiol.*, 2005, **56**, 1495–1517.
- 328 S. J. Coulthurst, A. M. L. Barnard and G. P. C. Salmond, *Nat. Rev. Microbiol.*, 2005, **3**, 295–306.
- 329 K. Bush and P. A. Bradford, *Cold Spring Harbor Perspect.* Med., 2016, 6, a025247.
- 330 S. J. McGowan, M. Sebaihia, L. E. Porter, G. S. A. B. Stewart, P. Williams, B. W. Bycroft and G. P. C. Salmond, *Mol. Microbiol.*, 1996, 22, 415–426.
- 331 W. L. Parker, M. L. Rathnum, J. Scott Wells, W. H. Trejo, P. A. Principe and R. B. Sykes, *J. Antibiot.*, 1982, 35, 653–660.
- 332 B. W. Bycroft and C. Maslen, *J. Antibiot.*, 1988, XLI, 1231– 1241.
- 333 G. B. Elion and G. H. Hitchings, J. Am. Chem. Soc., 1955, 77, 1676.
- 334 P. N. Munshi, M. Lubin and J. R. Bertino, *Oncologist*, 2014, 19, 760–765.
- 335 M. Stanulla and H. J. Schünemann, *Lancet*, 2006, 368, 1304– 1305.
- 336 G. B. Elion, Biosci. Rep., 1989, 9, 509-529.
- 337 A. Litomska, K. Ishida, K. L. Dunbar, M. Boettger, S. Coyne and C. Hertweck, *Angew. Chem., Int. Ed.*, 2018, 57, 11574–11578.
- 338 D. L. Pruess, M. Kellett and T. C. Demny, J. Antibiot., 1971, 24, 328–329.
- 339 S. Coyne, C. Chizzali, M. N. A. Khalil, A. Litomska, K. Richter, L. Beerhues and C. Hertweck, *Angew. Chem.*, *Int. Ed.*, 2013, 52, 10564–10568.
- 340 K. A. Black and P. C. Dos Santos, *Biochim. Biophys. Acta, Mol. Cell Res.*, 2015, **1853**, 1470–1480.
- 341 A. Wensing, M. Gernold, S. Jock, R. Jansen and K. Geider, Mol. Genet. Genomics, 2014, 289, 215–223.
- 342 B. A. Harris, D. A. Weigent and J. A. Nelson, *Biochem. Pharmacol.*, 1979, **28**, 1169–1173.
- 343 J. E. Heinze, T. Mitani, K. E. Rich and E. Freese, *Biochim. Biophys. Acta, Nucleic Acids Protein Synth.*, 1978, **521**, 16–26.

- 344 H. G. Mandel, R. G. Latimer and M. Riis, *Biochem. Pharmacol.*, 1965, **14**, 66–682.
- 345 B. M. Mehta and D. J. Hutchison, Ann. N. Y. Acad. Sci., 1975, 255, 559–563.
- 346 T. Kusumi, I. Ohtani, K. Nishiyama and H. Kakisawa, *Tetrahedron Lett.*, 1987, **28**, 3981–3984.
- 347 C. Ross, K. Scherlach, F. Kloss and C. Hertweck, *Angew. Chem., Int. Ed.*, 2014, **53**, 7794–7798.
- 348 E. Haldón, M. C. Nicasio and P. J. Pérez, Org. Biomol. Chem., 2015, 13, 9528–9550.
- 349 L. V. Flórez, K. Scherlach, P. Gaube, C. Ross, E. Sitte, C. Hermes, A. Rodrigues, C. Hertweck and M. Kaltenpoth, *Nat. Commun.*, 2017, 8, 1–9.
- 350 X. Zhu, J. Liu and W. Zhang, *Nat. Chem. Biol.*, 2015, **11**, 115–120.
- 351 M. Yamaguchi, H. J. Park, S. Ishizuka, K. Omata and M. Hirama, J. Med. Chem., 1995, 38, 5015–5022.
- 352 A. L. K. Shi Shun and R. R. Tykwinski, Angew. Chem., Int. Ed., 2006, 45, 1034–1057.
- 353 M. Yamaguchi, H. J. Park and M. Hirama, *Chem. Lett.*, 1997, 535–536.
- 354 E. Mühlberg, F. Umstätter, C. Kleist, C. Domhan, W. Mier and P. Uhl, *Can. J. Microbiol.*, 2020, **66**, 11–16.
- 355 R. S. Mandal, S. Saha and S. Das, *Genomics, Proteomics Bioinf.*, 2015, **13**, 148–158.
- 356 M. R. Wilson, L. Zha and E. P. Balskus, J. Biol. Chem., 2017, 292, 8546–8552.
- 357 W. K. Mousa, B. Athar, N. J. Merwin and N. A. Magarvey, *Nat. Prod. Rep.*, 2017, 34, 1302–1331.
- 358 M. S. Donia, P. Cimermancic, C. J. Schulze, L. C. W. Brown, J. Martin, M. Mitreva, J. Clardy, R. G. Linington and M. A. Fischbach, *Cell*, 2014, **158**, 1402–1414.
- 359 A. Bouslimani, L. M. Sanchez, N. Garg and P. C. Dorrestein, *Nat. Prod. Rep.*, 2014, **31**, 718–729.
- 360 D. Krug and R. Müller, Nat. Prod. Rep., 2014, 31, 768-783.
- 361 J. N. Tabudravu, L. Pellissier, A. J. Smith, K. Subko, C. Autréau, K. Feussner, D. Hardy, D. Butler, R. Kidd, E. J. Milton, H. Deng, R. Ebel, M. Salonna, C. Gissi, F. Montesanto, S. M. Kelly, B. F. Milne, G. Cimpan and M. Jaspars, *J. Nat. Prod.*, 2019, **82**, 211–220.
- 362 R. Zhang, X. Li, X. Zhang, H. Qin and W. Xiao, *Nat. Prod. Rep.*, 2020, DOI: 10.1039/d0np00043d.
- 363 R. Reher, H. W. Kim, C. Zhang, H. H. Mao, M. Wang, L. F. Nothias, A. M. Caraballo-Rodriguez, E. Glukhov, B. Teke, T. Leao, K. L. Alexander, B. M. Duggan, E. L. Van Everbroeck, P. C. Dorrestein, G. W. Cottrell and W. H. Gerwick, *J. Am. Chem. Soc.*, 2020, 142, 4114–4120.