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Bioactive natural products from Bacteroidetes

Stephan Brinkmann, [a] Marius S. Spohn [a] and Till F. Schäberle *[abc]

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Bacteria representing the phylum Bacteroidetes produce a diverse range of natural products, including polyketides, peptides and lactams. Here, we discuss unique aspects of the bioactive compounds discovered thus far, and the corresponding biosynthetic pathways if known, providing a comprehensive overview of the Bacteroidetes as a natural product reservoir.

[a]*Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Branch for Bioresources, 35392 Giessen, Germany. E-mail: stephan.brinkmann@ime.fraunhofer.de; marius.spohn@ime.fraunhofer.de; till.f.schaeberle@agrar.uni-giessen.de*

[b]*Institute for Insect Biotechnology, Justus Liebig University of Giessen, 35392 Giessen, Germany*

[c]*German Centre for Infection Research (DZIF), Partner Site Giessen-Marburg-Langen, Giessen, Germany*

1 Introduction

Antimicrobial resistance (AMR) is a serious threat to modern medicine and agriculture.[1] Bacteria and fungi resistant to many or most antimicrobials have dire socioeconomic consequences for human health and global food security.[2] The threat of AMR is exacerbated by the limited number of candidate antibiotics entering the development pipeline and thus the declining number of approvals for new anti-infective drugs.[3]

The AMR crisis is being addressed by efforts to discover natural products that can serve as new anti-infective leads, and microbes are a rich source of such compounds.[4] However, natural product research is hindered by high rediscovery rates, especially in classical producers such as Actinobacteria, Firmicutes and Myxobacteria – despite the strong genetic potential for the biosynthesis of novel molecules.[5] This limitation can be circumvented by focusing on underexplored bacterial taxa.

Members of the phylum Bacteroidetes are well-known, abundant and widely distributed sources of natural products, and many members are easy to cultivate. They colonize all types of

habitats, including soil, ocean, freshwater, and the gastrointestinal tracts of animals.[6] The phylum comprises six classes of gram-negative, non-spore forming, chemo-organotrophic bacteria. Gastrointestinal tracts harbor species from the mostly anaerobic class *Bacteroidia*, whereas classes *Chitinophagia*, *Cytophagia*, *Flavobacteriia*, *Saprospiria* and *Sphingobacteriia* are mainly found in the environment.[7] All of them are non-motile (or motile by gliding)[8] bacteria that can degrade polymeric organic matter.[6] A few are pathogenic[9] or endosymbiotic.[10] Their promoter structures feature a unique consensus sequence[11] recognized by the core RNA polymerase and an unusual primary sigma factor.[11,12] The strong genetic potential for the biosynthesis of natural products[13,14] is enriched in a few genera (*e.g.*, *Chitinophaga* and *Pedobacter*) and less abundant in the anaerobic class *Bacteroidia*.[14] However, the number of compounds isolated thus far is rather low. Known products are structurally diverse and some are potential antibiotic leads, such as the (iso)pedopeptins that can inhibit drug-resistant gram-negative bacteria including World Health Organization (WHO) top-priority carbapenem-resistant pathogens, and the dipeptide TAN-1057, with activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains.

In this review, we comprehensively discuss the bioactivity and biosynthesis of natural products that have already been isolated from Bacteroidetes.

2 Macrolides

Macrolides are hydrophobic polyketides characterized by a large macrocyclic lactone ring decorated with variable side chains and groups. One of the better-known macrolides is erythromycin,[15] which has bacteriostatic activity based on the inhibition of bacterial protein biosynthesis.[16] Macrolides also show antifungal, antiparasitic, antiviral, cytotoxic and immunosuppressive activities.[17]

2.1 Elansolids

The elansolids are unique macrolides produced by *Chitinophaga sancti* FxGBF13 (DSM 21134)[18–20] and *Chitinophaga pinensis* DSM 2588.[21] They share a bicyclo[4.3.0]nonane core originating from an intramolecular Diels–Alder cycloaddition (Fig. 1).[22]

Elansolids A1 (**1**) and A2 (**2**) are atropisomers featuring a 19-membered macrolactone ring. They are derived from the precursor elansolid A3 (**3**) by lactonization *via* a intramolecular Michael addition of the quinone methide group.[20,22] The side products elansolids B1–3 (**4–6**) and D1–2 (**7–8**) are instead derived from the highly reactive quinone methide moiety in elansolid A3.[20,21] The cultivation of *C. sancti* DSM 21134 in complex media containing different soy peptones, or synthetic medium supplemented with anthranilic acid, yielded elansolid C1 (**9**).[23] This is derived *via* a nucleophilic addition of the anthranilic acid amino group to the *p*-quinone methide carbon of elansolid A3.[23]

Stephan Brinkmann received his bachelor's degree in Biotechnology and Instrumentation Engineering from the Bielefeld University of Applied Sciences in 2014 and his master's degree in Molecular Biotechnology from the University Bielefeld in 2017. In his following PhD thesis, he developed microfluidic-based high-throughput approaches for the cultivation and screening of microorganisms for their potential to produce bioactive natural products and also analyzed the Bacteroidetes phylum for its potential to biosynthesize natural products using omics technologies at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Branch for Bioresources, in Giessen, Germany.

Marius Spohn received his master's degree in Microbiology in 2012 from the University of Tübingen. After his PhD studies focusing on the investigation of regulatory circuit's controlling microbial natural products biosynthesis and a short postdoctoral position he joined in 2016 the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME). Working as the group leader for molecular microbiology, he continues his research on microbial natural products in joint projects with partners from pharma-, agro- and veterinary industry.

Till F. Schäberle studied biology and at the age of 29, he received his PhD from the University of Tübingen, Germany at the Microbiology/Biotechnology department. In 2009, he moved to the University of Bonn, Germany where he worked as a postdoc and subsequently was heading his own working group within the Institute for Pharmaceutical Biology. Currently, he is professor at the Justus-Liebig-University Giessen and department head at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Branch Bioresources. His natural product (NP) research groups applies genetic, biochemical, bioinformatic and chemical analytical methods for NP biosynthesis/discovery.

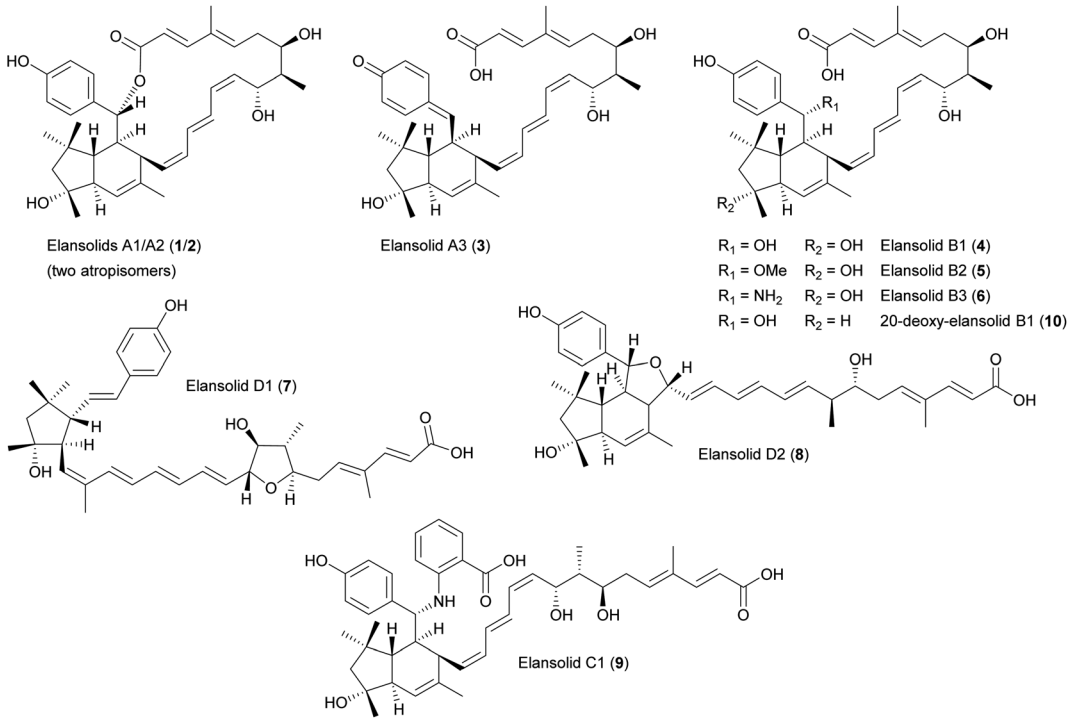


Fig. 1 Chemical structures of elansolids (**1**–**10**).

The elansolids are produced by *trans*-acyltransferase (AT) polyketide synthases (PKSs) (Fig. 2A).[21,22] The biosynthetic gene cluster (BGC) of *C. sancti* was identified by scanning a cosmid library.[22] In parallel, a closely related set of genes (84.6% nucleotide sequence identity) was identified in *C. pinensis* DSM 2588, facilitating the isolation of elansolid D2 (**8**) and the deduction of its biosynthetic pathway.[21] Elansolid A3 is probably the mature pathway product (Fig. 2B).[21,22] The two *trans*-AT type I polyketide BGCs consist of either 18 or 19 genes (*ela*/*els* PKS) with their assembly line including six multimodular PKS proteins and two *trans*-ATs.[21,22] We also screened 600 publically available Bacteroidetes genomes *in silico* to examine the sequential and compositional similarity of their BGCs, revealing *Chitinophaga* sp. YR627 as a third strain carrying a BGC highly similar to the known elansolid BGCs.[14] Interestingly, the comparison of all three BGCs suggests the presence of an additional gene encoding a major facilitator superfamily 1 protein, which may transport elansolids across membranes.

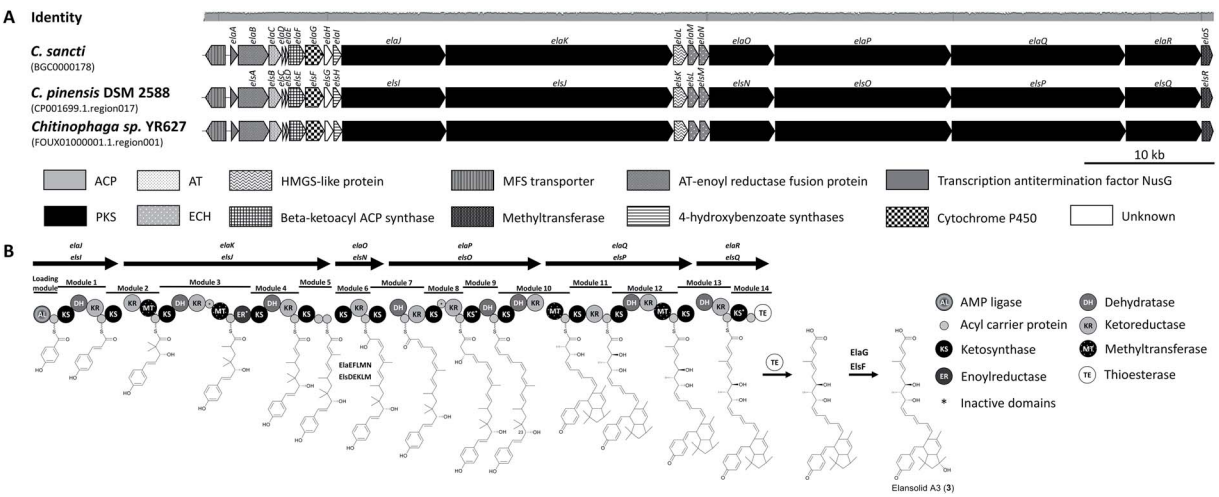


Fig. 2 Elansolid biosynthetic gene clusters (MIBiG BGC0000178) and pathway. (A) Elansolid BCGs found in genomes of three *Chitinophaga* strains. Identity represents the nucleotide alignment of all five BGCs using MAFFT.[156] (B) Model for elansolid biosynthesis.[21,22] The genes are indicated by arrows. The genes are denominated differently in the two source publications, so both names are given. The timing of the dehydration centered at C23 and subsequent IMDA reaction cannot be deduced with certainty. ACP, acyl carrier protein; PKS, polyketide synthase; AT, acyltransferase; MFS, major facilitator superfamily; HMGS, hydroxymethylglutaryl-CoA synthase; ECH, enoyl-CoA hydratase/isomerase.

Major facilitator superfamily proteins are membrane efflux pumps that contribute to the transport of various substances, including macrolides.[24]

Elansolids A1 (**1**) and A2 (**2**) are atropisomers, but only the latter is active against gram-positive bacteria, with minimal inhibitory concentrations (MICs) of 0.2–64 μg mL^{-1}. This product also showed moderate cytotoxicity against L929 mouse fibroblasts, B104 rat cells, SH-SY5Y human neuroblastoma cells, and HeLa cells.[18–21] Furthermore, the highly reactive *p*-quinone methide elansolid A3 (**3**) can be stabilized as an ammonium salt, which showed antibacterial and cytotoxic activity identical to elansolid A2 (**2**).[20] This reactivity was used to synthesize an elansolid library from crude extracts *via* Michael-type conjugate addition, using 21 different nucleophiles. None of these precursor-directed derivatives was more potent than elansolids A2 (**2**) and A3 (**3**), but all were less cytotoxic than elansolid A2 (**2**).[23]

Elansolids A2 (**2**) and C1 (**9**) inhibit the secretion-deficient *Escherichia coli* Δ*tolC* mutant when combined with the membrane-permeabilizing cationic cyclic peptide polymyxin B nonapeptide (PMBN) at 2 or 8 μg mL^{-1},[25] indicating that a molecular target is also present in gram-negative bacteria but protection is achieved by its outer membrane.

The total chemical synthesis of elansolid B1 (**4**)[26] was followed by the second-generation total synthesis of the same compound and the first synthesis of elansolid B2 (**5**).[27] Further studies to elucidate the biosynthesis of elansolids[28,29] revealed a further polyketide, 20-deoxy-elansolid B1 (**10**), with activity similar to elansolid A2 (**2**), showing that the hydroxyl group at C20 is not essential for antibacterial activity.[28]

2.2 YL-02905-A and YL-02905-B

Two *Cytophaga* strains were found to produce four macrolactones with activity against *S. aureus*.[30–32] First reported in a patent,[30] the two 22-membered macrolide ring antibiotics YL-02905-A (also named YM-32890-B, **11**) and YL-02905-B (also named YM-32890-A, **12**) were isolated from *Cytophaga* sp. YL-02905S.[31] Their natural isomers (**13** and **14**) complement this group.[32] The four macrolactones differ in the aliphatic chain attached to the macrolactone ring at C21 (Fig. 3). Accordingly, molecules **11** and **13** possess a conjugated tetrene system whereas **12** and **14** contain a conjugated triene system, separated by a methylene carbon at C28.[31,32] Compound **12** undergoes tautomerization to form **11** (ref. 32) and is generally unstable under light and aerobic conditions.[31] Only compounds **12** and **14** displayed activity against *S. aureus* KCTC 1927 with MICs of ~10–20 μg mL^{-1}.[32] These data reflect the importance of the conjugation pattern of the double bonds along the aliphatic chain.[32] The biosynthesis or total synthesis of these compounds has yet to be reported.

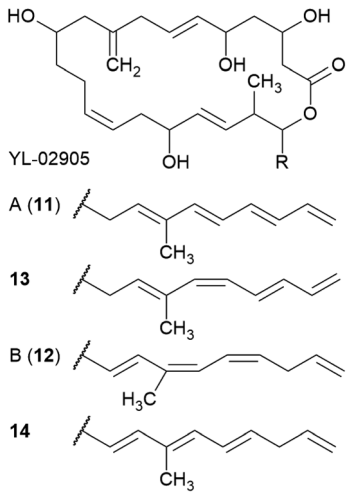


Fig. 3 Chemical structures of YL-02905 A (**11**) and B (**12**) as well as their structural isomers **13** and **14**.

3 Peptides

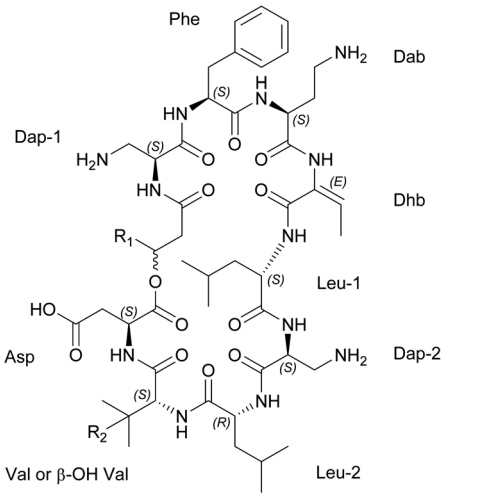
Peptide natural products are valuable sources of anti-infective leads and are either synthesized ribosomally or assembled by multi-modular non-ribosomal peptide synthetases (NRPSs), allowing the incorporation of non-proteinogenic amino acids. Optional (multi)cyclization and extensive post-translational modifications result in remarkable structural diversity.[33]

3.1 Cyclic depsipeptides

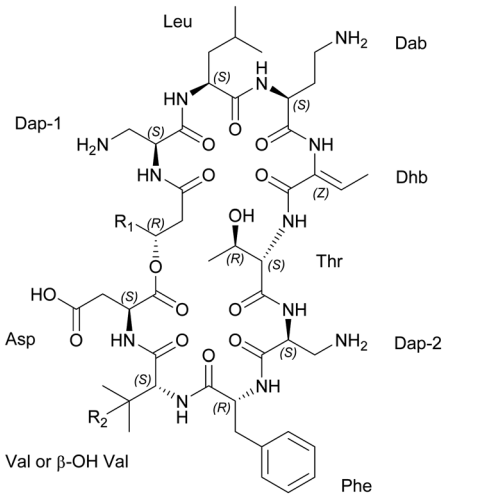
More than 1300 diverse natural cyclic depsipeptides have been described, and are characterized by the presence of ester and amide bonds that contribute to a range of biological properties.[34,35] Most cyclic depsipeptides are synthesized by modular NRPSs,[36] but some (*e.g.*, microviridins) are synthesized ribosomally.[37]

3.1.1 (Iso)pedopeptins. Natural products that inhibit lipopolysaccharide (LPS) binding to the receptor CD14 are useful leads against sepsis and septic shock. Screening for such products led to the discovery of pedopeptins A–C (**15–17**, patented as B-12489 A–C; Fig. 4).[38–40] These cyclic lipodepsipeptides (CLPs) were isolated from the culture broth of *Pedobacter* sp. SANK 72003.[39,40] They share three cationic amino acids – two diaminopropionic acid (Dap) and one diaminobutyric acid (Dab) residues – among a constituent of nine, including 2-amino-2-butenoic acid (dehydrobutyrine, Dhb) and β-hydroxy valine (β-OH-Val), as well as an aliphatic chain.[40,41] UHPLC-MS/MS analysis revealed that similar cyclic and linear peptides are also produced by several other *Pedobacter* strains.[42] Detailed investigation of the strain *Pedobacter cryoconitis* UP508 led to the discovery of isopedopeptins A–H (**18–25**; Fig. 4).[42–44] These are similar to the pedopeptins, but Leu-2 and Phe are exchanged, Thr replaces Leu-1, and the acyl moieties are distinct.[44]

Pedopeptins A (**15**), B (**16**) and C (**17**) inhibit the binding of LPS to CD14 with half-maximal inhibitory concentrations (IC_{50}) of 20, 11 and 47 nM, respectively. LPS-induced cytokine release in cell-based *in vitro* assays revealed dose-dependent inhibition, with IC_{50} values of 0.08–0.33 μM (~1–10 μg mL^{-1}). However, pedopeptins A and B were also cytotoxic at 100 and 30 μg mL^{-1}, respectively. Antimicrobial testing of all three peptides revealed MICs of 2–4 μg mL^{-1} against two *E. coli* strains. Interestingly, only



Pedopeptin A (**15**)	R_1=3-hydroxy-7-methyloctanoyl	R_2=OH
Pedopeptin B (**16**)	R_1=3-hydroxy-7-methyloctanoyl	R_2=H
Pedopeptin C (**17**)	R_1=3-hydroxydecanoyl	R_2=OH



Isopedopeptin A (**18**)	R_1=6-methylheptyl	R_2=OH
Isopedopeptin B (**19**)	R_1=heptyl	R_2=OH
Isopedopeptin C (**20**)	R_1=2-nonenyl	R_2=OH
Isopedopeptin D (**21**)	R_1=heptyl	R_2=H
Isopedopeptin E (**22**)	R_1=6-methylheptyl	R_2=H
Isopedopeptin F (**23**)	R_1=8-methylnonyl	R_2=OH
Isopedopeptin G (**24**)	R_1=8-methyl-4-nonenyl	R_2=H
Isopedopeptin H (**25**)	R_1=8-methyl-2-nonenyl	R_2=H

Fig. 4 Chemical structures of pedopeptins A–C (**15–17**) and isopedopeptins A–H (**18–25**). Dap, diaminopropionic acid; Dab, diaminobutyric acid; Dhb, dehydrobutyrine; β-OH-Val, β-hydroxy valine.

pedopeptin B showed strong activity (4 μg mL^{-1}) against two gram-positive bacteria: *S. aureus* ATCC 6538P and *S. epidermis* ATCC 14990.[39] All isopedopeptins show activity against a broad panel of gram-negative bacteria, including carbapenem-resistant strains on the WHO top-priority list. Isopedopeptin B (**19**) is the most potent representative, with MICs as low as 1 μg mL^{-1}. In contrast to the pedopeptins, isopedopeptins show minimal activity against gram-positive bacteria (*e.g.*, MIC = 16 μg mL^{-1} against *Bacillus cereus* CCUG7414), and none against fungi. Furthermore, isopedopeptins are cytotoxic against HepG2 liver cells (IC_{50} = 9–50 μM or 10–56 μg mL^{-1}) and are hemolytic (0.8–52.3%). Isopedopeptin B was therefore the most promising candidate due to its potent antibacterial activity and acceptable cytotoxicity.[44]

The mode of action (MoA) of the cationic pedopeptins is thought to involve interactions with lipid layers due to structural similarities with other cationic cyclic peptides such as polymyxin B and colistin, which are known to interact with anionic LPS.[39,45] Interestingly, the polycationic isopedopeptin B (**19**) showed activity against colistin-resistant strains of *Acinetobacter baumannii*, *E. coli*, and *Klebsiella pneumoniae* with a very low frequency of resistance ($<3 \times 10^{-9}$).[44] Using an *in vitro* bacterial membrane system based on unilamellar liposomes prepared from *E. coli* phospholipid extracts, isopedopeptin F (**23**) was shown to cause membrane leakage with a half-maximal effective concentration (EC_{50}) of 2 μM, a similar concentration to other cyclic peptides such as polymyxin B (EC_{50} = 1 μM) and LL-37 (EC_{50} = 0.6 μM).[44]

A putative BGC for the pedopeptins has been identified in the pedopeptin producer strain *Pedobacter lusitanus* NL19 (Fig. 5).[46] The NRPS is composed of a large and a small subunit. Genes encoding diaminobutyrate-2-oxoglutarate transaminase and oxygenase (cytochrome P450 family) are found nearby, and may be responsible for the supply of Dab as well as Val hydroxylation. The expression of both NRPS genes correlates with the production of pedopeptins. Furthermore, within this BGC, the C5 domain shows similarity to C-domains that modify incorporated amino acids. In this case, it should catalyze the dehydration of Thr (incorporated by A-domain 4) to Dhb. Genes encoding glutamate synthase and dehydrogenase were also found in the genome, and may be responsible for Dap biosynthesis.[46] A BGC for the isopedopeptins has yet to be described. However, the discovery of additional (iso)pedopeptin analogs in several other *Pedobacter* extracts indicates the existence of related BGCs, suggesting that small changes in A-domain specificity and/or module rearrangement within the pedopeptin BGC may facilitate the biosynthesis of various natural analogs, including the isopedopeptins.[42] The total synthesis of these cyclic peptides has not yet been reported.

3.1.2 Chitinopeptins A–D. The chitinopeptins are CLPs produced by *Chitinophaga* species. Chitinopeptins A (**26**) and B

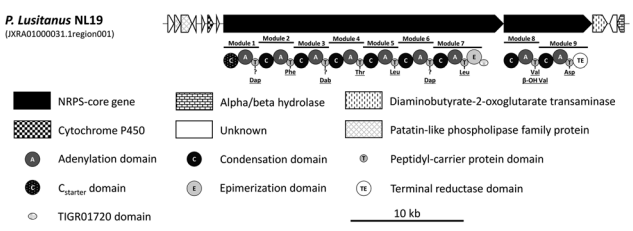


Fig. 5 Pedopeptin biosynthetic gene cluster. Non-ribosomal peptide synthetase biosynthetic gene cluster and assembly line responsible for the biosynthesis of pedopeptins. *In silico* predicted amino acids are indicated in the first row and those confirmed by NMR spectroscopy are underlined. Dap, diaminopropionic acid; Dab, diaminobutyric acid; β-OH Val, β-hydroxy valine.

(**27**) were isolated from *C. eiseniae* DSM 22224, whereas chitinopeptins C1, C2, D1 and D2 (**28–31**) were isolated from *C. flava* KCTC 62435. They share many non-proteinogenic amino acids such as Dap and *N*-methyl-Val, and a high degree of β-hydroxylation (Asp, Phe and Ile residues). The tetradecalipodepsipeptides **26** (2,9-dimethyl-3-amino decanoic acid) and **27** (3-amino-9-methyl decanoic acid) differ in the fatty acid residue attached to the peptide core. In contrast, the pentadecalipodepsipeptides (**28–31**) carry a 3-hydroxy-9-methyl decanoic acid residue and an additional Dap residue inserted between the fatty acid and the cyclized peptide chain, and the Leu-2 present in molecules **26** and **27** is replaced with Ile (**28** and **29**) or Val (**30** and **31**). The constitution of the Dap-2 residue (peptide bond between the α or β amino groups and the carbonyl group of Lys) distinguishes between molecules **28** and **29** as well as between molecules **30** and **31** (Fig. 6).[14]

Chitinopeptins inhibit gram-negative and gram-positive bacteria such as *Moraxella catarrhalis* (MIC = 2 μg mL^{-1}) and *Bacillus subtilis* (MIC = 4 μg mL^{-1}) as well as the yeast *Candida albicans* (MICs ≥ 4 μg mL^{-1}). MICs of ≥ 16 μg mL^{-1} were observed against *A. baumannii*, *Micrococcus luteus* and *Zymoseptoria tritici*. Interestingly, the chitinopeptins bind iron, which in the case of chitinopeptin A (**26**) reduces but does not abolish its activity.[14]

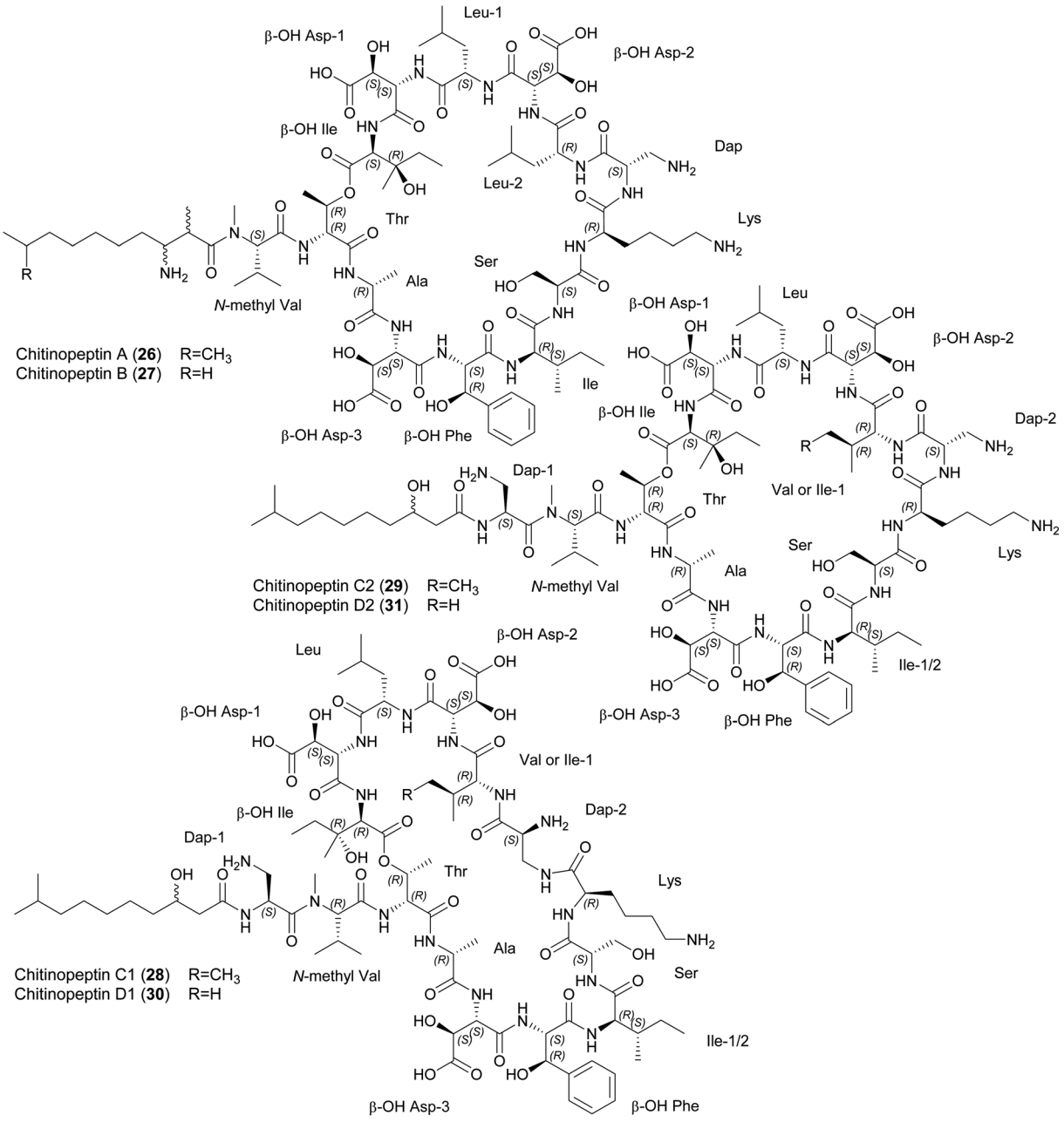


Fig. 6 Chemical structures of chitinopeptins A–D (**26–31**). β-OH Asp, β-hydroxy aspartic acid; β-OH Phe, β-hydroxy phenylalanine; Dap, diaminopropionic acid; β-OH Ile, β-hydroxy isoleucine.

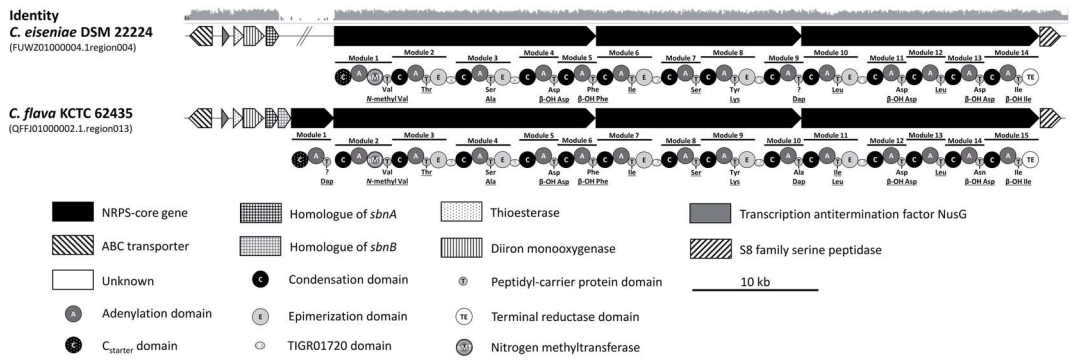


Fig. 7 Chitinopeptin biosynthetic gene clusters. Non-ribosomal peptide synthetase biosynthetic gene clusters and assembly lines responsible for the biosynthesis of chitinopeptins. *In silico* predicted amino acids are indicated and those confirmed by NMR spectroscopy are underlined. β-OH Asp, β-hydroxy aspartic acid; β-OH Phe, β-hydroxy phenylalanine; Dap, diaminopropionic acid; β-OH Ile, β-hydroxy isoleucine.

Putative NRPS-BGCs have been identified for the synthesis of these CLPs (Fig. 7). The number and predicted substrate specificity of the A-domains, the overall composition of the NRPS assembly line, and genes encoding enzymes responsible for precursor supply and post-assembly modifications matched the structural composition of the chitinopeptins. A BGC-similarity networking approach revealed the presence of homologous BGCs in *C. oryziterrae* JCM16595 and *C. niastensis* DSM 24859.[14] The total synthesis of chitinopeptins has yet to be disclosed. The synthesis of the β-hydroxylated amino acids Asp, Phe and Ile has been described as an attempt to elucidate the absolute configuration of these amino acids in all chitinopeptin structures.[14]

3.1.3 Katanosins A and B. Two groups independently elucidated the structure of the same cyclic depsipeptide antibiotic while searching for novel cell wall inhibitors. This molecule was isolated from the producer strain *Cytophaga* PBJ-5356 and was named katanosin B (**32**)[47,48] but was also isolated from *Lysobacter* sp. ATCC 53042 and was named lysobactin.[49–51] *Cytophaga* PBJ-5356 also produces the analog katanosin A (**33**), which is almost identical to katanosin B but has a Val residue replaced with Ile.[47,48] Both compounds consist of 11 amino acids, six of which are non-proteinogenic, including β-hydroxylated Phe, Asn and Leu (Fig. 8).[48,51] They show moderate activity against some gram-negative bacteria and strong activity against a broad panel of gram-positive bacteria including MRSA (MIC = 0.39 μg mL^{-1}) and vancomycin-resistant enterococci (MIC = 0.78 μg mL^{-1}).[47,49,50]

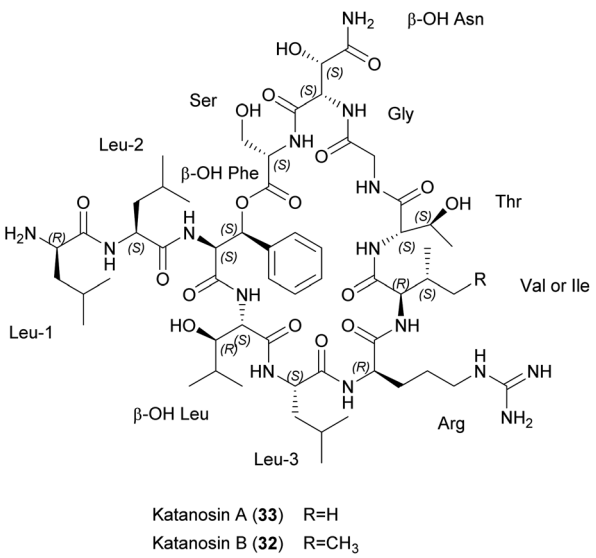


Fig. 8 Chemical structures of katanosins A (**33**) and B (**32**). β-OH Phe, β-hydroxy phenylalanine; β-OH Asn, β-hydroxy aspargine; β-OH Leu, β-hydroxy leucine.

Both molecules target peptidoglycan synthesis. To gain insight into the MoA, staphylococcal transglycosylation and its preceding reactions were studied by incorporating [^{14}C]GlcNAc into *S. aureus* peptidoglycan in whole cells, as well as nascent peptidoglycan formation *in vitro*. Both peptides inhibit nascent peptidoglycan synthesis, but only katanosin B also inhibits lipid intermediate formation.[52] Lipid II was identified as the cellular target of katanosin B.[53] The MoA of katanosins A and B differs from that of vancomycin and the peptides show a low frequency of resistance, making them suitable for further development. Accordingly, katanosin B and its method of production have been patented.[54]

A cloning study with a *Lysobacter* sp. ATCC 53042 genomic library revealed a partial NRPS gene (4.6 kb) as part of the BGC.[55] The complete BGC containing two multi-modular NRPSs (*lyb*A and *lyb*B) was discovered by genome sequencing (Fig. 9).[56] The termination module showed a tandem thioesterase architecture and both thioesterases were biochemically characterized *in vitro*. Peptide cyclization and simultaneous release is only mediated by the penultimate thioesterase. The second thioesterase fulfills a proofreading function involving the deacylation of miss-primed peptidyl carrier proteins.[56] Synthetic access was established based on modular, solution-phase approaches[57] and by solid-phase peptide syntheses.[58]

3.1.4 YM-47141 and YM-47142. The two macrocyclic depsipeptides YM-47141 (**34**) and YM-47142 (**35**), carrying a hydrated vicinal tricarbonyl moiety, were isolated from *Flexibacter* sp. Q17897.[59,60] Their structure comprises a peptide chain containing the non-proteinogenic amino acid 2,3-dioxo-4-amino-6-methylheptanoic acid (Dah), which has not been found

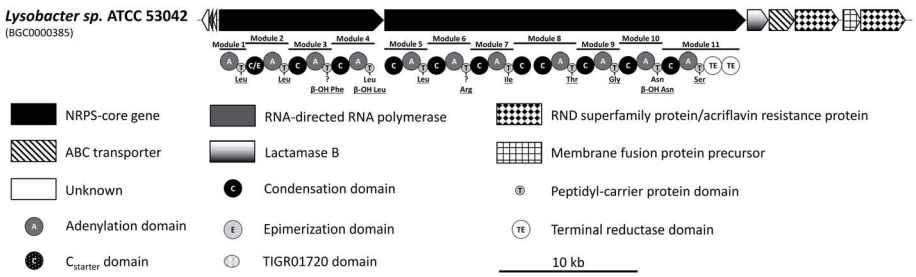


Fig. 9 Lysobactin/katanosin biosynthetic gene cluster (MIBiG BGC0000385). Non-ribosomal peptide synthetase biosynthetic gene cluster and assembly line responsible for the biosynthesis of lysobactin/katanosins. *In silico* predicted amino acids are indicated and those confirmed by NMR spectroscopy are underlined. β-OH Phe, β-hydroxy phenylalanine; β-OH Leu, β-hydroxy leucine; β-OH Asn, β-hydroxy asparagine.

before in microbial natural products. Five of the six remaining amino acids were found to have the L-configuration, in addition to D-Ala. The isovaleryl moiety in YM-47141 (**34**) is replaced by a phenylacetyl group in YM-47142 (**35**) (Fig. 10).[60] Total synthesis of both compounds conformed the L-configuration of Dah.[61] Both compounds inhibited human leukocyte elastase (HLE) with IC_{50} values of 15 and 30 μM, respectively.[59] YM-47141 (**34**) was also tested against porcine pancreatic elastase (PPE; $IC_{50} = 0.39$ μM), and was shown to inhibit other serine proteases, such as α-chymotrypsin and cathepsin G.[62] The BGC for these compounds has yet to be reported.

3.2 Short linear peptides

Short linear peptides also display remarkably diverse biological activities with biomedical and cosmeceutical applications.[63] They tend to have a short *in vivo* half-life[64] and many are immunogenic,[65] but generally they are highly selective, can cross biological membranes, and cost-effective synthesis has been reported.[63]

3.2.1 TAN-1057 A–D. The dipeptide-like metabolites TAN-1057 A (**36**) and B (**37**) (Fig. 11) are diastereomers first described by researchers at Takeda Chemical Industries Ltd.[66–68] They are produced by *Flexibacter* sp. PK-74 and PK-176,[67] along with diastereomers TAN-1057 C (**38**) and D (**39**). TAN-1057 A and B spontaneously epimerize in aqueous solution, whereas TAN-1057 C and D only transform into TAN-1057 A and B, respectively.[66,68] All four compounds show strong activity against staphylococci, including MRSA strains, but they are less active against gram-negative bacteria.[67] Subcutaneous treatment of MRSA-infected mice revealed an effective dose (ED_{50}) of ≥ 0.026 mg kg^{-1}, which is more potent than vancomycin (ED_{50} = 2.3 mg kg^{-1}). However, both TAN-1057 A and B are cytotoxic, with median lethal doses (LD_{50}) in mice of 50 mg kg^{-1} (intraperitoneal) and 100 mg kg^{-1} (intravenous).[67] This has been addressed by total synthesis to investigate the structure–activity relationship (SAR).[69] A synthetic derivative with a methyl group on the proximal guanidine nitrogen was less potent (10-fold lower MIC) but the ED_{100} was < 0.5 mg kg^{-1} (intravenous).[70]

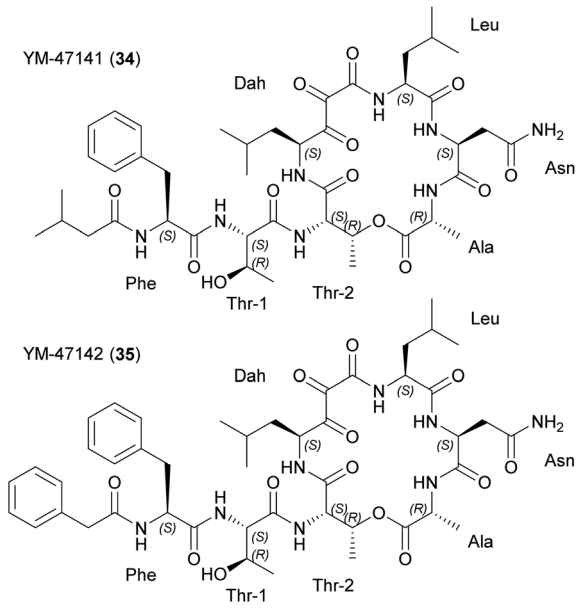


Fig. 10 Chemical structures of YM-47141 (**34**) and YM-47142 (**35**). Dah, 2,3-dioxo-4-amino-6-methylheptanoic acid.

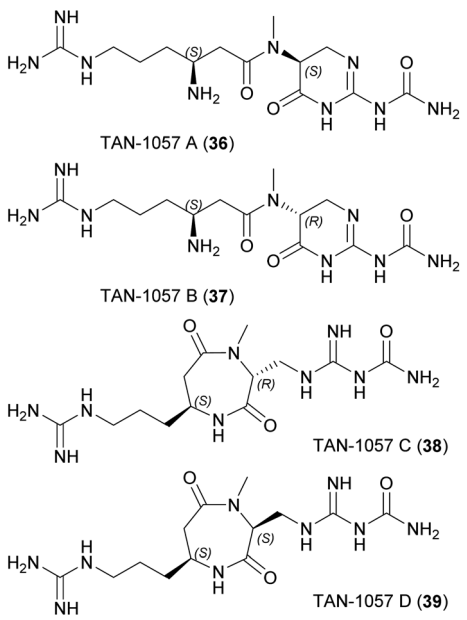


Fig. 11 Chemical structures of TAN-1057 A–D (**36–39**).

MoA studies with the TAN-1057 series showed a negative effect on protein synthesis at the elongation step in whole-cell and cell-free translation assays.[67,71] More detailed analysis revealed the target was the peptidyl transferase of the 50S ribosomal subunit, but the binding site appears to differ from that of other inhibitors.[67,71,72] Resistance studies indicated a rapid response based on changes in the transport of cellular dipeptides, followed by ribosomal alterations later on.[71,73] A BGC congruent with these dipeptide-like metabolites has yet to be identified.

3.2.2 Falcitidin and pentacitidins A and B. The linear *N*-acyl tetrapeptide falcitidin (**40**) was isolated from *Chitinophaga* sp. Y23, and features an unusual C-terminal amidated proline residue (Fig. 12).[74] Together with its synthetic derivatives, it represents a new class of cysteine protease inhibitors. In contrast to classical inhibitors, a reactive group such as an aldehyde that binds the active site Cys residue has not been identified.[74,75] Metabolic networking analysis revealed the presence of more than 30 natural analogs of falcitidin, including further falcitidin-like tetrapeptides.[76] They are produced by various *Chitinophaga* strains and two of these analogs were isolated from *Chitinophaga eiseniae* DSM 22224. Pentacitidins A (**41**) and B (**42**) are falcitidin-like pentapeptides with Phe as an additional C-terminal amino acid with an aldehyde group (phenylalaninal, H-Phe-al; Fig. 12). NMR spectroscopy revealed that both pentacitidins were purified as diastereomers for the Phe residue.[76] Interestingly, a pentapeptide and its corresponding falcitidin tetrapeptide analog with a C-terminal amidated proline were detected by UHPLC-MS, following the NMR analysis of pure pentacitidins, suggesting that falcitidin-like tetrapeptides are the degradation products of the mature natural products – the pentacitidins.[76] Further studies are needed to test this hypothesis and elucidate the degradation mechanism.

Falcitidin displays falcipain-2 enzyme activity *in vitro* (IC_{50} = 6 μM)[74] but lacks whole-cell activity against *Plasmodium falciparum* 3D7 (IC_{50} > 10 μM).[75] A liquid-phase synthesis approach produced an *N*-acyl trifluoromethyl derivative with a hydrophobic *N*-trityl group on the imidazole residue. In contrast to the isolated natural product, this molecule exhibits whole-cell antimalarial activity (IC_{50} = 0.14 μM).[75] Pentacitidins were produced by solid-phase peptide synthesis.[76] Aldehydes are electrophilic warheads that form covalent-reversible hemithioacetal adducts with catalytic Cys residues and covalent-reversible hemiacetal adducts with Ser residues. Testing pentacitidins against human cysteine proteases (cathepsins B and L) and related proteases from parasites (falcipain-2/3 and rhodesain) revealed selective inhibition of the parasitic enzymes with sub-micromolar IC_{50} values. In addition, the natural products were active against α-chymotrypsin. Docking studies showed favorable distances between the catalytic Cys residue and the pentacitidin aldehyde in its L-configuration.[76]

A pentacitidin BGC was tentatively identified, consisting of a single NRPS gene (18.5 kb) with five A-domains and a C-starter unit (Fig. 13).[76] It is congruent with the pentapeptide structure, strengthening the degradation hypothesis. The BGC contains a terminal reductase domain probably involved in the formation of the C-terminal aldehyde and alcohol analogs *via* reductive release. These alcohol analogs of pentacitidins were detected by molecular networking analysis and synthesized as pentacitidin intermediates, but showed no bioactivity.[76] Similar BGCs were found in the genomes of four other *Chitinophaga* strains.[76]

3.3 Ribosomally synthesized and post-translationally modified peptides (RiPPs)

There are more than 20 subclasses of RiPPs, and scientific interest in these peptides has increased in recent decades.[77] A few RiPPs have already been isolated from Bacteroidetes, and many more can be expected due to development of new genome mining tools for RiPP discovery.[78]

3.3.1 Lantipeptides. Lantipeptides are RiPPs containing the thioether cross-linked amino acids lanthionine and/or methyllanthionine. Most are produced by gram-positive bacteria, including the natural food preservative nisin from *Lactococcus* species.[79] Only a few such molecules have been isolated from gram-negative bacteria but genome analysis of the Bacteroidetes has revealed abundant putative lantipeptide BGCs.[78]

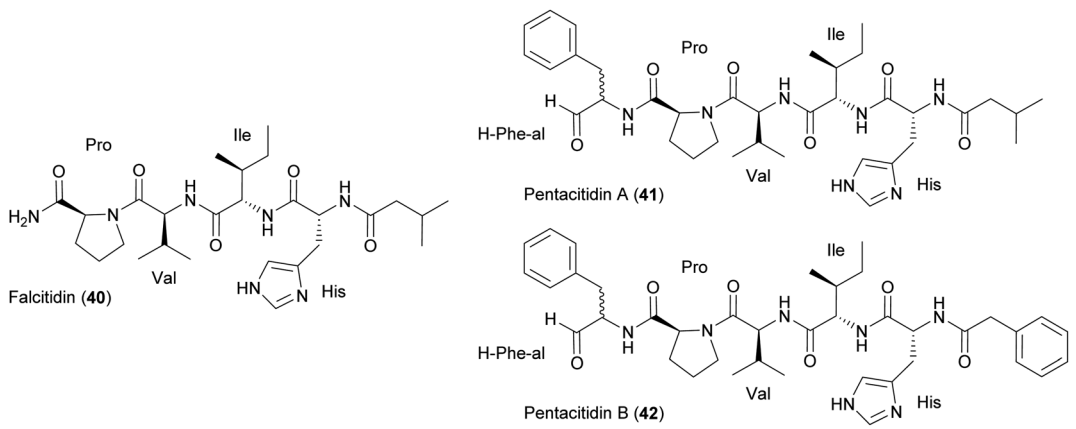


Fig. 12 Chemical structures of falcitidin (**40**) as well as pentacitidins A (**41**) and B (**42**).

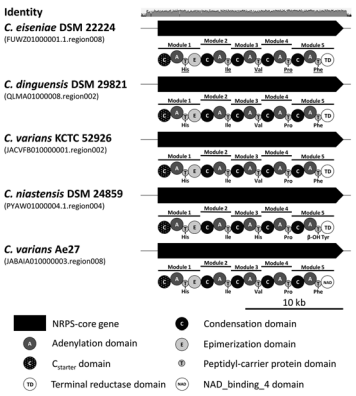


Fig. 13 Pentacitidin biosynthetic gene clusters. Non-ribosomal peptide synthetase biosynthetic gene clusters and assembly lines responsible for the biosynthesis of pentacitidins. *In silico* predicted amino acids are indicated and those confirmed by NMR spectroscopy are underlined. Identity represents the nucleotide alignment of all five BGCs using MAFFT.[156] β-OH Tyr, β-hydroxy tyrosine.

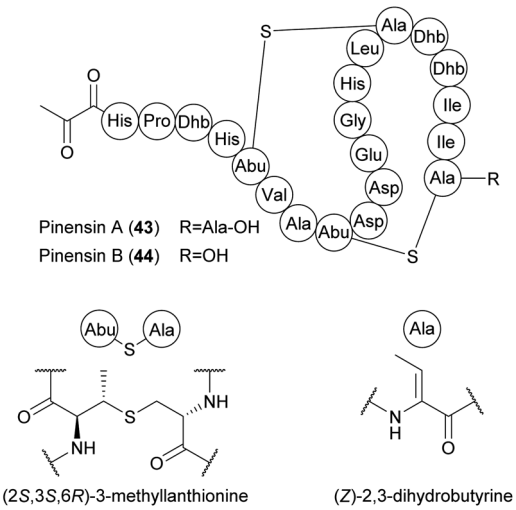


Fig. 14 Chemical structures of pinensins A (**43**) and B (**44**). Dhb, dehydrobutyrine; Abu, α-aminobutyric acid.

3.3.1.1 Pinensins A and B. Pinensins A (**43**) and B (**44**) are produced by *C. pinensis* DSM 28390. These two cyclic lantipeptides contain 22 and 21 amino acids, respectively, with a rare N-terminal pyruvic acid. Both incorporate two 3-methyllanthionine-bridged ring systems with an unusual (2*S*,3*S*,6*R*)-stereochemical configuration (Fig. 14).[80]

As a 1 : 1 mixture, pinensins A and B exhibit broad activity against filamentous fungi and yeast (MIC > 5 μg mL^{-1}) but only weak antibacterial activity. These lantipeptides also inhibit the growth of higher eukaryotic cells (IC_{50} > 10 μM). The pinensin BGC predicted *in silico* consists of at least five genes encoding the lantipeptide precursor peptide (PinA), two dehydratases (PinB1 and PinB2), one lanthionine synthetase (PinC) and PinT, required for export and cleavage of the precursor peptide (Fig. 15A). An unusual feature of pinensin biosynthesis is the split dehydratase, with functional domains catalyzing glutamylation and elimination encoded by separate open reading frames.[78,80] The precursor peptide PinA has 22 residues like pinensin A, suggesting that pinensin A is converted into pinensin B by the post-translational removal of the terminal Ala residue, but the enzyme responsible remains elusive.[80] Pinensin-like BGCs have also been identified in the genomes of other *Chitinophaga*,[14] *Cryseobacterium*, *Elizabethkingia*, *Pedobacter* and *Sinomicrobium* strains.[78] Predicted variations in the amino acid sequence of the core peptides indicate a yet undiscovered structural diversity of pinensin-type lantipeptides. A total synthesis approach for these peptides has not been reported.

3.3.1.2 PedA peptides. The computational prediction of precursor peptide sequences revealed several BGCs that may provide novel lathipeptides.[78] A BGC from *Pedobacter lusitanus* NL19 encoding two precursor peptides (Fig. 15B) could not be elicited in the native producer, so a heterologous expression strategy was implemented to gain access to these compounds. Co-expression of precursor peptide genes *pedA*15.1 and *pedA*15.2 in *E. coli*, together with their modifying enzymes, led to the discovery of the PedA15 peptides.[81] Only the structure of PedA15.1 (**45**) was elucidated in detail by NMR analysis (Fig. 16). This contains a novel 14-membered bis-lanthionine ring system in the LL-lanthionine stereochemical configuration. The 4-fold or 5-fold dehydrated peptide carries three lanthionine bridges between Dha7 and Cys16 nested with the Cys8–Dha15 ring, and a final ring between Dha18 and Cys21. This lantipeptide ring pattern has not been described in other lantipeptides. PedA15.1 carries further dehydrated but non-cyclized amino acids as well as a Ser that escaped dehydration in the heterologous host. The biosynthetic machinery of *E. coli* thus allows the production of modified peptides, but the heterologous products may not be

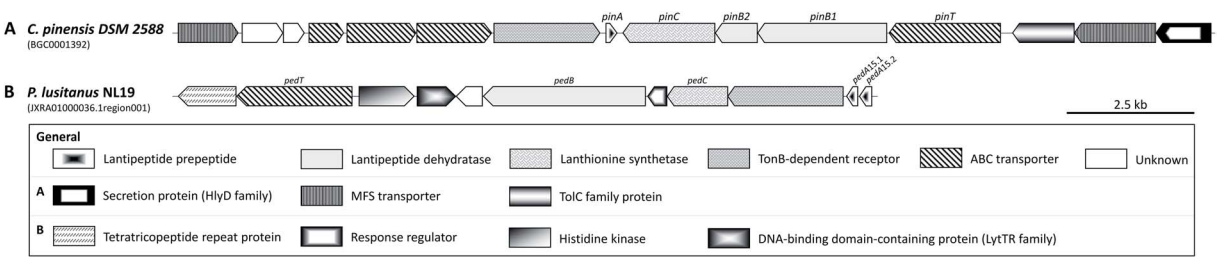


Fig. 15 Biosynthetic gene clusters of the ribosomally synthesized and post-translationally modified peptides pinensin (MIBiG BGC0001392) (A) and pedA15.1 (B).

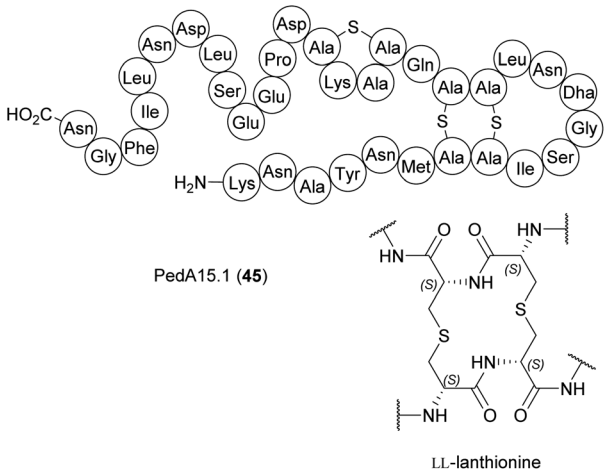


Fig. 16 Chemical structure of PedA15.1 (**45**). Dha, dehydroalanine.

the same as those synthesized in the native host, potentially explaining why PedA15.1 and PedA15.2 lack antibacterial and antifungal activity.[81] As for the pinensins, the total synthesis of PedA15.1 has not been described.

3.3.2 Bacteriocin-like peptide FR901451. The elastase inhibitor FR901451 (**46**) was isolated from *Flexibacter* sp. 758.[82] The tri-macrocyclic architecture of this patented compound[83] (Fig. 17) is based on 11 proteinogenic L-amino acids that are cyclized *via* two lactone bonds and one amide bond.[82,84] A substrate-competitive mode of inhibition was observed against PPE and HLE, with *in vitro* IC_{50} values of 0.27 and 0.23 μM, respectively.[82] In hamsters, FR901451 prevented HLE-induced lung hemorrhage *in vivo* and modulated PPE-induced changes to respiratory mechanics.[85] Crystallographic studies confirmed that FR901451 interacts with the substrate-binding site of PPE.[84] The BGC is unknown and total synthesis has not been reported for this compound. However, a BGC has been reported for the similar protease inhibitors microviridins B and J. A ribosomally synthesized precursor peptide is modified by two cyclases related to GRASP-like ligases that introduce two ester bonds and one amide bond[37]

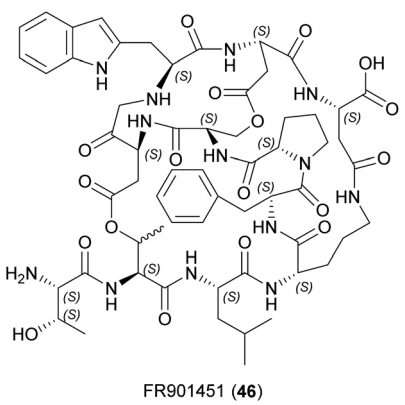


Fig. 17 Chemical structure of FR901451 (**46**).

4 Polyketide – non-ribosomal peptide hybrids

Modular PKS and NRPS systems present analogous assembly lines with similar mechanisms used to incorporate building blocks in the nascent chain. Genes encoding enzymes involved in either polyketide or non-ribosomal peptide synthesis can be combined, resulting in hybrid natural products that greatly expand the potential chemical diversity.[86] One example is the immunosuppressant rapamycin, with a pipecolate group in its polyketide skeleton.[87] Several of these complex polyketide–amino acid/peptide hybrids, featuring both acyl and aminoacyl building blocks, have been described in Bacteroidetes.

4.1 Ariakemicins A and B

Ariakemicins A (**47**) and B (**48**) were isolated from a marine gliding bacterium of the genus *Rapidithrix* and were described as an inseparable mixture.[88] The linear structures carry on one side an aromatic moiety substituted with one hydroxyl and one methoxy group, and on the other a methylated oxazol moiety decorated with a primary amide. The two compounds differ in the location of the double bond, which is found in position Δ^{12} and Δ^{11} in ariakemicins A and B, respectively (Fig. 18). The ariakemicin mixture was shown to inhibit gram-positive bacteria, with MICs ranging from 0.46 μg mL^{-1} against *S. aureus* IFO12732 to 83 μg mL^{-1} against *B. subtilis* IFO3134, but was inactive against gram-negative bacteria and *C. albicans*. Cytotoxicity was observed against human lung cancer cell line A549 and baby hamster kidney (BHK) cells with IC_{50} values of 25 and 15 μg mL^{-1}, respectively.[88] We were able to dereplicate ariakemicin A in extracts from *Rapidithrix thailandica* s80 (ref. 89) and subsequent isolation yielded pure ariakemicin A (**47**). This was active against *E. coli* ATCC25922 Δ*tolC* (our unpublished results), indicating that a molecular target for this compound is also present in gram-negative bacteria. One drawback of the ariakemicins is their rapid decomposition and loss of activity.[88] This may also explain why these compounds have not yet been synthesized.

5 Quinolines

The heterocyclic aromatic compound quinoline was first extracted from coal tar in the 18th century, and its structural motif can be found in many plant alkaloids, including quinine. However, there are also prominent bacterial examples, such as the antifungal compound pyrrolnitrin in *Pseudomonas* spp.[90]

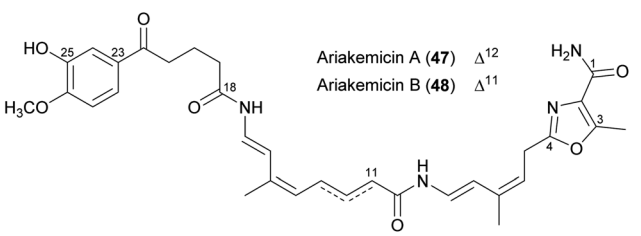


Fig. 18 Chemical structures of ariakemicins A (**47**) and B (**48**).

This aminophenylpyrrole alkaloid became a lead structure for the development of foliar fungicides. Additional structural variants have been isolated mainly from marine bacteria of the order *Cytophagales*.

5.1 G1499-2

The first quinoline isolated from the genus *Cytophaga* was 3-methyl-2-(2-pentylidenecyclopropyl)-4-quinolinone, also known as G1499-2 (**49**) (Fig. 19).[91] It has weak activity against a few bacteria, such as *Flavobacterium* sp. 1980, a marine species, and no toxicity in mice. The BGC is unknown and total synthesis has not been reported for this compound.

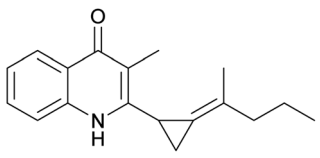
5.2 Marinoquinolines and marinoazepinones

Several natural products featuring a rarely described 3*H*-pyrollo [2,3-*c*]quinoline ring system have also been identified (Fig. 20), beginning with marinoquinoline A (**50**), which was isolated from *Rapidithrix thailandica*.[92] This product is an acetylcholinesterase inhibitor with an IC_{50} value of 4.9 μM.[93] Five derivatives known as marinoquinolines B–F (**51–55**) are produced by *Ohtaekwangia kribbensis*.[94] All five showed toxicity against three cancer cell lines in the low micromolar range (0.3–8.0 μg mL^{-1}) and two (**51** and **55**) showed potent activity against *P. falciparum* K1 (IC_{50} = 1.8 and 1.7 μM, respectively).

Several more marine Bacteroidetes were isolated by cultivation in seawater-based media, leading to the discovery of marinoquinolines G–K (**56–60**).[95] Furthermore, the prolific producer strain *Mooreia alkaloidigena* CNX-216^T synthesizes the structurally-related marinoazepinones A (**61**) and B (**62**), as well as other alkaloids.

To investigate quinoline biosynthesis in Bacteroidetes, the BGCs predicted *in silico* were expressed in *E. coli* along with other key enzymes for *in vitro* assays.[96] This revealed that only a single enzyme-catalyzed reaction is essential, in which tryptophan (**63**) is converted to 3-(2′-aminophenyl)-pyrrole (**64**), which is another potent acetylcholinesterase inhibitor already isolated from a *Rapidithrix* strain.[97] This active intermediate can be coupled to a variety of aldehydes *via* a spontaneous Pictet–Spengler-like reaction under physiological conditions, producing diverse products using a minimal complement of enzymes (Fig. 21).

The synthesis of a pyrroloquinoline system has been achieved using several approaches,[98–102] including the synthesis of four different marinoquinolines *via* Heck–Matsuda arylation and Pictet–Spengler cyclisation.[100] Brønsted acid-promoted arene-ynamide cyclization was applied to produce high yields



G1499-2 (**49**)

Fig. 19 Chemical structure of G1499-2 (**49**).

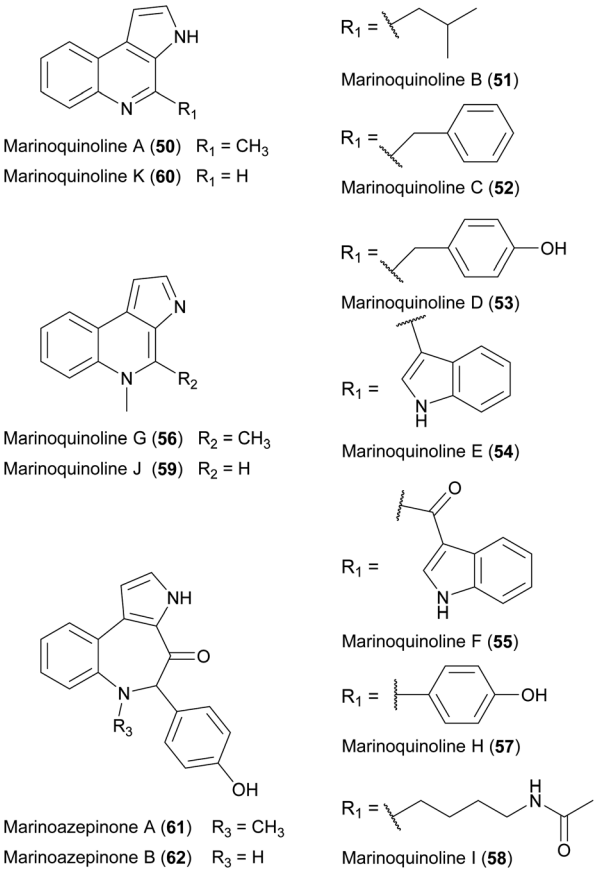


Fig. 20 Chemical structures of marinoquinolines A–K (**50–60**) as well as marinoazepinones A (**61**) and B (**62**).

of marinoquinolines A (**50**) and C (**52**).[101] More recently, the total synthesis of marinoquinoline A was achieved in four steps starting with the palladium-catalyzed Ullmann coupling of *o*-bromonitrobenzene to iodinated pyrrole.[102] Also, an intramolecular Diels–Alder oxazole cycloaddition reaction was used to produce marinoquinoline A from oxazole with an overall yield of 12%.[99]

6 Lactams

The clinical introduction of penicillin in the 1940s is a milestone in medical history. Even today, β-lactam antibiotics are

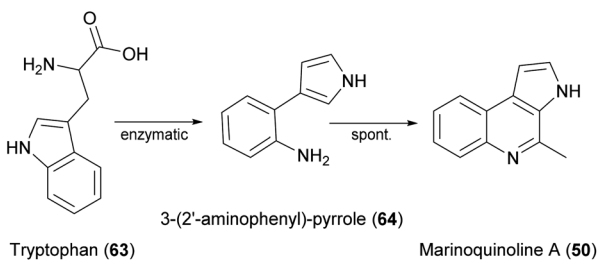


Fig. 21 Tryptophan (**63**) is enzymatically converted to 3-(2′-aminophenyl)-pyrrole (**64**). This intermediate reacts spontaneously with a variety of aldehydes, resulting in marinoquinolines such as marinoquinoline A (**50**).

ranked among the most important antimicrobials. Following the penicillins, further β-lactam antibiotic classes (*e.g.*, cephalosporins, carbapenems and clavams) were discovered in fungi and actinomycetes. By expanding the search to include Proteobacteria and Bacteroidetes, the global β-lactam discovery effort culminated in the description of the monobactams in the early 1980s.[103] All reports on lactams from Bacteroidetes date back to a short period between 1982 and 1987, so there is not yet any information about the genetic or metabolic basis of such compounds and their total synthesis has not been reported.

6.1 Monobactams

6.1.1 SQ 28332 (also known as PB-5582-A). The Squibb Institute for Medical Research used a β-lactamase reporter assay to detect compounds with intact β-lactam rings regardless of their antibacterial activity. Many new β-lactams were identified in this manner, including several monobactams that lack an annulated ring system.[103] SQ 28332 or PB-5582-A (**65**) was the first β-lactam isolated from a Bacteroidetes strain (*Flexibacter* sp. ATCC 35208). It is a desmethoxy monobactam, in which the terminal β-lactam ring is attached to a branched tetrapeptide comprising D-alanine, *N*-methyl-L-serine, glycine and L-2,3-diaminopropionic acid, with an *N*-terminal D-glyceric acid (Fig. 22). This compound showed weak antimicrobial activity against gram-positive bacteria (MIC = 6–25 μg mL^{-1}) and no activity against gram-negative bacteria. Enzymatic analysis confirmed resistance against narrow-spectrum β-lactamases (P99 and TEM-2) but slight susceptibility to extended-spectrum β-lactamase K1. SQ 28332 is a weak inhibitor of the P99 cephalosporinase and does not inhibit TEM-2 or K1.[104]

6.1.2 SQ 28502 and SQ 28503. The Squibb screening assay also identified desmethoxy monobactams decorated with an oligopeptide side chain (SQ 28502 and SQ 28503), which were isolated from *Flexibacter* sp. ATCC 35103. These compounds are more complex than SQ 28332 and their molecular weight exceeds 1400,[105] but their precise structures are not yet known. Both compounds resist hydrolysis by β-lactamases TEM-2, K1 and P99 and indeed strongly inhibited P99 in a time- and concentration-dependent manner (TEM-2 and K1 were not inhibited). SQ 28502 and SQ 28503 showed weak activity against both gram-positive and gram-negative bacteria with SQ 28503 showing the greatest potency.[105]

6.1.3 PB-5266 A–C. Another series of desmethoxy monobactams was isolated from *Cytophaga* sp. ATCC 43843.[106,107] The three compounds PB-5266 A–C (**66–68**, Fig. 23) are structurally similar to SQ 28332 (**65**), but the terminal β-lactam ring is attached to a dipeptide, decorated with an N-terminal D-glyceric acid. All three structures also contain a dehydroasparagine residue. Structural variation can be observed in the second amino acid of the dipeptide, which is an Ala in **66**, Ser in **67** and Gly in **68**. The three compounds weakly inhibit an *E. coli* mutant with increased sensitivity to β-lactam antibiotics.[106]

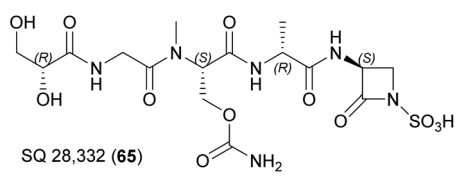


Fig. 22 Chemical structure of SQ 28332 (**63**).

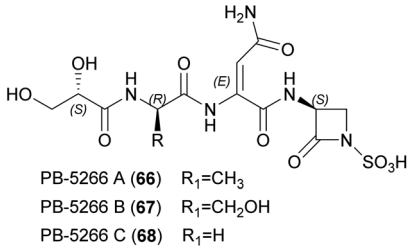


Fig. 23 Chemical structures of PB-5266 A–C (**64–66**).

6.1.4 Formadicins A–D. Formadicins A–D (**69–72**, Fig. 24) are produced by *Flexibacter alginoliquefaciens* sp. nov. YK-49.[108] Similar to the nocardicidin-type monocyclic β-lactams, the β-lactam ring of the formadicins includes a phenylacetic acid group, contrasting with formadicins from the other Bacteroidetes monobactams (which are *N*-sulfonated). In addition, formadicins A (**69**) and B (**70**) are glycosylated β-lactams, carrying a β-D-glucuronic acid. This sugar, attached to the phenylacetic acid, is not present in formadicins C (**71**) or D (**72**). The formadicins also contain a rare formylamino group, from which they get their name. This group is bound directly to the β-lactam ring in formadicins A (**69**) and C (**71**) but to the side chain in formadicins B (**70**) and D (**72**).[109] Similar substitutions on the β-lactam ring confer resistance to hydrolysis by β-lactamase, as exemplified by the methoxylated cephamycins. Accordingly, formadicins A (**69**) and C (**71**) were strongly resistant to hydrolysis by penicillinases and cephalosporinases, but formadicins B (**70**) and D (**72**) were not.[108] The formadicins showed antibacterial activity in a narrow spectrum, particularly against *Pseudomonas*, *Proteus* and *Alcaligenes* species. Formacidin C (**71**) was the most potent (MIC ≥ 3.13 μg mL^{-1}). Competitive binding with labeled benzylpenicillin revealed that formadicins A (**69**) and C (**71**) bind with stronger affinity than the other formadicins to *Pseudomonas aeruginosa* penicillin-binding proteins 1a and 1b and offered greater protection to mice against infections with *E. coli* and *Proteus vulgaris*.[109]

6.2 Cephems

6.2.1 Desacetoxycephalosporin C. Desacetoxycephalosporin C (**73**, Fig. 25) is an intermediate in the

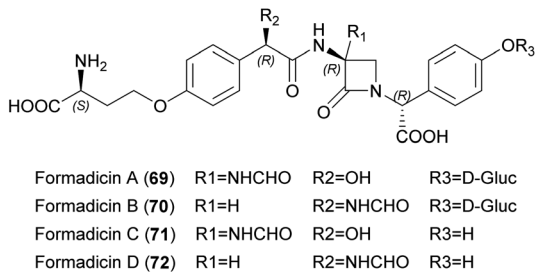


Fig. 24 Chemical structures of formadicins A–D (**67–70**).

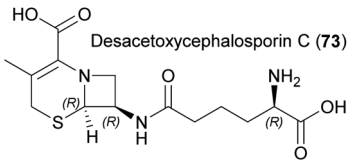


Fig. 25 Chemical structure of desacetoxycephalosporin C (**71**).

synthesis of cephalosporin C produced by several fungi and *Streptomycetes* strains. Its synthesis requires the enzyme deacetoxycephalosporin-C synthase, which converts penicillins into cephalosporins.[110] This intermediate was also found in the culture broth of *Flavobacterium* sp. SC 12,154, revealing that Bacteroidetes can also synthesize more complex β-lactams, including those with annulated ring systems.[111]

6.2.2 7a-Formylaminocephalosporins (SQ 28516 and SQ 28517). *Flavobacterium* sp. SC 12154 also produces the 7a-substituted cephalosporins SQ 28516 (**74**) and SQ 28517 (**75**).[112] These β-lactam ring substitutions increase resistance to β-lactamase hydrolysis. The β-lactam ring in these compounds also features a rare formylamino structure (Fig. 26) like that found in the monocyclic formadicins (**69** and **71**). SQ 28516 and SQ 28517 are structurally unstable, so they were isolated as acetylated derivatives. The proposed structure of SQ 28516 carries a carboxylic group, whereas the minor fermentation product SQ 28517 is cationic at acidic pH and probably carries an amide instead. SQ 28516 showed no antibacterial activity and its ability to inhibit β-lactamase has not been reported.

6.2.3 Chitinovorins A–D. Chitinovorins are 7a-formylaminocephems in which an α-aminoadipic acid group is attached to the β-lactam ring. Chitinovorins A–D (**76–79**) differ in the nature of the substitutions in their six-membered dihydrothiazine ring (Fig. 27). Chitinovorin C (**78**) is the simplest, featuring a single hydroxymethyl group. In the other chitinovorins, this is expanded by an ester-linked peptide. In chitinovorin A, the side chain consists of a guanidine-containing building block and an Ala residue, whereas in chitinovorin B this expands to two Ala residues (**77**).[113] Chitinovorin D (**79**) is the largest, with two additional N-terminal constituents.[114] Chitinovorins A (**76**) and B (**77**) showed very weak antimicrobial activity (MIC = 25–50 μg mL^{-1}),[113] and this was even lower for chitinovorins C (**78**) and D (**79**).[114]

7 Additional Bacteroidetes natural products

7.1 Pigments

The best-known Bacteroidetes pigments are flexirubin-like compounds,[115] which are phylogenetically conserved and were used as chemotaxonomic markers for the phylum (formerly known as the *Cytophaga–Flavobacterium–Bacteroides* group) upon their discovery in *Chitinophaga filiformis* (formerly *Flexibacter elegans* Fx e1).[116] The total synthesis[117] and biosynthesis[118] of these pigments has been reviewed.[115] Flexirubin analogs have been evaluated for their antioxidant activity.[119] Other pigments, found in many Bacteroidetes, include carotenoids such as saproxanthin, myxol, zeaxanthin, flexixanthin and deoxyflexixanthin, some of which also possess antioxidant activity.[120] The flexixanthin BGC has been identified.[121]

7.2 Siderophores

Siderophores are iron-chelating compounds. Hydroxamate siderophores (tenacibactins A–D and bisucaberin B) have been isolated from *Tenacibaculum* spp.,[122,123] and fulvivirgamides from *Fulvivirga* sp. W222.[124] The bisucaberin B and fulvivirgamid BGCs have been experimentally validated.[123–125]

7.3 Lipids

Chryseobacterium spp. produce sulfonolipids (sulfobacin A and B) that are von Willebrand factor (vWF) receptor antagonists[126] and potential anti-inflammatory[127] and anticarcinogenic[128] leads. Flavocristamides A and B (whereby flavocristamide B is structurally identical to the before mentioned sulfobacin A) were isolated from a marine *Flavobacterium* species and were shown to inhibit DNA polymerase α.[129] Other sulfonolipids (the capnoids capnine and *N*-acylcapnine)[130] were shown to be required for gliding motility in *Capnocytophaga* spp.[131] The *N*-acetylated sulfonolipids RIF-1 and RIF-2 (rosette inducing factors) and IOR-1 (inhibitor of rosettes) isolated from *Algoriphagus machipongonensis* were shown to influence morphogenesis in the choanoflagellate *Salpingoeca rosetta*.[132] The total synthesis[133] and biosynthesis[134] of most of these sulfonolipids has been investigated.

Other than sulfonolipids, the major lipids isolated from Bacteroidetes include lipoamino acids (LAAs), phospholipids

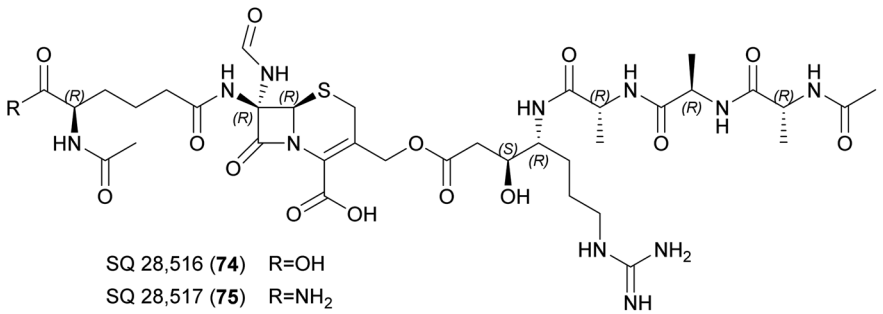


Fig. 26 Chemical structures of SQ 28516 (**72**) and SQ 28517 (**73**).

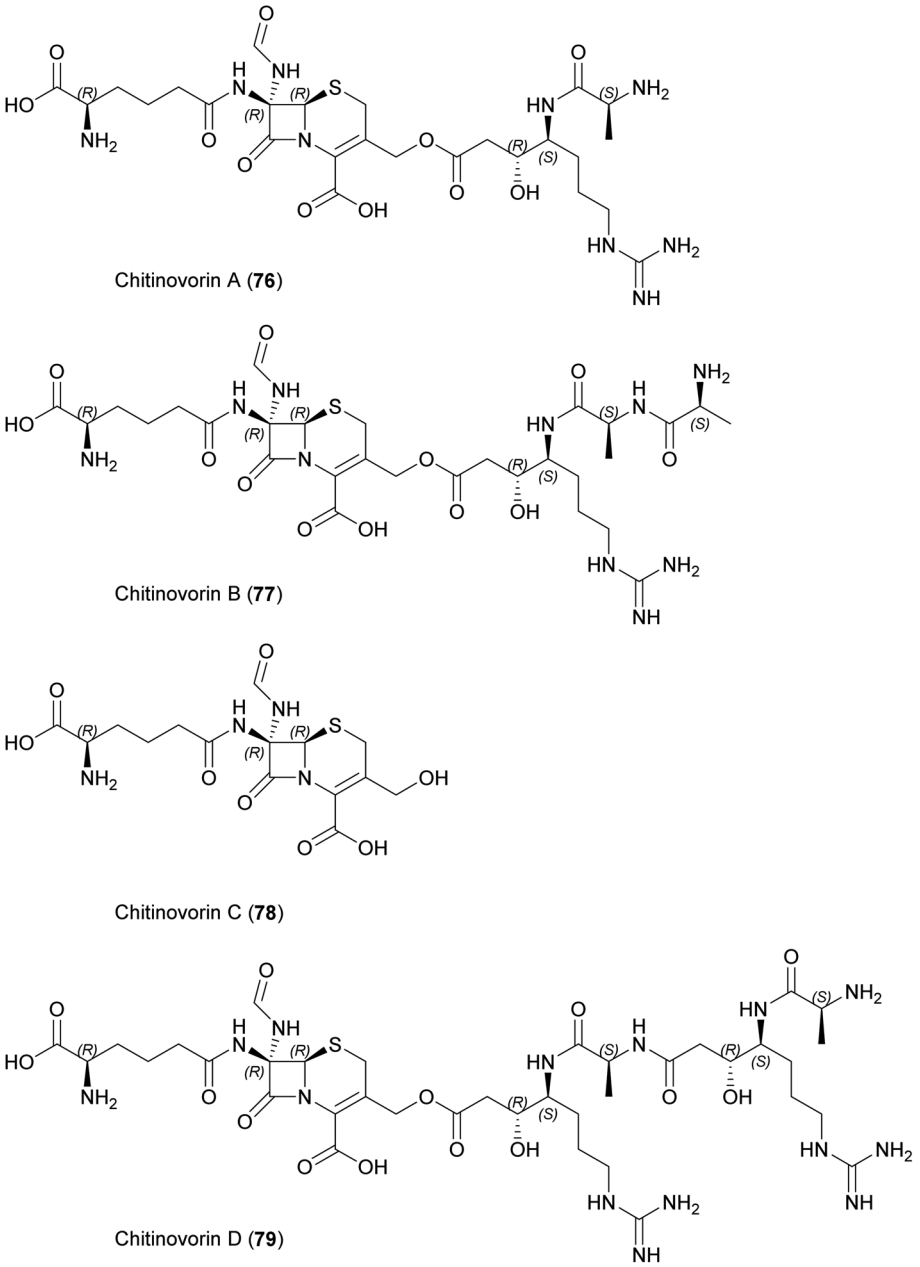


Fig. 27 Chemical structures of chitinovorins A–D (74–77).

and *N*-acyl amino acids (NAAAs). LAAs consist of a single de-esterified fatty acid or two fatty acids linked by esterification at C3 with different degrees of unsaturation. The hydrophobic fatty acid part is linked *via* an amide to Gly, Ser and ornithine, or a combination of two or three of these amino acids. The physicochemical properties and bioactivities differ significantly between stereoisomers in many cases, including WB-3559 A–D;[135] the topostins B553, B567 (also known as cytolipin), D640 and D654 (also known as WB-3559D);[136–138] and flavolipin[139,140] (also known as lipid 654 (ref. 141 and 142).[143] In terms of biological properties, these LAAs act as mammalian DNA topoisomerase I inhibitors,[136,137] macrophage activators,[144] promoters of hemagglutination,[140] bacterial virulence factors,[145,146] N-type calcium channel blockers,[147] and ligands for Toll-like receptor 2.[141,142,148] Accordingly, they are linked to the development of two chronic inflammatory diseases (periodontitis and atherosclerosis[146]), multiple sclerosis,[149] as well as showing antimicrobial activities.[150]

7.4 Others

Other diverse compounds produced by Bacteroidetes include indole analogs. For example, *Cytophaga* strain AM13.1 produces 2,5-bis(3-indolylmethyl)pyrazine and pharacine, a natural *p*-cyclophane.[151] *Cytophaga johnsonae* AJ 12589 produces resorcinin, which stimulates the growth of NIH 3T3 mouse fibroblasts.[152] Furthermore, four neoverrucosane diterpenoids were

identified as products of *Saprospira grandis* ATCC 23116,[153] whereas the algal morphogen thallusin is produced by a *Zobellia* strain and has been synthesized.[154]

8 Concluding remarks

The phylum Bacteroidetes produces a wide range of metabolites that highlight its status as a valuable resource for natural products research. Although the isolation of interesting compounds from Bacteroidetes traces back to the golden era of natural product discovery (1950–1960), the total number of chemical entities is still remarkably low.

The recent identification of novel natural products is exemplified by the isopedopeptins and chitinopeptins, supporting the hypothesis that the natural product diversity in this phylum has yet to be exploited comprehensively. Of outstanding significance are the isopedopeptins, which show potent activity against gram-negative bacteria on the WHO's top-priority list, a low frequency of resistance, and acceptable levels of cytotoxicity, making them promising new candidate antibiotics.[38,44] The β-lactams are a more historical (but still extremely relevant) class of natural products that were isolated from Bacteroidetes in the 1980s. Their clinical use as antibiotics that inhibit bacterial penicillin-binding proteins and also act as β-lactamase inhibitors with the potential to break AMR is of great importance to international healthcare. Resistance to β-lactam antibiotics and fluoroquinolones is a major societal burden that accounts for more than 70% of all deaths attributed to AMR.[1] The β-lactams are still an essential drug class and a cornerstone of the current antibiotics market. Therefore, we must develop new solutions to counteract the steady loss of existing products. The naturally evolved strategy of combining β-lactam antibiotics with a β-lactamase inhibitor sharing the same warhead is therefore crucial to address the AMR crisis. There is a constant need for the development new β-lactamase inhibitors that maintain or even expand the antibacterial range of our clinical β-lactam/β-lactamase inhibitor combinations.[155] Although not directly pursued as a therapeutic strategy, we also consider β-lactams from the Bacteroidetes as very much part of this success story in learning from naturally evolved concepts and translating microbial diversity (and the corresponding structural and functional diversity of natural products) into life-saving drugs. Accordingly, the Bacteroidetes join other β-lactam producers such as the Actinobacteria, Proteobacteria and fungi.

Even compounds not followed up in the past such, as the highly active but unstable ariakemicin, may be suitable as candidates for repurposing strategies that aim to generate more applicable structures based on a natural scaffold. Following this approach, our recent omics studies revealed the immense genomic potential of this phylum that corresponds to uncharted chemical space, which differs significantly from the more widely investigated producer taxa.[14] In our opinion, this pronounced and unique biosynthetic capacity offers the potential for groundbreaking new compounds in the near future. Considering their historically proven role in the discovery of structurally diverse natural products, the Bacteroidetes have not received the attention they deserve during the latest renaissance in natural products research. It is therefore time to reconsider the phylum Bacteroidetes as a source for the discovery of interesting and valuable natural products to fill the future drug development pipeline.

9 Conflicts of interest

There are no conflicts to declare.

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