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Natural Products as Inspiration for the Development of Bacterial Antibiofilm Agents

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Abstract

Natural products have historically been a rich source of diverse chemical matter with numerous biological activities, and have played an important role in drug discovery in many areas including infectious disease. Synthetic and medicinal chemistry have been, and continue to be, important tools to realize the potential of natural products as therapeutics and as chemical probes. The formation of biofilms by bacteria in an infection setting plays a significant factor in the recalcitrance of many bacterial infections, conferring increased tolerance to many antibiotics and to the host immune response, and as yet there are no approved therapeutics for combatting biofilm-based bacterial infections. Small molecules that interfere with the ability of bacteria to form and maintain biofilms can overcome antibiotic tolerance conferred by the biofilm phenotype, and have the potential to form combination therapies with conventional antibiotics. Many natural products with anti-biofilm activity have been identified from plants, microbes, and marine life, including: ellagic acid glycosides, hamamelitannin, carolacton, skellamycins, promysalin, phenazines, bromoageliferin, flustramine C, meridianin D, and brominated furanones. Total synthesis and medicinal chemistry programs have facilitated structure confirmation, identification of critical structural motifs, better understanding of mechanistic pathways, and the development of more potent, more accessible, or more pharmacologically favorable derivatives of anti-biofilm natural products.

Graphical Abstract

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Conflicts of interest

Dr. C. Melander is co-founder of Agile Sciences, a biotechnology company seeking to commercialize anti-biofilm compounds.

Introduction

Natural products are molecules produced by a biological source, and include secondary metabolites, molecules that are not directly involved in growth, development or reproduction, and are not essential for survival under normal conditions. Instead, they typically provide competitive advantage, or mediate interaction between and/or defense against other organisms. The biosynthesis of natural products is often unique to a specific organism and the environment within which it resides. Although the number of building blocks are limited, the number of novel secondary metabolites is almost infinite.¹

Natural products have long been a source of therapeutic entities for a myriad of disease areas including: oncology, neurological disease, cardiovascular and metabolic disease, immunological and inflammatory diseases, and infectious disease.² Natural products have played a particularly predominant role in the identification of antibacterial compounds, from the discovery of penicillin, produced by fungi of the genus *Penicillium*, in 1928,³ to the approval of fidaxomicin, a first in class macrocyclic antibiotic produced by the actinomycete *Dactylosporangium aurantiacum*, in 2011⁴. Nature continues to be an important source of antibacterial agents; of the 126 small molecule antibacterial agents approved between January 1981 and September 2019, eleven were natural products, and 78 were natural product derivatives.⁵

Much of the reason for the utility of natural products stems from the fact that they typically occupy chemical space not usually found in synthetic-based screening libraries. They are typically chemically more complex than synthetic “drug-like” molecules, possessing a greater proportion of sp^3 -hybridized bridgehead atoms and chiral centers, a lower nitrogen but higher oxygen content on average, a greater number of aliphatic versus aromatic rings, and accordingly, a greater degree of three-dimensionality.⁶

Chemical synthesis has played an important role both in the development of natural products for biomedical applications, and in their use as chemical tools to answer biological questions and identify new targets. Total synthesis of natural products in some cases enables the

acquisition of sufficient quantities of material that cannot be accessed from the source organism. In other cases, total synthesis has been utilized to confirm the structure of natural products.^{7, 8} Synthetic chemistry has been used to identify structural motifs within the natural product that are required for activity, and medicinal chemistry campaigns have utilized this information to develop analogues with improved activity, reduced toxicity, more desirable pharmacological properties, or that are more readily accessible.

Many natural products have been investigated both for the ability to disrupt bacterial biofilms, or to sensitize biofilm cells to conventional antibiotics. These compounds continue to serve as inspiration for the development of novel antibiofilm agents with improved activity and pharmacological profiles. The development of synthetic strategies to access active natural products and their analogues is important if the potential of these scaffolds, both as chemical probes and therapeutic entities, is to be harnessed. In this review, we discuss the role of chemical synthesis in the identification and development of natural products for combatting bacterial biofilms. We give a brief background explaining biofilms and their role in infection and antibiotic resistance. We describe natural products isolated from plants, microbes, and marine organisms that have demonstrated anti-biofilm activity, and for select examples, describe how chemistry has played a role in their study, development and application. A summary of activities of the featured natural products is shown in Table 1. This review is not meant to be a comprehensive description of all natural product and natural product-derived compounds with anti-biofilm activity, several other reviews have covered this area,^{9–12} but is intended to be an analysis of the impact of chemical synthesis in both developing therapeutic strategies to overcome biofilm-based bacterial infections, and in answering fundamental biological questions about the biofilm phenomenon.

Bacterial biofilms

Biofilms are sessile, surface associated, heterogeneous structures formed by multicellular communities of bacteria (and other microorganisms).¹³ Bacteria form biofilms in many environments in response to a number of external and internal stimuli. The ability to form biofilms is a universal attribute of bacteria,¹⁴ and biofilms play a role in a number of infections including: infection of indwelling medical devices, wound infections, bacterial endocarditis, otitis media, dental carries, and lung infections of cystic fibrosis patients.⁹ Bacteria within a biofilm are better able to evade the host immune response, and can display upwards of 1000-fold decreased sensitivity to antibiotics compared to planktonic bacteria,¹⁵ which contributes to the recalcitrance of biofilm based infections towards traditional approaches to eradication. The antibiotic tolerance exhibited by bacteria residing within a biofilm can render antibiotics ineffective even against bacterial strains that do not harbour genetic resistance determinants. Tolerance differs from antibiotic resistance in that it involves a reduction in the rate of antibiotic-induced killing of a whole bacterial population in comparison to cultures of the strain from which the tolerant population was derived.¹⁶ It is important to note that cells displaying biofilm mediated antibiotic tolerance will exhibit susceptibility to the antibiotic upon dissociation from the biofilm.¹⁷

There are several factors responsible for the decreased sensitivity of biofilm cells to antibiotic therapy. One of the most significant factors is the presence of the biofilm matrix. This self-produced matrix, which is predominantly comprised of extracellular polymeric substances (EPS) including carbohydrates, proteins, lipids and extracellular DNA, forms the three-dimensional architecture of the biofilm, and performs several functions. These include: providing mechanical stability, mediating adhesion, enabling transportation of nutrients and waste within the biofilm, and providing protection against desiccation, ultraviolet radiation, some predators, metallic cations, host immune defences, and biocides. The matrix also limits penetration of certain antibiotic classes into the biofilm^{18–20} Another contributing factor to antibiotic tolerance in a biofilm setting is the fact that nutrient and oxygen levels within a biofilm vary both spatial and temporally, leading to varied rates of microbial growth. Given that most conventional antibiotics target biosynthetic processes that only occur in actively growing bacteria and display limited efficiency in killing non-multiplying bacteria, cells within the biofilm exhibiting reduced metabolic activity show greater tolerance to such antibiotics.^{18, 21–23} Bacterial cells within a biofilm also exhibit altered gene expression compared to planktonic cells, including genes that mediate antibiotic resistance such as efflux pumps.²⁴ Increased mutation rates and the presence of hypermutable strains as a result of oxidative stress in the biofilm environment,^{25, 26} and increased horizontal gene transfer as a result of high population density^{27, 28} additionally contribute to increased rates of resistance development and spread. Finally, the presence of persister cells, cells that are genetically homogeneous to the rest of the population yet phenotypically heterogeneous and exhibit antibiotic tolerance through a mechanism generally thought to be a result of adopting a state of dormancy in which they become metabolically inactive, is also thought to contribute to the lack of efficacy of many antibiotics toward biofilm-based infections.^{29–31} All the above mentioned tolerance mechanisms make the eradication of biofilm-based infections a considerably challenging endeavour.³²

Current clinical approaches for the treatment of biofilm-based bacterial infections are severely limited and dependent upon causative agent and infection site. Select antibiotics have demonstrated some activity against bacterial biofilms, for example polymyxin antibiotics such as colistin, which does not depend upon cells being metabolically active to effect bacterial killing. However, colistin typically has to be administered as part of a combination regimen due to rapid resistance acquisition by metabolically active cells within the biofilm.¹⁵ The RNA polymerase inhibitor rifampin has also been reported to have some activity against biofilm cells, namely staphylococcal biofilms,³³ but again is not recommended as monotherapy due to rapid selection of resistant mutants.³⁴ Other current treatment approaches include: removal of colonized foreign bodies, surgical debridement and aggressive antibiotic therapy,^{35, 36} drainage of abscesses, or long-term high dose combination antibiotic regimens. These approaches often still result only in biofilm reduction rather than eradication, and continued chronic suppressive treatment is required.^{35, 37} Another issue with the use of conventional antibiotics is the fact that several studies have shown that the presence of sub-inhibitory levels of several commonly prescribed antibiotics can induce *in vitro* biofilm formation. Such antibiotic levels occur not only at the Beginning and end of a dosing regimen, but importantly also in areas of the biofilm that antibiotics cannot readily access as a result of limited penetration.³⁸

Alternative approaches to combatting biofilms involve the identification and development of compounds that act specifically on biofilm cells, typically in one (or more) of three ways: 1) inhibition of biofilm formation (to be classified as an anti-biofilm compound this should ideally occur with no or minimal toxicity toward planktonic bacteria), 2) dispersion of pre-formed biofilms, and 3) killing of biofilm cells (either selectively or in addition to planktonic toxicity). Such compounds have the potential to act in an adjuvant manner and be paired with conventional antibiotics, as has been successfully achieved with the pairing of β -lactamase inhibitors with β -lactam antibiotics such as Augmentin, and would be a valuable therapeutic option to overcome biofilm-based infections. Many small molecules with anti-biofilm activity have been identified, either through rational design to interfere with one of the many processes essential for the formation and maintenance of biofilms, or as in the case of many of the natural products detailed in this review, through phenotypic screening.⁹

Many of the anti-biofilm small molecules for which the mechanism of action is known, including some natural products, act by interfering with one or more of the bacterial signaling and communication systems that regulate biofilm formation and maintenance.³⁹ Some of these are analogues of the native signaling molecules, while others are based upon distinct scaffolds. Probably the most well characterized bacterial communication systems are the quorum sensing (QS) systems utilized by bacterial communities to coordinate gene expression, and consequently phenotypes including biofilm formation, in response to changes in population density.⁴⁰ Different QS systems are employed by different bacterial species, such as acyl homoserine lactone (AHL) based systems found in Gram-negative bacteria,⁴¹ autoinducing peptides (AIP) based systems found in Gram-positive bacteria,^{42, 43} and the universal autoinducer-2 (AI-2) based system employed by both Gram-negative and Gram-positive bacteria.^{44, 45} The different QS systems act through the same basic premise, which is centered upon release and detection of small diffusible signaling molecules known as autoinducers. QS systems comprise two primary proteins: a synthase that produces the autoinducer in response to population changes and environmental signals, and a receptor protein that enacts a response (change in gene expression) upon binding of the autoinducer above a threshold level.⁴⁶ Both the synthase and receptor, as well as many auxiliary proteins involved in QS systems, represent potential targets for modulating biofilms. Two-component system (TCS) signaling also plays a role in biofilm formation and regulation (in addition to many other phenotypes) across many bacterial species by allowing the bacterium to sense and respond to environmental changes. TCS consist of a sensor histidine kinase that responds to an extracellular stimulus and, through a series of phosphorylation events, activates the cognate DNA-binding response regulator, leading to a change in gene expression.⁴⁷ Both the two primary proteins of TCS, and many auxiliary proteins can be targets for controlling biofilm formation. Other signaling pathways that are involved in biofilm regulation and have been targeted as an anti-biofilm strategy include: the diguanylate cyclase (DGC) and phosphodiesterase (PDE) pathway that utilizes the second messenger bis-(3'5')-cyclic di-guanylic acid (c-di-GMP),⁴⁸ indole signaling pathways,^{49–51} and long chain fatty acid signaling pathways.⁵²

Plant natural products as antibiofilm agents

Plants have long been a source of natural products with a myriad of biological activities. Most plants produce hundreds to thousands of unique phytoalexins and other secondary metabolites belonging to various chemical families including: flavonoids, phenols, terpenoids, furanoacetylenes, steroid glycoalkaloids, stilbenes, sulfur-containing compounds and indoles.⁵³ Plant natural products serve purposes such as self-defense, and interaction with other organisms in the environment.⁵⁴ The biological utility of phytochemicals has been recognized and exploited for centuries in the practice of traditional medicine, where plant extracts have been used to treat numerous ailments, and medicinal plant usage remains widespread today.⁵⁵

Despite the spectrum of biological activities that plant natural products exhibit, there are no approved plant-derived antibiotics - most plant-derived compounds have only weak antibiotic activity that is several orders of magnitudes weaker than that of microbially produced antibiotics.⁵⁶ Yet, particularly in their natural setting, plants are generally able to successfully mount a defense against infecting organisms. This is believed to be a result of the fact that many plant natural products possess anti-virulence properties, comprising the main category of anti-infective activity identified from this source.⁵⁴ Several plant natural products that exhibit antibiofilm activity have been reported, as described below.

Flavonoids are a large, structurally diverse group of polyphenolic natural products that, along with semi-synthetic and synthetic derivatives, have been extensively studied for their antibacterial activity.⁵⁷ Baicalin (5,6-dihydroxy-7-*O*-glucuronide flavone) **1** (Figure 1), isolated from the roots of *Scutellaria baicalensis*, has a long history of use in Chinese medicine and has been reported to increase the susceptibility of *Burkholderia cenocepacia* biofilms to tobramycin.^{58, 59} This activity was initially thought to be a result of QS inhibition,⁵⁸ but was later revealed to be due to modulation of the oxidative stress response. It was shown that baicalin affects multiple pathways, including oxidative phosphorylation, ultimately leading to increased reactive oxygen species (ROS) production in the presence of tobramycin, which accounts for increased tobramycin-mediated killing of biofilm cells.⁵⁹ Naringenin **2**, and quercetin **3**, are found in many plants and have been shown to act as QS antagonists in *Vibrio harveyi*, and to inhibit biofilm formation by *V. harveyi* and *Escherichia coli*.⁶⁰ Phloretin **4**, a dihydrochalcone intermediate of the biosynthetic pathway of flavonoids found in apple peel and strawberries,⁶¹ reduces biofilm formation by enterohemorrhagic *E. coli* (EHEC) without affecting either planktonic growth or biofilms of commensal *E. coli*, and is thought to act via the repression of AI-2 importer genes.⁶² Proanthocyanidins, represented by procyanidin A2 **5**, a class of flavonoids that are produced by cranberry plants, have been reported to inhibit biofilm formation by *Pseudomonas aeruginosa* and to potentiate gentamicin activity in a *Galleria mellonella* model of *P. aeruginosa* infection.⁶³

The stilbenoid resveratrol **6**, (found in the skin of grapes and berries) inhibits biofilm formation by *Vibrio cholerae*,⁶⁴ while this and the related oxyresveratrol **7** also inhibit biofilm formation by uropathogenic *E. coli* (UPEC) at low concentrations (10–50 µg/mL) by reducing fimbriae production and swarming motility.⁶⁵ The triterpene ursolic acid **8** (found in many plants, in particularly large quantities apple peel), inhibits *E. coli* biofilm formation

and upregulates genes involved in biofilm related processes such as chemotaxis and motility.⁶⁶ The diterpene 4-epi-pimaric acid **9**, which is produced by *Aralia cachemirica* L. (Araliaceae) inhibits biofilm formation by *Streptococcus mutans*, an oral pathogen implicated in dental carries and endocarditis.⁶⁷

Garlic extracts have been reported to increase the susceptibility of *P. aeruginosa* biofilms to tobramycin *in vitro*, and to clear *P. aeruginosa* lung infections *in vivo* when used prophylactically.⁶⁸ The active compounds are thought to be ajoenes **10** and **11**, which exert their effects through modulation of quorum sensing.^{69, 70} Another sulfur containing phytochemical that has been reported to display anti-biofilm activity is the isothiocyanate iberin **12**, produced by horseradish, which inhibits biofilm formation by *P. aeruginosa*, potentially by inhibiting the Gac/Rsm quorum sensing systems.⁷¹

Ellagic acid glycosides

Rubus ulmifolius Schott. (Rosaceae) is a plant that produces edible berries, and has also traditional medicine applications for the treatment of skin and soft tissue infections (SSTI).⁷² The anti-staphylococcal activity of *R. ulmifolius* was validated by Quave et al. as part of a study to evaluate extracts of south Italian plants for antibacterial and anti-biofilm activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The ethanolic extract of the roots of this plant were reported to inhibit biofilm formation with an IC₅₀ of 8 µg/mL, significantly lower than its minimum inhibitory concentration (MIC) of 512 µg/mL.⁷³ Subsequent bioassay-guided fractionation of the ethanolic root extract identified fraction 220D-F2, which inhibited biofilm formation across a panel of genotypically-diverse clinical *S. aureus* isolates and also enhanced the susceptibility of biofilms to the functionally distinct antibiotics daptomycin, clindamycin and oxacillin. This fraction was also reported to reduce pre-formed biofilms of *Streptococcus pneumoniae* as well as display antibiotic activity against planktonic cultures of this bacterium.⁷⁴ Fraction 220D-F2 was subject to LC-UV/MS/MS analysis to identify the active chemical architecture responsible for anti-biofilm activity, revealing the presence of ellagic acid **13** (Scheme 1) along with several ellagic acid derivatives, in addition to sapogenin-related compounds.⁷⁵

While mass spectrometry was able to confirm the presence of generic xylopyranoside or xylofuranoside ellagic acid as well as mannopyranoside ellagic acid, this technique is unable to determine the identity of the sugar, anomeric configurations, nor the site of glycosylation. To address this ambiguity, the Weinert and Quave labs synthesized **15** and **16**, and examined their anti-biofilm activity. Ellagic acid itself is extremely insoluble and somewhat unreactive, thus per-*O*-acetyl α-glycosyl iodide donors, generated from reaction of per-*O*-acetyl sugars with trimethylsilyl iodide (TMSI), were used for the preparation of ellagic acid glycosides. The regioselectivity of glycosylation was ensured by first protecting ellagic acid **13** as its per-*O*-*t*-butyldimethylsilyl ether **14** (Scheme 1), followed by selective tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF) mediated deprotection at the 3-position to afford the 3-monophenolate *in situ* (the selectivity is thought to be mediated by inductive effects of the proximal endocyclic lactone oxygen). Glycosylation of this intermediate with α-L-rhamnopyranosyl iodide or α-D-xylopyranosyl iodide afforded protected α-rhamnopyranoside ellagic acid and β-xylopyranoside ellagic acid. For the

formation of the xyloside derivative, it was necessary to carry out the reaction in the presence of tetrabutylammonium iodide (TBAI) to promote *in situ* anomerization of the sugar to the more reactive β -iodide which, following iodide displacement by the adjacent acetoxy group, ensured generation of solely the β -xyloside upon reaction of the acetoxonium intermediate with protected ellagic acid. De-Osilylation of the glycosides with K_2CO_3 in wet DMF followed by deacetylation with K_2CO_3 in MeOH/H₂O, followed by treatment with cation-exchange resin afforded ellagic acid 3-O-rhamnoside **15** and ellagic acid 3-O-xyloside **16** which were used as LCMS standards to confirm occurrence of both in the 220D-F2 extract.⁷²

Ellagic acid rhamnoside **15** inhibited biofilm formation with a 50% minimum biofilm inhibitory concentration (MBIC₅₀) of 64 μ g/mL, compared to 128 μ g/mL for ellagic acid and 25 μ g/mL for 220D-F2 against the strain used in this study, and exhibited lower growth inhibition than 220D-F2. Ellagic acid xyloside **16** exhibited similar biofilm inhibition activity to **15** but much more potent growth inhibition. The authors posit that the high anti-biofilm activity and limited growth inhibition of ellagic acid rhamnoside suggest that it is a key component of 220D-F2 in the context of antibiofilm activity. Confocal laser scanning microscopy (CLSM) revealed similar biofilm architecture and levels of attachment upon treatment with **15** to that observed following treatment with 220D-F2.⁷²

The ellagic acid glycoside scaffold has been further explored by the Townsend lab via a synthetic approach that centers around *de novo* ellagic acid formation utilizing a copper mediated Ullmann coupling and phenolic *O*-glycosylation. This approach can provide access to novel ellagic acid derivatives and potentially allow for incorporation of aryl moieties bearing unnatural substitutions. The synthesis was initiated by per-*O*-benzylation of methyl gallate **17** followed by electrophilic aromatic bromination with NBS. Ullmann coupling of the resultant aryl bromide in the presence of excess copper at elevated temperature delivered biaryl **18**. Debenzylation and subsequent bis-lactonization provided ellagic acid, which was per-*O*-silylated with triisopropylsilyl chloride to give **19**. As in the Weinert synthesis, the ellagic acid glycosyl acceptor was generated via selective de-*O*-silylation at the 3-position *in situ* followed by reaction of the quaternary ammonium salt of **19** under Gervay-Hague conditions with the desired glycosyl iodide and TBAI at elevated temperature. Finally, a one-pot, two-step global deprotection afforded both the previously reported ellagic acid xyloside and rhamnoside in addition to the arabinose derivative **20**.⁷⁶

The synthetic ellagic acid glycosides displayed modest antibiofilm activity against the Gram-positive pathogen *Streptococcus agalactiae* (Group B Streptococcus, GBS). Arabinoside **20** was the most active of the three, and was shown by scanning electron microscopy (SEM) to act via early-stage inhibition of cellular adhesion.⁷⁶

Hamamelitannin

Hamamelitannin (2',5-di-*O*-galloyl-D-hamamelose) **21** (Scheme 2 is a component of the bark of *Hamamelis virginiana* (witch hazel) that has been reported as an inhibitor of TNF-mediated endothelial cell death and DNA fragmentation,⁷⁷ and to display specific cytotoxic activity against colon cancer cells⁷⁸. Hamamelitannin was identified as an analogue of the *S*.

aureus quorum sensing inhibitor RNAIII-inhibiting peptide (RIP) through a virtual screen of commercially available small molecules.⁷⁹ RIP mediates biofilm formation by inhibiting phosphorylation of TRAP (target of RNA-III activating protein) and subsequent expression of the *agr* operon.⁸⁰ When combined with vancomycin, hamamelitannin has been reported to effect a significant increase in killing of biofilm cells *in vitro* in several biofilm model systems.

Hamamelitannin has also been reported to increase the susceptibility of *S. aureus* biofilm cells to antibiotics from other classes including: several β -lactams, daptomycin, linezolid, tobramycin, and fusidic acid without affecting their activity toward planktonic cells⁸¹. *In vivo*, the combination of hamamelitannin and vancomycin significantly enhanced survival compared to either compound alone in both *Caenorhabditis elegans* and *G. mellonella* infection models,⁵⁸ as well as in a mouse mammary gland infection model. Additionally, hamamelitannin has been shown to prevent device-associated infections in a rat graft model of both *S. aureus* and *S. epidermidis* infections.⁷⁹ The TraP receptor has been reported to be involved in the mechanism by which hamamelitannin sensitizes *S. aureus* biofilms, by affecting cell wall synthesis and decreasing its thickness, and by mediating extracellular DNA release.⁸¹

Hamamelitannin has been utilized as a scaffold for the development of novel vancomycin potentiators against staphylococcal biofilms. Given the absence of any structural information regarding the inhibitor–target interaction, much of the work has involved iterative bioactivity guided analogue design, exploiting synthetic and medicinal chemistry to not only identify compounds with greater activity, but also potentially providing information about target interactions.

Initially aiming to address the liabilities within the hamamelitannin scaffold with regards to therapeutic potential, namely the high polarity due to the large number of hydroxyl group, which might compromise bioavailability, the propensity of aromatic hydroxyl groups to undergo oxidation and glucuronidation, and the potential instability of the ester linkages, Vermote et al. synthesized a series of hamamelitannin analogues (Scheme 2).⁸² In this study, three modifications to the hamamelitannin scaffold were explored: isosteric replacement of the ester groups, systematic modification or elimination of the aromatic hydroxyl groups, and removal of the anomeric hydroxyl group to replace the hamamelose core with a tetrahydrofuran ring. Library synthesis was initiated from branched azidolactol **22** and proceeded through a three-step protection-benzylation-deprotection sequence to afford **23** (along with its anomer). Intermediate **23** was converted to the bis-azido compound **24** via methanesulfonyl activation of the 2' hydroxyl, followed by azide displacement. Staudinger reduction, EDC-mediated acylation of diamine **25**, and finally deprotection provided seven amido analogues with various hydroxyl patterns on the aromatic rings. Azidolactol **22** was also utilized to generate 51 total analogues, which in addition to replacement of the ester linkers of hamamelitannin with amide linkers, the anomeric hydroxyl was also removed (two analogues with identical acyl groups at the 2' and 5-positions, and 49 analogues with a benzamide at the 2'-position and varying acyl groups at the 5-position). Reduction of **22** to triol **27** and subsequent tosylation was followed by cyclization, which proceeds to give solely the *cis*-isomer **28**. Azide displacement of the tosylate followed by reduction of the

resulting bis-azido compound **29** and subsequent acylation of the diamine **30** allowed for the generation of two analogues that contained identical acyl groups at the 2' and 5 positions. In a divergent approach, phthalimide displacement of tosylate **28** formed **31**, in which the 2' and 5-nitrogen moieties could be orthogonally revealed. Hydrazine cleavage of the phthalimide was followed by benzylation to afford **32**, which underwent azide reduction and acylation to provide a library of 49 analogues.

The effect of the hamamelitannin analogues upon the susceptibility of *S. aureus* biofilms to vancomycin both under pretreatment and combination treatment regimens was determined, revealing that ester replacement with an amide moiety is well tolerated, as is removal of the anomeric hydroxyl, and finally that removal of phenolic groups is also well tolerated. The 5-orthochlorobenzamide derivative **33** was the most active compound to emerge from the *in vitro* assay, displaying EC₅₀ values (defined as the concentration needed to double the effect of antibiotic) of 0.389 μ M and 7.976 μ M for pretreatment and treatment of preformed biofilms respectively. By comparison, methyl isostere of the ortho-chloro phenyl moiety of **33** exhibited EC₅₀ values of 13.67 μ M and 82.62 μ M, and the parent hamamelitannin exhibited EC₅₀ values of 145.5 μ M and 165.1 μ M for pretreatment and treatment of preformed biofilms respectively. Compound **33** was subsequently shown to be stable in human plasma and in the presence of both rat and human S9 microsomal fractions, and did not display cytotoxicity in MRC-5 lung fibroblast cells up to a concentration of 128 μ M. Furthermore, in both a *C. elegans* and murine infection model, the combination of compound **33** with vancomycin or cephalexin respectively effected a significantly greater reduction in CFUs compared to antibiotic alone, and the effect was greater than that observed for hamamelitannin.⁸²

To gain further insight into the structure activity relationship (SAR) of the hamamelitannin scaffold, a library of 21 derivatives was next prepared, consisting of analogues in which the 5-amido group was replaced with alternative nitrogen-based linkers, and analogues with various substituents at the ortho position of the 5-phenyl ring. Replacement of the 5-amide linker with a sulfonamide group was found to be tolerated, as seen by the comparable activity observed between compounds **34** and **35** (Figure 2), but did not improve upon the activity of **33**. 5-Benzamide ortho substitution with other halides resulted in compounds with increased activity over compound **34**, but still less potent than compound **33**. Finally, the 5-*N*-methylated analogue of **34**, compound **36**, exhibited increased activity compared to compound **34**, which the authors attribute to an out-of-plane distortion of the benzamide moiety leading to better interaction with the binding site.⁸³ In a subsequent report that centered on delineating the SAR at the 2'-position of lead compound **33**, as well as the construction of hybrid molecules in which the most active moieties at the 2' and 5-positions were combined, led to the identification of phthalimide **37**, which exhibited greater potency than **33**, returning EC₅₀s of 0.26 μ M and 1.27 μ M for pretreatment and combination treatment respectively.⁸⁴

In a follow-up study with the aim of identifying compounds with improved synthetic accessibility and activity the same group investigated structural alternatives for the central tetrahydrofuran ring of **33**.⁸⁵ They screened a subset of the publicly available ZINC database using the 2-chloro-*N*-(2-methoxyethyl)benzamide moiety of **33** as a reference fragment, and

identified five potential central scaffolds. Two of those retained the tetrahydrofuran ring but either transposed the benzamidomethyl group to the 1-position (**38**), or deleted the hydroxyl group at the 3-position (**39**), the other three scaffolds replaced the tetrahydrofuran ring with either a substituted 1,4-dioxane (**40**), a 1,3-substituted morpholine (**41**) or a 2,4-substituted pyrrolidine (**42**). Deletion of the 3-hydroxyl group, or replacement of the tetrahydrofuran with a morpholine or pyrrolidine ring, resulted in a loss of activity (the latter was also reported in a recent study that examined the activity of *N*-substituted pyrrolidine analogues)⁸⁶. Only compounds **38** and **40** displayed the ability to potentiate vancomycin against *S. aureus* biofilms. Compound **38**, in which the benzamidomethyl group was moved to the 1-position delivered improved activity compared to compound **33** for the combination treatment of preformed biofilms with vancomycin, and comparable activity when administered as a pretreatment. Compound **40**, which contains a dioxane core, displayed comparable activity under both conditions.⁸⁵

Microbial natural products as antibiofilm agents

Many natural products produced by microorganisms have found application in a variety of therapeutic areas, including as immunosuppressive, anticancer, and anti-inflammatory agents. Furthermore, most antibiotics are, or are derived from, microbial secondary metabolites that are produced by bacteria and fungi as a means of competing for resources with other species in densely populated environments.⁸⁷ Microbial natural products have likely been highly successful as a source of antibiotic scaffolds because they are derived from millions of years of evolution, and natural selection has ensured that they not only possess high potency and selectivity, but also the necessary physicochemical properties to be able to penetrate the bacterial cell envelope, something synthetic compounds often lack.⁸⁸ It has been suggested that natural-product antibiotics, at sub inhibitory concentrations, may affect biofilm formation by neighboring microbes⁸⁹, and several non-antibiotic microbial natural products have proved effective as scaffolds for the identification of anti-biofilm compounds, as described below.

Walkmycin C **43** (Figure 3), produced by *Streptomyces* sp. strain MK632–100F11, causes the formation of abnormal *S. mutans* biofilms and a reduction in biofilm mass. Walkmycin C was identified from a screen for inhibitors of the TCS histidine kinase WalK in *Bacillus subtilis*, and has also been shown to inhibit autophosphorylation of WalK in *S. aureus* by targeting the conserved catalytic domain.⁹⁰ The anti-biofilm activity of this compound against *S. mutans* is a result of inhibition of autophosphorylation of the TCS histidine kinases VicK and CiaH, which play a role in sucrose-dependent biofilm formation.^{90, 91} Another *Streptomyces* secondary metabolite that has demonstrated anti-biofilm activity is streptorubin B **44**, a prodiginine produced by *Streptomyces* sp. strain MC11024. Streptorubin B was identified from a screen of actinomycete culture extracts and inhibits biofilm formation by *S. aureus* at concentrations as low as at 1 µg/mL.⁹² An enantioselective total synthesis of streptorubin B has been completed by the Thomson lab, which enabled confirmation of the absolute and relative stereochemistry of this natural product.⁹³

The plant pathogen *Xanthomonas campestris* produces cis-11methyl-2-dodecenoic acid **45**, known as diffusible signal factor (DSF)⁹⁴, which has been reported to inhibit biofilm

formation by *Bacillus cerus*.⁹⁵ Other DSF-family signaling molecules that have demonstrated anti-biofilm activity include cis-2-decenoic acid **46**, produced by *P. aeruginosa*, which disperses both its own biofilms and those of multiple other species including the human pathogens *E. coli*, *K. pneumoniae*, and *S. aureus*.⁹⁶ The *Burkholderia* diffusible signal factor (BDSF), cis-2-dodecenoic acid **47**, produced by *B. cenocepacia*, inhibits and disperse biofilms of *Francisella novicida*.⁹⁷

The simple ethyl *N*-(2-phenethyl) carbamate **48**, is produced by the marine bacterium SCRC3P79 (*Cytophaga* sp.), and was initially reported to exhibit anti-biofilm activity against another marine bacterium, *Rhodospirillum salexigens*.⁹⁸ This activity was subsequently shown to translate to several medically relevant pathogens including *S. aureus*, and analogue synthesis based upon this scaffold led to the identification of several compounds with increased activity, the most active of which being the menthyl derivative **49**.⁹⁹ Several metabolites isolated from the endophytic fungus *Eurotium chevalieri* KUFA 0006 have reported anti-biofilm activity, including the anthraquinones questinol **50** and acetylquestinol **51**, which inhibit biofilm formation by *E. coli*, and physcion **52**, which inhibits biofilm formation by *S. aureus* and *Enterococcus faecalis*.¹⁰⁰

Carolacton

Carolacton **53** (Scheme 3) is a macrolactone secondary metabolite produced by the myxobacterium *Sorangium cellulosum* and has been reported to selectively kill biofilm cells of *S. mutans*, exhibiting only minor effects on both planktonic cell growth of *S. mutans*¹⁰¹ and on other oral bacteria¹⁰². Carolacton has also been reported to exhibit growth inhibition of *S. pneumoniae*, and its target in this bacterium was determined to be the folate-dependent C1 metabolism enzyme FolD. Interestingly, carolacton has also been shown to inhibit the human FolD orthologs, MTHFD1 and MTHFD2 and inhibit the growth of several human cancer cell lines.¹⁰³

In *S. mutans*, carolacton affects several regulatory systems including the VicKRX and ComCDE two-component signal (TCS) transduction systems. VicKRX is required for the response to oxidative stress,¹⁰⁴ and also plays an essential role in biofilm formation and maintenance of cell wall and membrane integrity. ComCDE plays an essential role in several pathogenic behaviors including: biofilm formation, virulence, survival under acidic conditions, and genetic competence.¹⁰⁵ These effects are thought to be mediated through the serine/threonine protein kinase PknB, and it is hypothesized that carolacton effects membrane defects, which causes leakage of cytoplasmic content and subsequent cell death, during the pH decrease that occurs during biofilm growth of this bacterium.¹⁰⁵

The structure of carolacton was elucidated in 2010 by the Kirschning and Müller labs, during which chemical derivatization was utilized to aid in the assignment of configuration for all the stereogenic centers. Carolacton **53**, was treated with *p*-bromobenzoyl chloride, leading to macrolactonization between the C1 carboxy group and the C18 hydroxy group to form the bislactone **54**. Compound **54** was treated with both enantiomers of methoxy(trifluoromethyl)phenylacetyl chloride (MTPA-Cl), affording the diastereomeric esters (the (*R*)-diastereomer **55** is depicted in Scheme 3). Mosher analysis was applied and the absolute configuration at C17 was determined from the calculated δ SR values to be

(*R*), and relative configurations for the remaining stereogenic centers of **54** could then be established using a combination of extended NMR analyses and molecular modeling and confirmed by X-ray analysis once a crystal was obtained.¹⁰⁶

The first total synthesis of carolacton was published in 2012 by Schmidt and Kirschning and utilized several metal-mediated key transformations, including: Ley and Duthaler–Hafner aldol reactions and asymmetric Nozaki–Hiyama–Kishi and asymmetric Negishi–Fu CC coupling reactions.¹⁰⁷ Seeking a synthetic route that more readily allowed for exploration of the carolacton SAR, Hallside et al. reported a concise synthesis in which key elements include ring-closing metathesis followed by a selective olefin hydrogenation, and macrocycle formation by esterification (Scheme 3). The known gulonolactone-derived diol **56** was subject to oxidative cleavage to form aldehyde **57**, and a subsequent *Z*-selective Wittig olefination yielded alkene **58**. Benzoate removal followed by Swern oxidation afforded lactone **59**, which was treated with allylmagnesium bromide and CuBr·SMe₂ to effect an SN2' opening of the lactone and form **60** as a single diastereomer. The second subunit of the natural product was assembled by first carrying out enolization of the Evans β-ketoimide **61** under conditions favoring formation of the (*E*)isomer, followed by reaction with aldehyde **62** to form **63** in an inseparable mixture with a *dr* of 5:1.16. However, the subsequent three-step sequence of methylation, reduction, and oxidation–Wittig olefination allowed for isolation of **64** as a single diastereomer. Silylation to form **65**, followed by conversion of the ester to an aldehyde and application of Leighton's (*R,R*)-*trans* EZ-Crotyl Mix afforded **66** in a 5:1 *dr*. The two subunits **60** and **66** were then coupled via a Steglich esterification to give **67**, which underwent selective terminal alkene metathesis upon treatment with the second-generation Grubbs catalyst, forming **68**. Selective olefin reduction and concomitant PMB group removal was followed by TBS removal to provide diol **69**. Finally, simultaneous oxidation of the primary and secondary alcohols under Swern conditions, followed by a Lindgren–Pinnick oxidation yielded keto-acid **70**, and removal of the acetonide afforded carolacton **53**. This synthetic route allowed for the biological investigation of intermediates **69** and **70**, and while **70** was inactive, diol **69** was shown to significantly affected both the integrity and morphology of the biofilm matrix (albeit at higher concentrations than the natural product).¹⁰²

Both the Kirschning and Wuest labs have developed synthetic routes to analogues of carolacton to delineate the SAR of this scaffold, and to readily access simplified active analogues that could be used as chemical probes to further understanding of the mechanism of action of carolacton. In 2015 the Kirschning lab reported the synthesis of several new carolacton analogues, revealing important SAR data. Inversion of configuration at C9 abolished activity, while methyl ester **71** retained activity but the analogous C17 carbonyl derivative **72** did not, implying that (*R*)configured carbinol moiety at C17 is crucial. Additionally, the bicyclic bislactone derivative **73**¹⁰⁶ also retained activity, and it was subsequently shown that the activity of **71** and **73** was a result of enzymatic hydrolysis by *S. mutans* to the natural product; these derivatives essentially act in a prodrug-like manner.¹⁰⁸

In 2017, the Wuest lab reported several carolacton analogues, accessed through a diverted total synthesis (DTS) approach, that displayed a range of biological activities. Compound **74** inhibits biofilm formation, while compound **75** acts in a similar manner to carolacton, killing

cells during biofilm growth, while compound **76** inhibits biofilm maturation.¹⁰⁹ More recently, the same group reported that compound **77**, a further simplified carolacton analogue, inhibits biofilm formation and decreases biofilm cell viability to a greater degree than carolacton. This simplified analogue was employed in a proof of concept forward chemical genetic screen of *S. mutans* mutants to identify the CcpA signaling pathway as likely harboring the target of **77**.¹¹⁰

Skyllamycins

Skyllamycins B **78** and C **79** were identified as non-antibiotic inhibitors of *P. aeruginosa* biofilms in a high-throughput screen of 312 natural-product prefractions that utilized the redox-sensitive dye XTT to determine bacterial cellular metabolic activity in addition to with biofilm mass. The skyllamycin family of natural products are cyclic depsipeptides produced by *Streptomyces* sp. Skyllamycins B and C exhibited M respectively, and when co-dosed with azithromycin, were shown to eliminate biofilms and depress cellular metabolic activity.¹¹¹

The skyllamycins are highly modified and possess a number of unusual amino acids, including an extremely rare α -OH-glycine residue.¹¹² Giltrap et al. have reported a synthesis of the skyllamycins, which involved the initial preparation of a series of structurally simplified analogues and subsequent adaption of the route to afford skyllamycins B and C. The synthesis required synthesis of the (2*S*,3*S*)- β -OH-Phe monomer that began with Grignard addition of PhMgBr to (*R*)-Garner's aldehyde **80**, giving a mixture of diastereomers (Scheme 4). Swern oxidation to **81**, followed by diastereoselective DIBAL-H reduction yielded the desired anti-diastereomer **82**, which was treated with *p*-toluenesulfonic acid to remove the oxazolidine moiety and afford diol **83**. Selective oxidation of the primary alcohol was achieved using a TEMPO-mediated oxidation with NaOCl as the stoichiometric oxidant to form **84**. Subsequent exchange of amine protecting group delivered the protected (2*S*,3*S*)- β -OH-Phe **85**. The protected (2*S*,3*S*)- β -OH-O-Me-Tyr residue was also prepared from (*R*)-Garner's aldehyde **80**, and involved addition of the organo-lithium species generated from *para*-bromoanisole to give alcohol **86** as a mixture of diastereomers. Oxazolidine removal, oxidation, and swapping protecting group as above afforded **87**. Final protection of the hydroxyl using a novel pseudoproline protecting group strategy provided the fully protected β -OH-Tyr monomer **88**, which could be separated from its diastereomer by silica column chromatography. The final non-proteogenic amino acid, (2*R*,3*S*)- β -OH-Leu, was accessed in a similar manner, starting with the reaction between (*S*)-Garner's aldehyde **89** and ⁱPrMgBr to yield alcohol **90** with exclusive (3*S*)-stereochemistry. Removal of the oxazolidine moiety to give diol **91** was followed by selective oxidation of the primary alcohol. Finally, swapping of the amine protecting group, and protection as before afforded the fully protected β -OH-Leu monomer **92**.

Construction of the natural products was achieved by first assembling the linear peptide precursors **93** and **94** via Fmoc-solid phase peptide synthesis utilizing both conventional and microwave assisted HATU-mediated peptide coupling conditions. An on-resin esterification yielded the branched peptides, to which the two remaining residues were coupled. Cleavage from the resin and deprotection afforded the unprotected linear peptides **95** and **96**. The

linear peptides then underwent oxidative cleavage to aldehydes **97** and **98** by treatment with NaIO₄ under pH-controlled conditions and quenching with ethylene glycol. Finally, cyclization and concomitant formation of the α -OH-Gly residue afforded skylamycins B **78** and C **79**.¹¹²

The synthetic skylamycins, along with the simplified analogues synthesized during method development, were examined for antibiofilm activity against *P. aeruginosa*. It was revealed that removal of the hydroxyl group from the hydroxyglycine motif, removal of the β OH moieties from the three β -hydroxy amino acids, or elimination of the methyl group from the OMe-tyrosine had a significant impact upon activity, indicating that the skylamycin scaffold is amenable to manipulation, which could allow for the exploration of simplified analogues.¹¹²

Promysalin

Promysalin **99** was isolated from the saprotrophic soil bacterium *Pseudomonas putida* RW10S1, and reported to exhibit anti-bacterial activity against *P. aeruginosa* and other pseudomonads.¹¹³ Promysalin was later reported to disperse *P. aeruginosa* biofilms and to inhibit pyoverdine production in *P. putida* KT244.^{114, 115} It was subsequently determined that the biological target of promysalin in *P. aeruginosa* is succinate dehydrogenase (Sdh), which plays a key role in both the tricarboxylic acid cycle (TCA) and respiration.¹¹⁶ The compound also binds iron¹¹⁵. The postulated structure given in the initial disclosure lacked stereochemical assignment, and while bioinformatic analysis of the gene cluster indicated that the absolute configuration of the C16 stereocenter should be assigned as (*S*), the C2 and C8 stereocenters remained unassigned. Steele et. al developed a synthetic route to access the four possible diastereomers generated by these two centers, and were able to confirm the stereochemistry of promysalin as (*2R,8R,16S*).¹¹⁴

Synthesis of the promysalin diastereomers commenced with construction of the myristic acid fragment (Scheme 5). The known compound (–)-**100**, formed from 5-hexenoic acid and the phenylalanine-derived Evans oxazolidinone, underwent diastereoselective oxidation using the Davis oxaziridine, and was then silyl protected to provide (–)-**101**. Subsequent crossmetathesis with homoallylic alcohol (+)-**102** or (–)-**102** in the presence of Grubbs catalyst C711, followed by hydrogenation then ammonolysis, afforded diastereomers (+)-**103a** and (+)-**103b**, respectively. In the same manner, myristic acid diastereomers (–)-**103c** and (–)-**103d** were synthesized from the enantiomeric starting material (+)-**100**.

The proline–salicylate fragment was accessed from SEM protected methyl salicylate **104**, which was subjected to ester hydrolysis, HATU mediated coupling with trans-4-L-hydroxyproline methyl ester, and Dess–Martin oxidation to provide (+)-**105**. Regioselective conversion to the enamine followed by reduction using a modified Stille reaction and then ester hydrolysis provided the proline–salicylate coupling fragment (–)-**106**. Compound (–)-**106** was then subject to EDC-mediated esterification with each of the four myristic acid diastereomers which, followed by deprotection under mild conditions that employed 1 M TBAF in THF with DMPU as a cosolvent, afforded the four promysalin diastereomers (–)-**107a-d**.¹¹⁴ ¹H and ¹³C NMR spectral data for compound **107a** best aligned with that of

the isolated material, and this diastereomer also exhibited the greatest anti-biofilm activity, exhibiting IC₅₀ values of 1 μ M and 125 nM against PAO1 and PA14 respectively, and dispersing biofilms of both strains at 6.25 and 12.5 μ M, leading the authors to conclude that the structure of the natural product is that of (–)-**107a**.

In a follow-up study aiming to elucidate the chemical moieties responsible for the species-specific activity of promysalin, the Wuest lab synthesized a series of promysalin analogues using another diverted total synthesis (DTS) approach. They determined that removal of the proline ring double bond, replacement of the proline with a piperidine ring, or substitutions on the proline ring other than fluorination (compound **108** IC₅₀s 7.7 and 0.019 μ M against PAO1 and PA14 respectively), led to a reduction or complete loss of activity. Alterations to the salicylate moiety, including varying the position, removal, replacement, or esterification of the phenol were not well tolerated. Side chain modifications however, did produce active analogues including alkene **109**, and de-hydroxy analogue **110**, which demonstrated equipotent activity to the natural product. Compound **110** is more synthetically accessible than promysalin, requiring two less steps, one less protecting group and avoiding the necessity of a chiral auxiliary.¹¹⁵

Halogenated Phenazines

Phenazines, a class of secondary metabolites identified in both Gram positive and Gram-negative bacteria,¹¹⁷ have demonstrated antibiofilm activity in addition to antibiotic, antifungal, and anticancer activities.^{118, 119} Murine model studies have demonstrated that production of pyocyanin **111** (Figure 5) by *P. aeruginosa* regulates the release of the EPS component extracellular DNA.¹²⁰ Studies have shown that, in cystic fibrosis (CF), young patients initially get infected by *S. aureus* and later *P. aeruginosa* co-infects CF patients and competes with *S. aureus* through phenazine mediated generation of reactive oxygen species (ROS).¹²¹ Phenazines also participate in iron acquisition, energy-generation, redox homeostasis and even act as signaling molecules, prolonging the originators lifespan in comparison to non-phenazine-producing bacteria.¹¹⁹ The discovery that bacteria utilize the redox-active nature of phenazines as a means of competing with other microbes within their environment led their investigation as potential antibacterial agents.

The Huigens lab synthesized and screened a panel of halogenated phenazines and their ester analogues as potential antimicrobial agents.¹²² Compared to pyocyanin (MIC = 50 μ M), halogenated phenazines **112** (MIC = 6.25 μ M) and **113** (MIC = 50 μ M) demonstrated comparable or improved antibacterial activity against *S. aureus* and *S. epidermidis*. To investigate the hypothesis that *P. aeruginosa* in chronic CF infection could be eradicating *S. aureus* biofilms, the potential of halogenated phenazines as antibiofilm agents was examined. Diverse 1-methoxyphenazine scaffolds were accessed through either a Wohl-Aue reaction or condensation of quinone with symmetric phenylenediamines, yielding halogenated phenazines with diversity at the 2-, 4-, 7-, and 8- positions.^{122–126} Using a Calgary Biofilm Device (CBD) assay, multiple biofilm eradicators against Gram-positive bacteria were identified, with compound **114** being the most potent biofilm-eradicator against Gram -positive bacteria (MBEC = 9.38 μ M, 2.35 μ M, and 0.20 μ M against MRSA, methicillin-resistant *S. epidermidis* (MRSE), and vancomycin resistant *Enterococcus*

faecium (VRE) respectively). It was revealed that a bromine at the 2-position is essential for both planktonic and biofilm-eradicating activities, with compound **115** exhibiting four- to 32-fold higher MBECs than **114**.¹²⁴ Halogens at the 7- and 8- position were also found to enhance biofilm-eradication activity against Gram-positive bacteria.

In a subsequent report, that broadened the synthetic scope, a two-step Buchwald–Hartwig cross-coupling and reductive-cyclization was utilized to construct multiple 1-methoxyphenazines.¹²⁷ Additionally, using the potent biofilm eradicator **116** (MBECs 4.69 μ M, 37.5 μ M, and 0.59 μ M against MRSA, MRSE, and VRE respectively) as a starting point, several prodrugs were synthesized with the aim of enhancing water-solubility (e.g. carbonate analogue **117**) or selectivity against bacteria (e.g. alkylloxycarbonyloxymethyl analogue **118**). While both **117** and **118** retained biofilm eradication activity, carbonate **117** showed enhanced planktonic activity against MRSA, compared to parent **116** which was thought to be a result of enhanced membrane permeability. Hemolysis assay, metal-chelation studies, and RNA transcription analysis, indicate that HPs could eradicate biofilms via binding iron (II) and thereby inducing iron starvation.^{123, 128}

Following these findings, similar metal-chelating scaffolds including halogenated quinolines and nitroxolines were investigated, revealing that the 2-position of halogenated quinolines plays an important role in tuning activity against Gram-positive bacteria.¹²⁹ After generating a series of halogenated quinolines using microwave assisted Friedländer synthesis,¹³⁰ the 2-position was rapidly diversified via alkylation and reductive-amination chemistry.^{131, 132} While several halogenated quinolines demonstrated planktonic toxicity, compound **119** exhibited potent biofilm-eradication activity against *S. aureus*, *S. epidermidis*, and *E. faecium* (MBECs 7.8 μ M, 5.9 μ M and 1.0 μ M against MRSA, MRSE and VRE respectively) without significant HeLa-cell-cytotoxicity or human red blood cell (RBC) lysis. Attempts to improve water-solubility of this scaffold, through the introduction of polar groups at the 2-position utilizing Williamson ether synthesis with compound **120**, resulted in a reduction in biofilm eradication activity (MBECs 200 μ M, 75 μ M and 2.35 μ M against MRSA, MRSE and VRE respectively).¹³³

Marine natural products as antibiofilm agents

The marine environment encompasses tremendous biodiversity and marine organisms have long been a rich source of bioactive compounds.¹³⁴ It is thought that the unique conditions found in marine environments are responsible for the production of molecules bearing significant diversity in terms of both structural and functional features, which consequently exhibit a high degree of bioactivity.¹³⁵ In particular, the sessile nature of marine invertebrates such as sponges and corals has driven the evolution of chemical defense systems to evade predation and prevent biofouling. Marine natural product derived drugs with a broad spectrum of therapeutic applications including: cancer, anti-viral, chronic pain, and hypertriglyceridemia have been approved, and many others are in development.¹³⁶ The study of marine natural products is hampered by the fact that typically only small quantities can be isolated from the source organism, and synthetic methods to access these natural products are therefore highly valuable if the potential of this rich source of chemical space is to be realized.

Examples of marine natural products that have demonstrated anti-biofilm activity include: the diterpene alkaloid (–)-ageloxime D **121** (Figure 6), which was isolated from the marine sponge *Agelas nakamurai*, and inhibits biofilm formation by *S. epidermidis* without affecting bacterial growth.¹³⁷ Another diterpene, darwinolide, **122**, which was isolated from the Antarctic sponge *Dendrilla membranosa*, inhibits MRSA biofilm formation with an IC₅₀ value of 33.2 μM, four-fold lower than its MIC.¹³⁸ Total synthesis of darwinolide was recently reported by Siemon et al.¹³⁹ The tryptamine derivative bufotenine **123**, isolated from the Mediterranean gorgonian *Paramuricea clavate*, has been reported to exhibit anti-adhesion activity against the marine bacterium *Pseudoalteromonas* spp D41.¹⁴⁰ Synoxazolidinones are brominated guanidinium oxazolidinones isolated from the sub-arctic ascidian *Synoicum pulmonaria*. Synoxazolidinone A **124** exhibits moderate anti-bacterial activity against Gram-positive bacteria,¹⁴¹ while synoxazolidinone C **125** has been reported to inhibit adhesion by several species of marine bacteria.¹⁴² Synthetic analogues of synoxazolidinone A reported by the Pierce lab, compounds **126** and **127**, inhibit MRSA biofilm formation with low or sub micromolar IC₅₀ values (0.78 and 1.2 μM respectively), while compound **126** disperses pre-formed biofilms with an EC₅₀ of 4.7 μM.¹⁴³

Bromoageliferin

Bromoageliferin **128** (Scheme 6) belongs to the pyrrole-imidazole class of alkaloids, which has been exclusively isolated from marine sponges¹⁴⁴, and has been reported to reduce voltage-dependent calcium entry in PC12 cells.¹⁴⁵ The structurally simple oroidin **129** (Figure 7), is a key intermediate in the biosynthesis of many of the more complex pyrrole-imidazole alkaloids, and was first isolated from *Agelas oroides* in 1971,¹⁴⁶ (it has subsequently been isolated from several other sponges), while bromoageliferin was isolated from *Agelas conifera*.¹⁴⁷ Oroidin and bromoageliferin were reported to inhibit the growth of *R. salexigens* SCRC 113 by Yamada et al. in 1997.⁹⁸ Both bromoageliferin and the related pyrrole-imidazole alkaloid sceptrin **130** inhibit biofilm formation by the commonly encountered human pathogens MRSA and *Acinetobacter baumannii* with IC₅₀s of 10.6 μM and 28.3 μM respectively.¹⁴⁸

Following the first enantioselective total synthesis of ageliferin by the Baran lab in 2007, via a vinyl cyclobutane rearrangement of sceptrin,¹⁴⁹ the Chen lab developed a biomimetic synthesis of the ageliferins that allowed for flexible introduction of the pyrrole side chains thus allowing synthesis of dibromoageliferin.¹⁵⁰ The synthesis was initiated by coupling azidoimidazole **131** with allylic ester **132** to give **133** (Scheme 6), which was then subject to oxidative cyclization via treatment with Mn(OAc)₃ in acetic acid at 50–60 °C, or with Mn(piccolinate)₃ in methanol at 90 °C to form the ageliferin core skeleton **134**. Optimal yield of **135** was achieved upon using crude **134**, as a mixture of C9' diastereomers, that upon decarboxylation yielded a single (cis) diastereomer, which was subsequently mesylated to deliver **135**. Conversion to the iodide and finally displacement with sodium azide gave **136**. The orthogonally masked amine groups of this key intermediate allowed for the selective introduction of the differing pyrrole units of bromoageliferin. The dibromopyrrole was introduced first at the N7 position through Staudinger reduction of the azide and coupling with the dibromopyrrole to form **137**. Selective coupling to the less hindered N7 position was achieved with careful control of reaction time. Introduction of the monobromopyrrole

unit at the N7' position was achieved through Boc-deprotection and coupling under the same conditions as before to form **138**. Construction of the second 2-AI group was carried out by installation of a guanidine group on **138**. This was followed by oxidation of the hydroxyl group with 2-iodoxybenzoic acid (IBX) to the aldehyde which underwent intramolecular cyclization to form **139**. Following correction of the C9' configuration of **139** through a slow equilibration under acidic conditions (60–72 h, d.r. > 10:1), the C10' carbonyl group was removed by sequential reductions to afford **140**. Removal of the BOM protecting group was achieved through a two-pot process consisting of conversion to the corresponding chloromethyl derivative (BCl₃) followed by cleavage with ammonium hydroxide. Finally, hydrolysis of the triphenylphosphine imide group with hydrochloric acid afforded bromoageliferin **128**.¹⁵⁰

Both bromoageliferin and the simple pyrrole-imidazole alkaloid oroidin **129** have been employed as templates for the design of a number of classes of simplified, more easily accessible analogues, many of which exhibit anti-biofilm activity across a broad spectrum of bacteria. The first indication that simplified bromoageliferin analogues could modulate bacterial biofilms occurred with the report that *trans*-bromoageliferin analogue (TAGE) **141** and *cis*-bromoageliferin (CAGE) **142** inhibited biofilm formation by *P. aeruginosa* (IC₅₀'s of 100 µM against PAO1).¹⁵¹ Structural modification led to the identification of the di-brominated acylpyrrole containing analogue **143**, which inhibits the formation of biofilms by *P. aeruginosa* more potently than does TAGE, and also inhibits biofilm formation by *A. baumannii*, *Bordetella bronchiseptica* and *S. aureus*.¹⁵² These analogues retain both the bicyclic core of the natural product, along with the 2-aminoimidazole (2-AI) subunit. The 2-AI subunit would prove to be particularly important in conferring anti-biofilm activity, and there have been numerous subsequent reports of 2-AI containing compounds that demonstrate anti-biofilm activity. Simplification of the chiral bicyclic structure found in bromoageliferin produced a series of 2-aminobenzimidazole (2-ABI) analogues that exhibit anti-biofilm activity against Gram-positive, Gram-negative, and mycobacteria. Compound **144** inhibits biofilm formation by *S. aureus*, *E. faecium* and *S. epidermidis* with low micromolar IC₅₀s and was shown to act via a zinc dependent mechanism³¹, as was 2-ABI **145**, for the inhibition of biofilm formation by MRSA¹⁵³. In 2012 it was reported that a homologue of **145**, compound **146**, inhibits biofilm formation by *Mycobacterium smegmatis*.¹⁵⁴ Analogue synthesis identified compound **147**, which in addition to inhibiting biofilm formation by *M. smegmatis*¹⁵⁵, also inhibits biofilm formation by *Salmonella enterica*.¹⁵⁶ Additionally, the simple 2-ABI 5,6-dimethylbenzimidazole (DMABI) **148** was reported by the Blackwell lab to potently inhibit biofilm formation by *P. aeruginosa*, potentially through interference with the *P. aeruginosa* Las and Rhl QS systems.¹⁵⁷

Several classes of oroidin-like compounds have also been synthesized and examined for anti-biofilm activity. Dihydrosventrin (DHS) **149** inhibits biofilm formation by *P. aeruginosa*, *A. baumannii*, and *B. bronchiseptica*¹⁵⁸, while the *para*-bromophenyl derivative **150**, exhibits increased anti-biofilm activity against *A. baumannii* as compared to DHS.¹⁵⁹ Compound **151** inhibits and disperse biofilms of both *P. aeruginosa* and *A. baumannii*,¹⁶⁰ and a biotinylated analogue of **151** was used to identify the target of this class of compounds

in *A. baumannii* as the response regulator BfmR (of the BfmRS TCS),¹⁶¹ which plays an important role in biofilm formation¹⁶² in this bacterium.

The related 2-AI **152**, inhibits biofilm formation by *S. mutans* and downregulates the histidine kinase ComD in this bacterium,^{163, 164} while several aryl 2-AI compounds including **153** bind both BfmR and the *Francisella spp.* response regulator QseB, and inhibit biofilm formation by both species.^{165, 166} The Van der Eycken and Steenackers labs have also conducted medicinal chemistry programs on the 2-AI scaffold, and reported a series of aryl 2-AIs including **154** that exhibit anti-biofilm activity against *P. aeruginosa* and *S. typhimurium*.^{167, 168} A series of tri-substituted 2-AI compounds, including **155**, synthesized as analogues of Naamine A **156**, also displayed antibiofilm properties against the same two bacteria.¹⁶⁹ Inspired by the dimeric nature of naturally occurring 2-AI containing compounds, these same groups investigated the activity of 5-aryl-2AIs dimers linked by an alkyl chain between either the *N1*-position or the *N2*-positions. They observed an increase in anti-biofilm activity for *N1,N1*-linked dimers compared to the respective monomers against *S. typhimurium*, *E. coli* and *S. aureus*, while certain *N2,N2*-linked dimers additionally displayed increased biofilm activity against *P. aeruginosa* compared to the respective monomers. For example, dimer **157** exhibited IC₅₀ values of 0.3, 10.1, 5.4, and 8.9 μ M against *S. typhimurium*, *P. aeruginosa*, *E. coli*, and *S. aureus* respectively compared to values of 84.4, 15, 75.5, and >800 μ M respectively for the monomer.¹⁷⁰

Expanding upon the key 2-AI and 2-ABI moieties contained in the compounds described above, libraries of compounds possessing the related 2-aminopyrimidine and 2-aminoquinazoline moieties have also been examined. 2-aminopyrimidine **158** inhibits biofilm formation by MRSA¹⁷¹ and *M. smegmatis*,¹⁵⁴ while 2-aminoquinazoline **159** inhibits biofilm formation by *M. smegmatis*.¹⁷²

Flustramine C

Flustramine alkaloids, a family of indole-derived secondary metabolites isolated from the bryozoan *Flustra foliacea*,^{173, 174} were reported by Riedel et al. to possess antimicrobial activity against marine bacterial strains, suggesting a role combating fouling organisms.¹⁷⁵ While most flustramines do not exhibit significant activity against terrestrial bacteria, some derivatives were reported to antagonize AHL-regulated QS systems,¹⁷⁵ which inspired the investigation of the flustramine scaffold for the identification of antibiofilm agents.

Following total syntheses of flustramine C **160** by the Sakamoto¹⁷⁶ and Funk¹⁷⁷ groups, Lindel et al. reported a synthetic approach from the related flustramine alkaloid deformylflustrabromine **161** via a Danishefsky inverse prenylation (Scheme 7).¹⁷⁸ The synthesis initiated with the conversion of *N*-methyltryptamine **162** to *N*-formyl-*N*-methyltryptamine **163** followed by treatment with *t*-BuOCl and freshly prepared prenyl-9BBN to effect the inverse prenylation and provide 1,1-dimethylallyl indole **164**. Compound **164** was monobrominated at the 6-position via treatment with NBS in AcOH/HCO₂H, to form the natural product flustrabromine **165**, which was converted to deformylflustrabromine **161** via hydrolysis of the formamide moiety with sodium hydroxide. The key step in the conversion of deformylflustrabromine **161** to flustramine C **160** was the

1,2-rearrangement of the inverse prenyl group, which was successfully achieved via treatment with one equivalent of NBS.¹⁷⁸

The Lindel synthetic route was utilized to investigate the antibiofilm activity of flustramine family members, revealing that flustramine C modestly inhibited biofilm formation by *A. baumannii* (30% at 100 μ M).¹⁷⁹ The synthesis of analogues of flustramine C in which the inverse prenyl moiety was replaced by a propargyl group, thus allowing for diversification via click chemistry, led to the identification of several compounds that displayed anti-biofilm activity against several species. Compounds **166-168** (Figure 8) inhibited biofilm formation by MRSA with IC₅₀ values of 86 μ M, 25 μ M, and 5.4 μ M respectively, although toxicity toward planktonic bacteria increased as chain length increased. Modest inhibition of biofilm formation by *E. coli* and *A. baumannii* (**166** and **167** only), without planktonic toxicity was also observed. Aromatic analogues **169-171** demonstrated a similar activity trend to the aliphatic analogues, in which anti-biofilm activity and planktonic toxicity increased with increasing chain length, and compounds **169-171** inhibited biofilm formation by both methicillin sensitive *S. aureus* (IC₅₀ values of 32.0 μ M, 12.5 μ M, and 6.6 μ M respectively) and MRSA (IC₅₀ values of 13.5 μ M, 6.6 μ M, and 3.4 μ M respectively). Compound **172**, a furindoline analogue of **170**, was synthesized using similar chemistry, and inhibited biofilm formation by MRSA with an IC₅₀ of 18.7 μ M in a non-toxic manner.¹⁷⁹ Subsequently, related series of flustramine C analogues in which a propargyl group was installed at the at 3-position of indole, with or without bromination of the indole, and again derivatized via click chemistry were reported by Minvielle et al.¹⁸⁰ Compound **173** inhibited biofilm formation by *A. baumannii*, *S. aureus* and MRSA, yet interestingly promoted *E. coli* biofilm formation, while compound **174** modestly inhibited biofilm formation by *S. aureus* yet its 2-bromo analogue **175** promoted formation.

Desformylflustrabromine **161** has also been investigated as a scaffold for the identification of compounds with anti-biofilm activity. Desformylflustrabromine itself exhibits moderate inhibition of biofilm formation by *E. coli* and *S. aureus*, however it also exhibits toxicity toward planktonic bacteria. A comprehensive SAR study of this scaffold revealed that the C-6 bromine and the C-2 reverse prenyl group are essential for anti-biofilm activity. Alkylation of the indole nitrogen was tolerated, while activity could be increased through modulation of the *N*-methyl moiety.^{181, 182} Compounds **176** (IC₅₀ 53 μ M and 5.9 μ M against *E. coli* and *S. aureus* respectively) and **177** (IC₅₀ 15.6 μ M and 7.7 μ M against *E. coli* and *S. aureus* respectively) were found to be highly active non-toxic inhibitors of biofilm formation. Compound **178**, in which the inverse prenyl group was transposed to the indole nitrogen, demonstrated moderate biofilm-inhibition activity (IC₅₀ 80 μ M and 65 μ M against *E. coli* and *S. aureus* respectively). The three-carbon homologue of **176**, compound **179**, exhibited potent biofilm-inhibition activity (IC₅₀ 16.3 μ M and 13.4 μ M against *E. coli* and *S. aureus* respectively), demonstrating the tunability of the alkyl-linker. Mechanistic studies with **176** revealed that, analogous to indole itself, the activity of compound **176** is dependent on temperature, TnaA, and the transcriptional regulator SdiA, suggesting that **176** may be modulating biofilm formation via indole-based signaling pathways.¹⁸¹

Meridianin D

The meridianins are secondary metabolites isolated from the marine invertebrate *Aplidium meridianum*,¹⁸³ that have demonstrated a number of biological activities,^{184–186} including inhibition of CDKs, GSK-3, PKA and other protein kinases at low micromolar concentration,¹⁸⁷ and antibacterial activity against *S. aureus*¹⁸⁶ and *M. tuberculosis*.¹⁸⁸ Given that meridianin D **180** (Scheme 8) possesses both an indole and 2-aminopyrimidine moiety, it was investigated as a scaffold for the identification of novel anti-biofilm compounds. Huggins et al. utilized the synthetic approach developed by Brederick¹⁸⁹ to access meridianin D and analogues.¹⁹⁰ Synthesis of meridianin D commenced with acylation of 6-bromo indole **181** at the 3-position using acetyl chloride and tin chloride to afford **182**, followed by tosyl protection of the indolic nitrogen giving **183**. The enaminone **184** was prepared by reaction with DMF/dimethylformamide-dimethylacetal (DMF-DMA), and finally cyclization and deprotection of the enaminone in 2-methoxyethanol using potassium carbonate and guanidine hydrochloride provided meridianin D **180**, which inhibited biofilm formation by MRSA with an IC₅₀ value of 87.4 µM.¹⁹⁰ SAR exploration centered on varying the position and identity of the halogen on the indole ring, alkylation of the indole nitrogen, alkylation of the exocyclic nitrogen of the 2-AP, and replacement of the 2-AP with a 2-AI. Transposition of the bromine moiety from the 6-position in the natural product to the 5-position in compound **185** (Figure 9) led to increased activity, with this compound exhibiting an IC₅₀ value of 17.9 µM for the inhibition of biofilm formation by MRSA. Compound **185** also modestly dispersed pre-formed MRSA biofilms, with an EC₅₀ value of 138.4 µM, which was lowered by 33.4% when dosed with vancomycin at a concentration that does not affect the biofilm as a standalone treatment. Alkylation of the exocyclic amine of **185** with methyl (**186**) or ethyl (**187**) led to an increase in dispersal activity albeit with a tandem increase in toxicity and diminished synergy with vancomycin, while benzylation at this position (**188**) increased inhibition activity (IC₅₀ 9.62 µM) but abolished dispersal activity. Replacement of the 2-aminopyrimidine with a 2-AI in both meridianin D and compound **185** resulted in increased planktonic toxicity, and no antibiofilm activity below toxic concentrations. The meridianin D analogue library was also investigated for its effect on mycobacterium biofilms, which revealed that the natural product exhibited moderate dispersal activity (EC₅₀ 51.0 µM) but more potent inhibition (IC₅₀ 21.5 µM) against *M. smegmatis*.¹⁹¹ Several analogues displayed increased activity compared to meridianin D, including 2AI containing **189**, which inhibited biofilm formation with an IC₅₀ of 7.5 µM, and dispersed pre-formed biofilms with an EC₅₀ of 27.9 µM. As mentioned above, compound **189** did not disperse MRSA biofilms, indicating that synthetic manipulation of the meridianin scaffold can be utilized to tune selectivity.

Brominated furanones

Halogenated furanones are secondary metabolites isolated from the marine macro-alga *Delisea pulchra*, first reported by The Roche Research Institute of Marine Pharmacology,¹⁹² and found to have broad-spectrum antimicrobial as well as biofilm-inhibition activities,^{193–195} while some brominated furanones have been reported to display cytotoxic effects toward human neuroblastoma SK-N-SH cells¹⁹⁶. Halogenated furanones share structural similarities with AHLs and have been shown to inhibit quorum sensing through competitive

interaction with LuxR-type receptors.^{197, 198} The exocyclic vinyl bromide has been reported to be an essential structural feature, mediating activity through nucleophilic attack and subsequent degradation of the target protein.¹⁹⁸

Sulfuric acid mediated synthesis of halogenated furanones from bromo-substituted levulinic acid was first reported by Sims et al.,¹⁹⁹ and later optimized by Steinberg et al.²⁰⁰ The synthesis involves condensation of ethyl acetoacetates with ethyl 2-bromoalkanoates, subsequent hydrolysis and decarboxylation of the diester in presence of sodium hydroxide, followed by reaction with molecular bromine. Furanone **190** (Figure 10) was reported to inhibit QS in *P. aeruginosa* and to effect bacterial clearance in a pulmonary mouse model,^{198, 201, 202} however the therapeutic application of halogenated furanones is hindered by toxicity concerns, with high doses of **190** resulting in mortality in a fish model despite a lack of HeLa cell cytotoxicity.²⁰³ Janssens et al. synthesized **190** along with a series of analogues with varied alkyl chain lengths, and reported that **190** inhibits biofilm formation by *S. enterica* with an IC₅₀ of 10 µM, and exhibits mild planktonic toxicity (MIC 500 µM).²⁰⁴ The ethyl analogue **191** demonstrated moderate biofilm inhibition (IC₅₀ 50 µM) without any planktonic toxicity, while the isomer **192** was inactive in both assays.

To further investigate structure-activity of this class of compounds, Han et al. synthesized **193** starting from α-methyllevulinic acid, which was then converted to brominated analogues **194** and **195** using NBS.²⁰⁵ Interestingly, both **193** and its dibromo counterpart **194** displayed biofilm inhibition against *E. coli* without any planktonic toxicity. However, mono-bromo analogue **195**, while able to inhibit biofilm formation, also displayed toxicity. Chang et al. synthesized 5-hydroxyl-3,4-halogenated-5H-furan-2-ones via an acid catalyzed oxidative cyclization of furfural, which was used as a building block to access a series of alkyl analogues, ultimately identifying multiple compounds with moderate biofilm inhibition activity against *P. aeruginosa*.²⁰⁶ Computational investigation of hydrogen bonding interactions with LasR indicated that aryl esters may interact more strongly with the binding site than alkyl esters, and synthesis of several analogues identified compound **196**, which demonstrated 63% biofilm inhibition against *P. aeruginosa* at 1/16 MIC (32 µg/ml, MIC 512 µg/ml). Molecular docking revealed similar interactions between **196** and LasR to those of AHLs, which, coupled with the fact that **196** reduces pyocyanin production, indicates that **196** acts through a LasR depended mechanism. Compound **196**, which does not possess an exocyclic vinyl bromide, did not demonstrate any cytotoxicity MTT assay in RAW264.7 cells, which may enable circumvention of toxicity issues associated with the brominated furanone class of anti-biofilm compounds.

Conclusions and future perspectives

The ability of bacteria to form biofilms within an infection setting is a significant factor in the recalcitrance of such infections. Small molecules that interfere with the ability of bacteria to form and maintain biofilms during infection can reverse antibiotic tolerance conferred by the biofilm phenotype, and have the potential to form combination therapies with conventional antibiotics. Many of the compounds identified to date with such activity are natural product small molecules, isolated from various sources including plants, marine organisms, and other microbes. Total synthesis can confirm structure and provide material

for screening, while semi-synthesis and medicinal chemistry can identify natural product analogues with improved activity and the physicochemical properties necessary to advance compounds towards therapeutic feasibility. We have described the activity, synthesis and analogue development of several natural products from various sources. The plant derived hamamelitannin synergizes with vancomycin against biofilms of *S. aureus*, and synthesis has identified an analogue of this natural product displays 20-fold increased activity for the treatment of pre-formed biofilms. The marine sponge derived bromoageliferin and related pyrrole imidazole alkaloids inhibit biofilms of many bacterial species, while a synthetic analogue inhibits MRSA biofilm formation with over 10-fold increased potency, highlighting the utility of synthetic chemistry for optimizing biological activity.

Limited chemical space has yet been explored for the identification of natural product anti-biofilm compounds, and, given the predominance of natural product scaffolds in the antibiotic field, it should be assumed that there are many antibiofilm scaffolds still to be identified. The discovery and development of additional compounds will require efforts from multiple disciplines, and synthetic and medicinal chemistry will be vital if the potential of natural products for the treatment of biofilm-based infections is to be realized.

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Figure 1.
Natural products isolated from plants that have demonstrated anti-biofilm activity.

Figure 2.
Second generation hamamelitannin analogues.

Figure 3.
Microbial natural products that have demonstrated anti-biofilm activity.

Figure 4.
Carolacton analogues **71-77**.

Figure 5.
Halogenated phenazines and quinolones **101-120**.

Figure 6.
Marine natural products that have demonstrated anti-biofilm activity.

Figure 7.
Bromoageliferin inspired compounds with anti-biofilm activity.

Figure 8. Flustramine inspired compounds with anti-biofilm activity.

Figure 9.
Meridianin D analogues with anti-biofilm activity.

Figure 10.
Brominated furanones with anti-biofilm activity.

Scheme 1.
Synthesis of ellagic acid glycosides **15**, **16**, and **20**.

Scheme 2.

Hamamelitannin **21** and synthesis of first generation hamamelitannin analogues.

Scheme 3.
Total synthesis of carolacton **53**.

Scheme 4.

Total synthesis of skyllamycins B **78** and C **79**. Top: synthesis of non-proteogenic amino acids. Bottom: Construction of natural products.

Scheme 5.
Synthesis of promysalin **99** and structures of analogues **108-110**.

Scheme 6.
Total synthesis of bromoageliferin **128**.

Scheme 7. Synthesis of flustramine C **160** and deformatylustrabromine **161**.

Scheme 8.
Synthesis of meridianin D **180**.

Table 1.

Summary of natural product activity

Natural product or natural product class	Source	Antibiofilm activity			Mechanism	Any other biological activity
		Bacterial species	Activity	Analogue activity		
Ellagic acid glycosides 15, 16, 20	Plant (<i>Rubus ulmifolius</i> Schott.)	<i>S. aureus</i>	15 MBIC ₅₀ 64 µg/mL	-	Inhibits cellular adhesion	-
Hamaelittannin 21	Plant (<i>Hamaelis virginiana</i>)	<i>S. aureus</i>	EC ₅₀ 145.5 µM (pretreatment) 165.1 µM (treatment of preformed biofilms) with vancomycin	33 EC ₅₀ 0.389 µM (pretreatment) 7.976 µM (treatment of preformed biofilms) with vancomycin	Inhibits TRAP phosphorylation	Inhibits TNF-mediated endothelial cell death Cytotoxic activity colon cancer cells
Carolacton 53	Microbe (<i>Sorangium cellulosum</i>)	<i>S. mutans</i>	Decreases biofilm cell viability 3078% % at 62 nM - 2 µM	77 decreases biofilm cell viability 75–93% at 62 nM - 2 µM	Affects VicKRX and ComCDE via PknB	Inhibits human cancer cell lines via MTHFD1 and MTHFD2
Skyllamycins B 78 and C 79	Microbe (<i>Streptomyces</i> sp.)	<i>P. aeruginosa</i>	78 EC ₅₀ 30 µM 79 60 µM	-	Unknown	-
Promysalin 99	Microbe (<i>Pseudomonas putida</i>)	<i>P. aeruginosa</i>	IC ₅₀ 1 µM (PAOI) 125 nM (PA14) Disperses PAOI and PA14 biofilms at 6.25 µM	108 IC ₅₀ 7.7 µM (PAOI) 19 nM (PA14)	Binds Sdh	Inhibits pyoverdine production in <i>P. putida</i> KT2440
Phenazines	Microbe (various bacteria)	Gram-positive bacteria	-	114 MBEC 9.38, 2.35, 0.20 µM (MRSA, MRSE, VRE)	Potentially through binding iron (II)	-
Bromoageliferin 128	Marine (<i>Agelas conifera</i>)	Many	IC ₅₀ 10.6 µM (MRSA) 28.3 µM (<i>A. baumannii</i>)	144 IC ₅₀ 0.89 µM (MRSA) 28.3 µM 151 IC ₅₀ 26 µM (<i>A. baumannii</i>)	Binding response regulators	Reduces voltage-dependent calcium entry in PC12 cells
Flustramine C 160	Marine (<i>Flustra foliacea</i>)	Many	30% <i>A. baumannii</i> biofilm inhibition at 100 µM	179 IC ₅₀ 16.3 µM (<i>E. coli</i>) 13.4 µM (<i>S. aureus</i>)	Potentially through indole signaling pathways	-
Meridianin D 180	Marine (<i>Aplidium meridianum</i>)	<i>S. aureus</i> <i>M. smegmatis</i>	IC ₅₀ 87.4 µM (MRSA) IC ₅₀ 21.5 µM EC ₅₀ 51.0 µM (<i>M. smegmatis</i>)	188 IC ₅₀ 9.62 µM (MRSA) 189 IC ₅₀ 7.5 µM EC ₅₀ 27.9 µM (<i>M. smegmatis</i>)	Unknown	Inhibits multiple kinases
Brominated Furanones	Marine (<i>Delisea pulchra</i>)	<i>S. enterica</i>	-	190 IC ₅₀ 10 µM	QS Inhibition	Cytotoxic