Innovative Strategies for Combating Biofilm-Based Infections

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Roberta J. Melander and Christian Melander

1 Introduction

The identification of novel targets and compound-scaffolds to combat biofilms is a valuable tool in the fight against infectious diseases, many of which are now known to be biofilm-mediated. Continuing research into the environmental cues and genetic elements that play a part in the regulation of biofilms, such as quorum sensing, two-component signal transduction systems, cyclic-di-GMP signaling, and indole-signaling, along with an evolving knowledge of the composition of biofilms, has led to numerous innovative strategies for combating biofilms.

Strategies to inhibit biofilm formation, and/or eradicate established biofilms, have the potential to have a profound impact on human medicine by enhancing the efficacy of antibiotics that are otherwise ineffective against biofilm bacteria. Controlling biofilm development via non-microbicidal mechanisms should limit the pressure on bacteria to evolve resistance as compared to the selective pressures exerted by conventional bactericidal entities. Here, we aim to provide an overview of the various strategies that have been explored for the inhibition or eradication of bacterial biofilms, focusing on strategies that operate via non-microbicidal mechanisms. These approaches range from: effecting phenotype shifts through controlling cell-signaling pathways with small molecules, to enzymatic approaches to biofilm eradication by matrix degradation. Such strategies have the potential, when paired with conventional antibiotics that will kill planktonic bacteria, to eliminate established biofilm infections.

1.1 Biofilms

Biofilms, defined as highly organized surface-associated communities of bacteria encased within an extracellular matrix, play a significant role in infectious disease. Biofilms contribute to both pathogenesis and antibiotic/host immune resistance, and have a considerable impact on many fields, including medicine, food, environment, and industry. The impact of biofilms upon infectious disease is a particular cause for concern given the fact that The National Institutes of Health (NIH) estimates that 80 % of all bacterial infections occurring in the human body are biofilm related, and it is estimated that 17 million new biofilm infections arise each year in the U.S., resulting in up to 550,000 fatalities (Quave et al. 2012). Biofilms play a role in lung infections of cystic fibrosis (CF) patients, burn wound infections, ear infections, bacterial endocarditis, chronic wound infections, and tooth decay (Musk and Hergenrother 2006). In addition to increased mortality rates, biofilm mediated infections impart a

R.J. Melander • C. Melander (✉)
Department of Chemistry, North Carolina State University, Raleigh, NC, USA
e-mail: ccmeland@ncsu.edu

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considerable economic burden; with device related biofilm infections adding over one billion dollars to US hospitalization costs annually (Shirtliff and Leid 2009). The total economic impact of biofilms to the agricultural, engineering, and medical sectors of society has been estimated at billions of dollars annually (Davies 2003).

Bacteria within a biofilm present a number of characteristics that lead to difficulties in their eradication. They are phenotypically distinct from their planktonic counterparts, particularly in regard to growth rates and gene expression (Donlan and Costerton 2002). Biofilms are inherently insensitive to antibiotics, exhibiting upwards of 1000-fold higher resistance than planktonic bacteria (Rasmussen and Givskov 2006). Furthermore, the higher cell densities found within a biofilm (10^{11} CFU/mL compared to 10^{8} CFU/mL for bacteria in the planktonic state) vastly increase the opportunity for horizontal gene transfer, with transfer rates of up to 1,000-fold higher than for planktonic cells (Hausner and Wuertz 1999), leading to an increased likelihood of the emergence of strains with increased resistance or altered virulence profiles (Quave et al. 2012).

1.2 Effectiveness of Conventional Antibiotics in Eradicating Biofilms

There are many factors that lead to the increased resistance bacteria exhibit towards antibiotics while in a biofilm state. These include changes in gene expression related to antibiotic resistance, such as upregulation of genes responsible for efflux pumps (Davey and O'Toole 2000). The biofilm matrix, which is predominantly comprised of self-produced extracellular polymeric substances (EPS) including carbohydrates, proteins, lipids and extracellular DNA, affects penetration of certain antibiotic classes into the biofilm (Mah and O'Toole 2001; Flemming et al. 2007). The reduced growth rate exhibited by cells embedded deep within the biofilm that experience a lack of nutrients and oxygen also likely plays a role in resistance to antibiotics (Mah and O'Toole 2001), as bacterial cells with reduced metabolic activity are inherently more recalcitrant to antimicrobial therapies (Spoering and Lewis 2001). This reduced efficacy is due to the fact that almost all conventional antibiotics target one of five biosynthetic processes occurring in actively growing bacteria: the biosynthesis of proteins, RNA, DNA, peptidoglycan and folic acid (Hurdle et al. 2011). In the absence of genetic resistance mechanisms, conventional antibiotics efficiently kill growing and dividing bacterial cells, but are very inefficient at killing non-multiplying bacteria (Coates and Hu 2008), leading to minimal inhibitory concentrations (MICs) of conventional antibiotics against biofilm-residing bacteria being 100-1,000-fold higher than against planktonic bacteria (Hoiby et al. 2010a).

Despite this recalcitrance, certain conventional antibiotics have demonstrated activity against bacterial cells growing in the biofilm state. For example, rifampin has activity against staphylococcal biofilm cells, particularly when used in combination with one or more additional antibiotic including: fusidic acid, vancomycin and ciprofloxacin (Saginur et al. 2006). Colistin has demonstrated activity against non-dividing *Pseudomonas aeruginosa* biofilm cells that reside at the center of a biofilm; however metabolically active cells can acquire resistance to polymyxins and other cationic peptides rapidly due to modification of their outer membrane. Therefore, colistin appears to only be a viable option for treating *P. aeruginosa* biofilm infections when used in combination with another antibiotic such as ciprofloxacin (Hoiby et al. 2010a). Even more problematic is the fact that several studies have shown that the presence of sub-MIC levels of some antibiotics, as experienced at the beginning or end of a dosing regimen, or by cells deep within the biofilm throughout the regimen, can induce in vitro biofilm formation in a range of bacteria. Almost all the most commonly used antibiotic classes including: aminoglycosides, ß-lactams, fluoroquinolones, glycopeptides, rifamycins, tetracyclines exhibit this phenomenon. This has prompted a need to develop alternative strategies to combat biofilms (Kaplan 2011).

2 Small Molecule Strategies for Combating Biofilms

Several approaches to the design of small molecules that either inhibit the formation of biofilms, or cause established biofilms to disperse, have been investigated. Many of these approaches involve designing small molecules to interfere with the bacterial communication pathways that control the formation and maintenance of biofilms. Additionally, many natural products possess anti-biofilm activity and have been used as structural inspiration in medicinal chemistry programs to identify anti-biofilm compounds. These strategies are detailed below.

2.1 Disruption of Quorum Sensing Pathways

Quorum sensing (QS) involves intercellular communication between bacteria via the production of diffusible small molecules, and allows the community to make coordinated alterations in gene expression based upon population density (Camilli and Bassler 2006). There exist a number of different quorum sensing molecules that have been shown to play a role in the formation of biofilms in numerous species of bacteria. These include: acyl homoserine lactones (AHLs) in Gram-negative bacteria, autoinducer peptides (AIPs) employed by Gram positive bacteria, and autoinducer-2 (AI-2) molecules derived from 4,5-dihydroxy-2,3-pentanedione (DPD), which are interspecies signals employed by both Gram-negative and Gram-positive bacteria (Irie and Parsek 2008). The approach of interfering with QS pathways to modulate biofilm formation has been one of the most popular approaches to combating biofilms and a number of small molecules that modulate QS pathways have been developed.

Acyl Homoserine Lactones (AHLs)

AHLs are the predominant QS signal employed by Gram-negative bacteria, and more than 70 species of bacteria are known to communicate via AHL-mediated QS, with specificity mediated via variation in the length and oxidation state of the acyl side chain (for example, compounds **1**–**3**) (Fig. 6.1) (Amara et al. 2009). In the well-studied QS system of *Vibrio fisheri*, AHL synthesis occurs when the *luxI* gene is activated, producing the AHL synthase enzyme LuxI. When the AHL reaches a threshold intracellular concentration, it binds to the transcriptional activator LuxR, leading to activation of the *luxR* operon. AHLs freely diffuse in and out of bacterial cells allowing the AHL concentration to correlate to the bacterial concentration, which enables population density-based control of gene expression and therefore control of various processes such as biofilm formation and maintenance (Finch et al. 1998). Homologous systems to the LuxI and LuxR proteins exist in several Gram-negative bacteria, including LasI and LasR in the medically important biofilm-forming bacterium *P. aeruginosa* (Parsek and Greenberg 2000). The role of AHL based QS in biofilm development was first demonstrated by the discovery that a *P. aeruginosa*

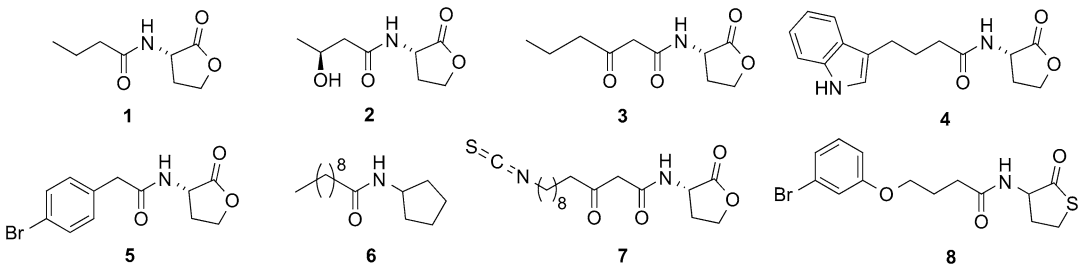


Fig. 6.1 Native AHLs from *P. aeruginosa* **1**, *V. harveyi* **2**, and *V. fisheri* **3**, and synthetic AHL analogues **5**–**8**

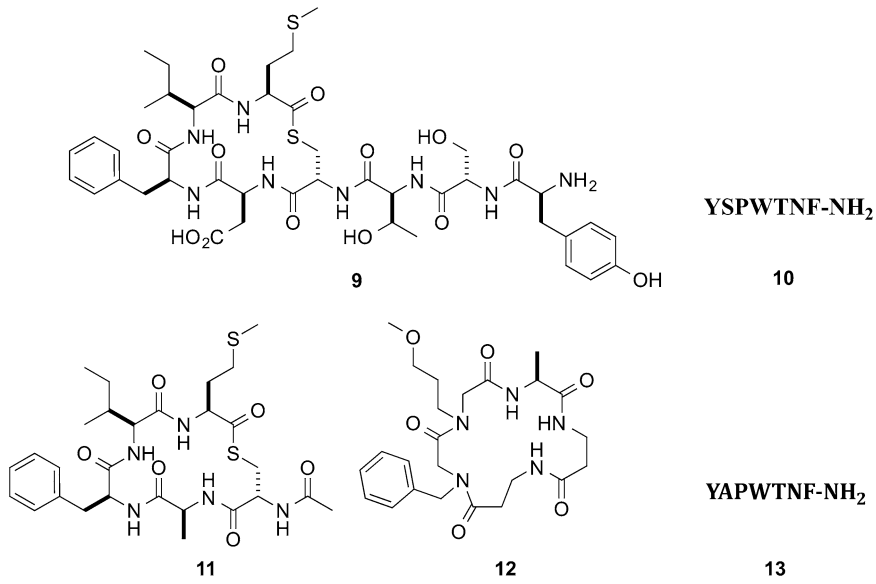


Fig. 6.2 *S. aureus* AIP-1 **9**, RIP **10**, and synthetic AIP analogues **11–13**

lasI mutant forms biofilms with altered morphology and reduced thickness compared to the wild type strain, and that the mutant biofilms are sensitive to biocides. Exogenous addition of the synthetic QS ligand to the mutant strain resulted in biofilms that resembled the wild-type and restoration of biocide resistance (Davies et al. 1998).

AHLs are prone to hydrolysis at physiological pH and the ring-opened product is QS inactive, and they have also been reported to possess immunomodulatory activity (Yates et al. 2002). This has led to the design of several synthetic AHL analogues with the aim of improving stability and in vivo properties. Recently, the Blackwell group have documented the synthesis and identification of a number of natural and unnatural AHLs with the ability to modulate QS in *P. aeruginosa* and *Agrobacterium tumefaciens,* and demonstrated that two of the most active AHL analogues inhibit biofilm formation in *P. aeruginosa* PAO1. Significant inhibition of biofilm formation was observed for a green fluorescent protein (GFP) producing PAO1 strain in the presence of synthetic AHLs **4** and **5** (Fig. 6.1) at 50 μM (Geske et al. 2005). The *N*-acyl cyclopentylamide analogue, C10-CPA **6**, completely prevents biofilm formation by GFP labeled *P. aeruginosa* PAO1 at 250 μM under flow conditions (Ishida et al. 2007).

A series of inhibitors of the *P. aeruginosa* transcriptional regulator LasR bearing electrophilic functional groups, including isothiocyanates, bromoacetamides, and chloroacetamides, were designed to react with Cys79 in the LasR binding pocket. Isothiocyanate containing ligands were able to covalently and selectively bind Cys79 and inhibit quorum sensing, with compound, **7** (Fig. 6.1), inhibiting PAO1 biofilm formation by almost 50 % at a concentration of 50 μM (Amara et al. 2009). More recently, the thiolactone **8** was reported by the Bassler group and reduces *P. aeruginosa* biofilm height by almost threefold at 100 μM, and brings about a reduction in nematode death in a *Caenorhabditis elegans P. aeruginosa* infection assay at 50 μM (O'Loughlin et al. 2013).

Autoinducing Peptides (AIPs)

QS in Gram-positive bacteria is predominantly mediated by autoinducing peptides (AIPs) (Lyon and Novick 2004). The accessory gene regulator (*agr*) operon, which plays an important role in

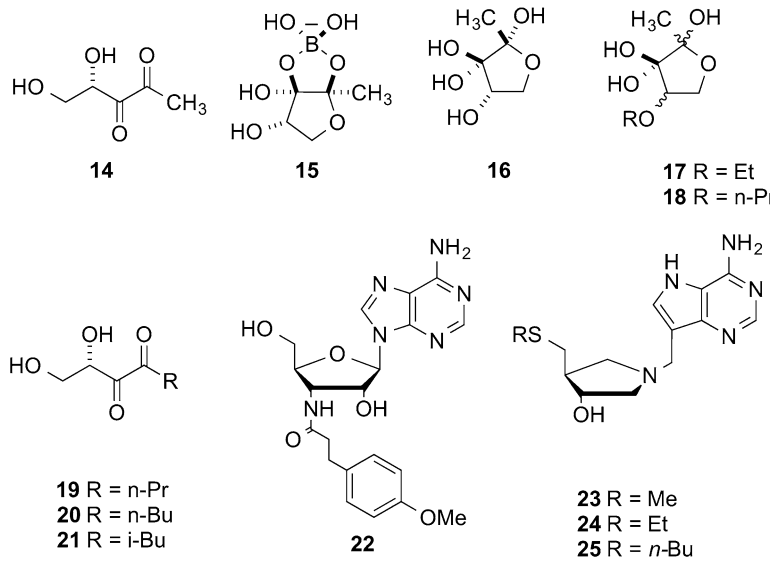


Fig. 6.3 DPD **14**, native AI-2 s from *V. harveyi* **15**, and *S. typhimurium* **16**, and synthetic compounds **17**–**25** that interfere with AI-2 QS

biofilm formation in *Staphylococcus aureus*, contains the *agrD* gene, which encodes AgrD, the precursor of the *S. aureus* AIP **9** (Fig. 6.2). The *S. aureus* AIP binds AgrC, a histidine kinase, leading to the expression of a small non-coding RNA known as RNA-III, which subsequently down-regulates genes that encode adhesins required for biofilm formation (Bordi and de Bentzmann 2011). The RNA-III activating protein (RAP) activates TRAP (target of RNA-III activating peptide) via phosphorylation, leading to increased cell adhesion and biofilm formation (Fux et al. 2003). The RNA-III inhibiting peptide (RIP) **10** inhibits phosphorylation of TRAP leading to reduced biofilm formation, and RIP has been investigated as an anti-biofilm agent, successfully preventing infections in multiple animal models (Giacometti et al. 2003). AIP analogues have also been investigated as anti-biofilm agents; the truncated peptide **11** is a potent AgrC QS antagonist (Chan et al. 2004), while the peptidomimetic **12** promotes biofilm formation in *S. aureus* (Fowler et al. 2008), and the synthetic RIP derivative FS3 **13** (Fig. 6.2) enhances the efficacy of tigecycline in a rat model of staphylococcal vascular graft infection (Simonetti et al. 2013).

Autoinducer-2 (AI-2)

Autoinducer-2 (AI-2) is the collective term for a number of autoinducing signals derived from DPD **14** (Fig. 6.3). The synthase that drives DPD production is conserved in over 55 bacterial species (Waters and Bassler 2005) and AI-2 has been proposed as a putative universal quorum sensing mechanism shared by both Gram-negative and Gram-positive bacteria. The structures of AI-2 from *Vibrio harveyi* (S-THMF borate **15**) and *Salmonella typhimurium* (R-THMF **16**) are depicted in Fig. 6.3 (Miller et al. 2004).

Several AI-2 analogues have been developed, including the C4-alkoxy-5-hydroxy-2,3-pentanediones **17** and **18** (Fig. 6.3), which activate the AI-2 pathway more potently than DPD, exhibiting submicromolar EC_{50} values in a *V. harveyi* reporter strain (Tsuchikama et al. 2012). Several C1-substituted DPD analogues act as antagonists of AI-2-based QS, with propyl-DPD **19** and butyl-DPD **20** exhibiting IC_{50} values ten-

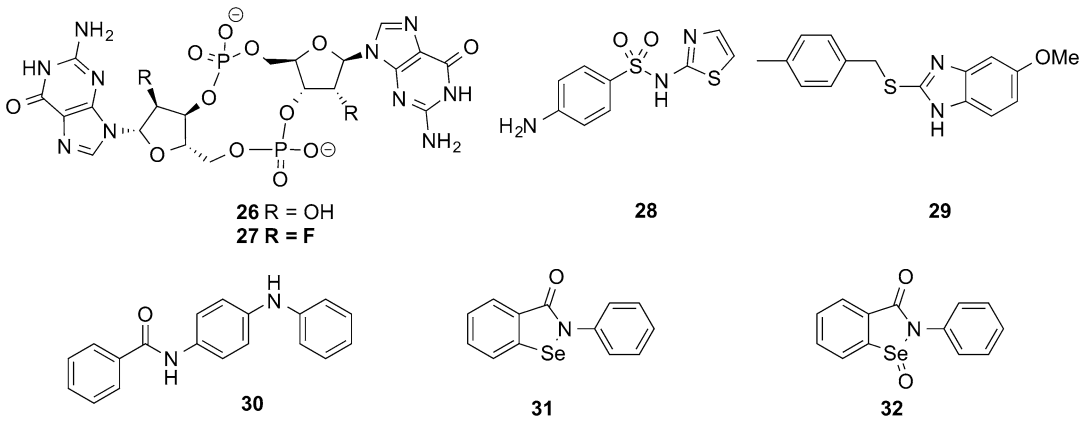


Fig. 6.4 c-di-GMP **26**, 2′-F-c-di-GMP **27**, and small molecules **28**–**32** that interfere with c-di-GMP signaling

fold below that of DPD (Lowery et al. 2008). Another C1-substituted analogue, isobutyl-DPD **21** significantly inhibits maturation of *Escherichia coli* biofilms and also brings about near complete clearance of pre-formed *E. coli* biofilms when administered in combination with gentamicin (Roy et al. 2013).

Another approach to interfering with AI-2 signaling is to design compounds that inhibit synthesis of the signal itself. The biosynthesis of DPD begins from *S*-adenosyl methionine (SAM), therefore a number of nucleoside analogues have been evaluated as potential inhibitors, with the finding that the adenosine analogue **22** (Fig. 6.3) blocks AI-2-based QS without affecting bacterial growth. This compound was subsequently shown to affect biofilm formation in several *Vibrio* species (Brackman et al. 2009). Another enzyme involved in the biosynthesis of both AI-2 and AHLs is 5′-methylthioadenosine nucleosidase (MTAN), which catalyzes the *N*-glycosyl hydrolysis of SAM and other adenosyl derivatives (Ronning et al. 2010; Gutierrez et al. 2009). Transition state analogues **23**, **24**, and **25** (Fig. 6.3) inhibit MTAN activity in cell lysates of a virulent *Vibrio cholerae* strain with nanomolar IC_{50} values, and inhibit QS induction in *V. harveyi* reporter strains. Analogues **23** and **25** also inhibit MTAN in *E. coli*, resulting in inhibition of AI-2 production, while compound **25** reduces biofilm formation in *E. coli* and *V. cholerae* by 18 and 71 % respectively at a concentration of 1 μM without inhibiting planktonic growth (Gutierrez et al. 2009).

2.2 Small Molecule Disruption of Other Signaling Pathways

c-di-GMP

Bis-(3′5′)-cyclic di-guanylic acid (c-di-GMP) **26** (Fig. 6.4) is a ubiquitous second messenger bacterial signaling molecule. Diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), which are responsible for the synthesis and breakdown of c-di-GMP, respectively (Yan and Chen 2010) are regulated by various environmental and intracellular signals such as oxygen, light, and small molecules, and play a role in the transition between the planktonic and biofilm lifestyle in *P. aeruginosa* (Tamayo et al. 2007). It has been observed that exopolysaccharide (a component of the biofilm matrix) synthesis is regulated by c-di-GMP in various proteobacterial species including *V. cholera, P. aeruginosa, Pseudomonas fluorescens, A. tumefaciens, E. coli,* and *Salmonella enterica* (Ryjenkov et al. 2005), while dispersion of bacteria from a mature biofilm is also thought to be regulated by c-di-GMP (Tamayo et al. 2007).

Interfering with c-di-GMP signaling is therefore another attractive target for the control of biofilm

formation. Modification of the 2′ position of c-di-GMP generates analogues with selectivities for different classes of c-di-GMP binding proteins, and the 2′-fluoro analogue **27** exhibits higher affinity for DGCs than does the native ligand (Zhou et al. 2013). Screening of a 1,120 compound library with known biological activities identified sulfathiazole **28** (Fig. 6.4), which inhibits DGC activity and inhibits *E. coli* biofilm formation with an IC_{50} value of 5.8 μM without significantly inhibiting bacterial growth (Antoniani et al. 2010). The benzimidazole **29** was identified from a high-throughput screen (HTS) of approximately 66,000 compounds and natural-product extracts from the Center for Chemical Genomics at the University of Michigan for compounds that affect induction of a *V. cholerae* c-di-GMP-inducible transcriptional fusion. Compound **29** is a broad-spectrum inhibitor of biofilm formation, significantly inhibiting biofilm formation by *P. aeruginosa* (CF-145), *Klebsiella pneumoniae, Erwinia amylovora,* and *Shigella boydii*, at 100 μM and by MRSA USA300, and *S. aureus* Newman at 25 μM, without affecting bacterial growth. IC_{50} values for inhibition of biofilm formation by *P. aeruginosa* and *V. cholerae* were reported to be 45.9 and 32.3 nM, respectively, however compound **29** did not disperse preformed biofilms (Sambanthamoorthy et al. 2011).

Another small molecule, compound **30**, was identified from a high-throughput screen for inhibitors of DGCs and shown to significantly reduce c-di-GMP levels in *V. cholerae* and inhibit biofilm formation by *V. cholerae* and *P. aeruginosa* (Sambanthamoorthy et al. 2012). In other studies, ebselen **31** and ebselen oxide **32** were shown to reduce DGC activity by covalently modifying cysteine residues, leading to inhibition of c-di-GMP-receptor binding and subsequent inhibition of biofilm formation in *P. aeruginosa* (Lieberman et al. 2014).

Indole Signaling

Indole is another putative universal intercellular signal molecule (Lee and Lee 2010) that plays a role in the control of many behaviors including biofilm formation (Lee et al. 2007b). Eighty-five species of bacteria have been documented to produce indole, with both indole-positive and indole-negative strains of bacteria altering various behaviors upon the extracellular presence of indole (Lee and Lee 2010). *E. coli* produces high concentrations (>600 μM) of extracellular indole when cultured in rich medium and indole has been shown to decrease biofilm formation in *E. coli* in a non-toxic manner (Lee et al. 2007a). Indole is readily converted by oxygenases found in several bacterial species to a number of oxidized indole derivatives, such as hydroxyindoles **33**–**35**, isatin **36**, and isoindigo **37** (Fig. 6.5), which have been investigated for their effects on biofilm formation. 5-Hydroxyindole **33** and 7-hydroxyindole **34** inhibit biofilm formation by enterohemorrhagic *E. coli* (EHEC) by 11-fold and 27-fold respectively at a concentration of 1 mM, compared to 18-fold inhibition by indole at the same concentration (Lee et al. 2007a). Indole-3-acetaldehyde, **38**, which is produced by the plant pathogen Rhodococcus sp. BFI 332, inhibits biofilm formation by EHEC without affecting planktonic growth, while the spent medium of *Rhodococcus* sp. BFI 332, from which **38** was identified, was shown to have an inhibitory effect on biofilm formation by two Staphylococcal species, *S. aureus and Staphylococcus epidermidis* (Wood et al. 2008).

The plant secondary metabolites 3-indolylacetonitrile (IAN) **39** and indole-3-carboxyaldehyde (I3CA) **40** (Fig. 6.5) reduce biofilm formation by *E. coli* O157:H7 by 11-fold and 24-fold respectively at 100 μg/mL, compared to a threefold reduction brought about by indole. In contrast to indole and the hydroxyindoles, which promote biofilm formation by *P. aeruginosa* PAO1, **39** and **40** weakly inhibit biofilm formation by this bacterium, effecting a 1.9-fold and 2.3-fold reduction in respectively (Lee et al. 2011a). A follow up study to identify indole derivatives with increased anti-virulence activity compared to IAN **39**, identified the simple synthetic indole derivatives, 7-fluoroindole (7FI) **41**, and 7-formylindole **42**, which reduce *P. aeruginosa* biofilm formation by fourfold and fivefold respectively at concentrations of 1 mM (Lee et al. 2012).

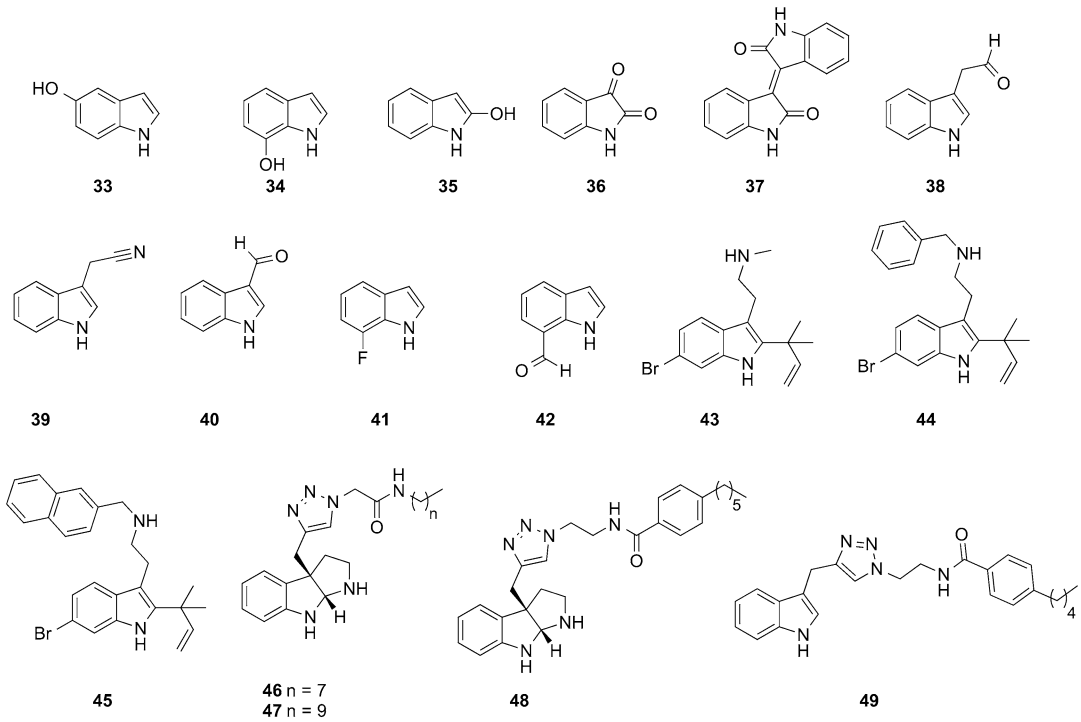


Fig. 6.5 Hydroxyindoles **33**–**35**, naturally occurring indole derivatives, **36**–**39**, and synthetic indole derivatives including simple derivatives **41** and **42** and flustramine derivatives **43**–**49**

The pyrroloindoline and indole containing monobrominated secondary metabolites from the bryozoan *Flustra foliacea* (Peters et al. 2003) have been used as scaffolds for the design of compounds to interfere with indole signaling. One metabolite, desformylflustrabromine (dFBr) **43** (Fig. 6.5) inhibits biofilm formation by *E. coli* and *S. aureus* with IC_{50} values of 70 μM and 174 μM respectively, however this compound exhibits microbicidal effects on planktonic growth at these concentrations (Bunders et al. 2011b). Synthetic manipulation of various regions of the dFBr scaffold led to the identification of **44,** which inhibits biofilm formation by *S. aureus* and *E. coli* with IC_{50} values of 5.9 μM and 53 μM respectively, and compound **45**, which inhibits biofilm formation by *S. aureus* and *E. coli* with IC_{50} values of 7.7 μM and 15.6 μM respectively. Both compounds elicit their effects through a non-microbicidal mechanism. Mechanistic studies with both compounds in wild-type and knockout *E. coli* strains revealed that the activity of these indole derivatives is dependent on the same factors as the activity of indole itself, namely temperature, the transcriptional regulator SdiA, and tryptophanase, suggesting that the observed anti-biofilm activity may be occurring through modulation of indole-based signaling pathways (Bunders et al. 2011b; Minvielle et al. 2013b). Other flustramine derived small molecules with non-microbicidal anti-biofilm activity include compound **46**, which inhibits biofilm formation by *Acinetobacter baumannii* with an IC_{50} value of 193 μM, compound **47**, which inhibits biofilm formation by *E. coli* with an IC_{50} value of 36 μM, and compounds **48** and **49**, which exhibit Gram-positive anti-biofilm activity, inhibiting biofilm formation by *S. aureus* (Bunders et al. 2011a; Minvielle et al. 2013a).

Two-Component Systems

Two component signal transduction systems (TCS) are a class of regulatory systems found mainly in prokaryotes that allow the organism to sense and

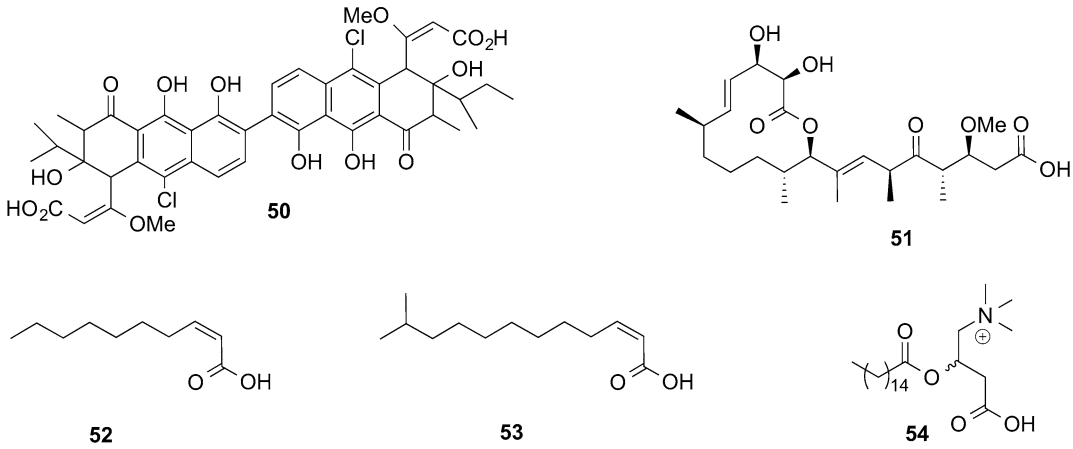


Fig. 6.6 Small molecules that interfere with two-component system signaling (**50** and **51**), and fatty acid derived small molecules that modulate biofilm formation (**52**–**54**)

respond to changes in their environment. The prototypical TCS consist of a histidine kinase and a response regulator. In response to an extracellular signal, the histidine kinase undergoes autophosphorylation at a conserved histidine residue. The phosphorylated histidine kinase subsequently transfers the phosphate group to a conserved aspartate residue on the response regulator that results in activation of an effector domain and ultimately leads to the response, which typically involves altered gene expression (Stock et al. 2000). TCS regulate the expression of genes that control numerous behaviors including biofilm formation and maintenance (Gotoh et al. 2010). Examples of TCS involved in biofilm regulation include, AgrAC (*S. aureus*) (Gotoh et al. 2010) LytSR (*S. aureus*) (Sharma-Kuinkel et al. 2009), BfmRS (*A. baumannii*) (Tomaras et al. 2008) BfmRS (*P. aeruginosa*) (Petrova et al. 2011), GacAS (*P. aeruginosa*) (Parkins et al. 2001) and VicRK (*S. mutans*) (Reck et al. 2011).

The role of TCS in the control of biofilm formation and maintenance, and the fact that it has been demonstrated that both the histidine kinase and response regulator are targetable by small molecules makes them attractive targets for the development of anti-biofilm compounds (Worthington et al. 2013). Walkmycin C **50** (Fig. 6.6), a member of the walkmycin family of natural products produced by *Streptomyces* sp. strain MK632-100 F11, inhibits autophosphorylation of the histidine kinase WalK (YycG) from both *B. subtilis and S. aureus,* and was subsequently shown to inhibit the in vitro autophosphorylation activity of purified VicK (an orthologue of WalK that is involved in sucrose dependant biofilm formation) from *Streptococcus mutans* with an IC_{50} of 2.87 μM. At sub-MIC levels, walkmycin C causes the formation of abnormal biofilms and a reduction in biofilm mass (37.6 % of the control at 0.63 μg/mL) in *S. aureus* (Eguchi et al. 2011). Walkmycin C also inhibits the histidine kinase CiaH in *S. mutans*, which plays a role in sucrose-dependent biofilm formation, with an IC_{50} of 4.87 μM (Qi et al. 2004). The bacterial secondary metabolite carolacton **51**, affects the viability of *S. mutans* biofilms at nanomolar concentrations, killing bacterial cells that are within a biofilm state while exhibiting only minor effects on the growth of planktonic bacteria. Carolacton was subsequently shown to affect the expression of several TCS in *S. mutans* biofilm cells, two of which (VicKRX and ComDE) were shown to play essential roles in the response to carolacton. It was shown that this response is mediated through the serine/threonine protein kinase PknB, which the authors posited could be due to phosphorylation of VicR by PknB Although the effect of carolacton upon *S. mutans* biofilms is microbicidal, the selectivity toward

biofilm bacteria over planktonic bacteria represents an alternative strategy for the small molecule control of biofilms and validates targeting of TCS as a means of controlling bacterial biofilms (Reck et al. 2011; Kunze et al. 2010).

Other Signaling Molecules

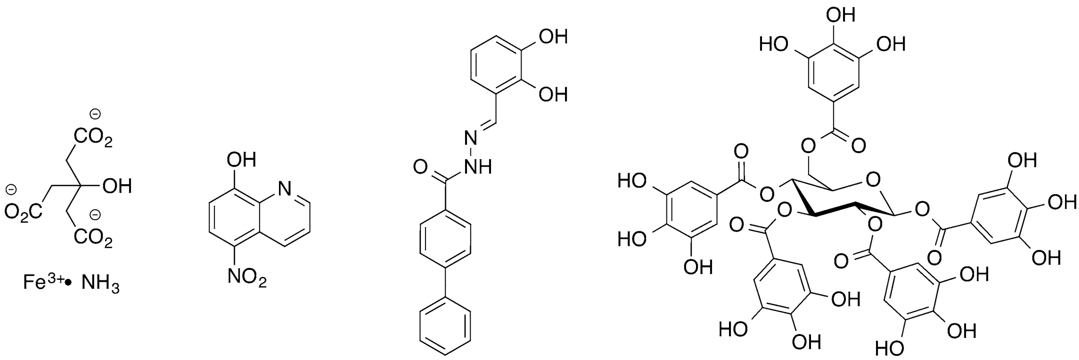
The fatty acid signaling molecule, cis-2-decenoic acid **52** (Fig. 6.6), which is produced by *P. aeruginosa,* has been shown to induce the dispersion of established biofilms of several bacterial species when assayed using microcolony disaggregation and measurement of the number of cells released into the bulk culture medium as the readout of biofilm dispersion (Davies and Marques 2009). The ability of cis-2-decenoic acid **52** to disperse pre-formed biofilms of *A. baumannii* and *S. aureus* has also been assayed with a crystal violet reporter assay, which measures the biofilm mass remaining as a function of compound concentration. However, under these conditions less than 10 % dispersion was observed at a concentration of 400 μM (Su et al. 2011). Another fatty acid, cis-11-methyl-2-dodecenoic acid **53**, known as diffusible signal factor (DSF), produced by *Xanthomonas campestris*, is able to disaggregate cell flocs formed by *X. campestris* (Dow et al. 2003). The fatty acid-derived molecule palmitoyl-DL-carnitine **54** (Fig. 6.6) inhibits biofilm formation by *P. aeruginosa* PAO1, *E. coli*, and *Listeria monocytogenes* with IC_{50} values of 13.2, 3.00 and 5.85 μM respectively, but does not disperse preformed biofilms (Wenderska et al. 2011; Nguyen et al. 2012).

Certain D-amino acids produced by bacteria in the stationary phase are thought to be a native signal for biofilm disassembly in *B. subtilis,* and exhibit inhibitory effects upon biofilm formation by several bacteria including *B. subtilis, S. aureus* and *P. aeruginosa*. This activity is thought to be a result of disruption of the connection between an extracellular matrix protein and the bacterial cell, which is posited to prevent growth of initial foci into larger assemblies of cells. D-Tyrosine, D-leucine, D-tryptophan, and D-methionine synergistically inhibit biofilm formation by *B. subtilis*, exhibiting activity at 10 nM in combination, while only displaying activity at micromolar to millimolar concentrations when administered alone. The analogous L-amino acids, and other D-amino acids including D-alanine and D-phenylalanine were inactive. Against *S. aureus*, the combination of D-phenylalanine, D-proline, and D-tyrosine exhibit greater biofilm inhibitory activity than the combination of D-tyrosine, D-leucine, D-tryptophan, and D-methionine (Kolodkin-Gal et al. 2010; Hochbaum et al. 2011).

2.3 Metal Ions and Chelation

Some metal ions play an important role in biofilm formation, stimulating cell–cell adhesion and aggregation (Abraham et al. 2012), and the effect of metal ion chelation upon biofilm formation has been investigated as a strategy to combat biofilms. Ethylenediaminetetraacetic acid (EDTA) was shown to disperse *P. aeruginosa* biofilms and result in 1,000-fold enhanced killing by gentamicin (Banin et al. 2006), while a combination of EDTA and minocycline effectively reduced in vitro and ex vivo staphylococcal catheter colonization (Raad et al. 2003).

A screen of 4,500 compounds belonging to the University of Illinois Marvel Library Compound Collection (MLCC) led to the identification of ferric ammonium citrate (FAC) **55** (Fig. 6.7) as a non-toxic inhibitor of *P. aeruginosa* PA14 biofilms, exhibiting an IC_{50} value of approximately 60 μM. No toxicity was observed even upwards of 500 μM and it was shown that neither the ammonium nor citrate ions were responsible for the observed anti-biofilm activity. The subsequent analysis of other iron salts (ferric chloride, ferric sulfate, ferrous sulfate) revealed biofilm inhibitory profiles that were comparable to that of FAC and it was also demonstrated that upon switching to an iron-rich growth media established *P. aeruginosa* biofilms could be disrupted and cleared in continuous flow experiments (Musk et al. 2005). High iron levels have been shown to suppress extracellular DNA (eDNA) release and structural development of biofilms in *P. aeruginosa*, and it is thought that



55 **56** **57** **58**

Fig. 6.7 Small molecules **55**–**58** that modulate biofilm formation by chelating metal ions

biofilm formation by *P. aeruginosa* can only occur in a narrow iron concentration range (1–100 μM) (Yang et al. 2007). Iron chelated by either picolinic acid or acetohydroxamic acid prevents biofilm formation by *P. aeruginosa* PA14 and also by 20 clinical isolates of *P. aeruginosa* (Musk and Hergenrother 2008). Additionally, at sub-MIC levels the antibiotic nitroxoline **56** inhibits *de novo P. aeruginosa* biofilm synthesis with little microbicidal activity toward planktonic bacteria. The thickness of preformed biofilms was also reduced by 40 % by nitroxoline at 8 μg/mL, and iron and zinc complexation was shown to underlie the activity of nitroxoline (Sobke et al. 2012).

A high-throughput screen of 66,095 small molecules by Junker and Clardy identified several compounds that inhibit PAO1 biofilms with IC_{50} values below 20 μM, the structures of some of which lead the authors to posit that they act via metal chelation. The most active compound identified, **57** (Fig. 6.7), exhibits an IC_{50} value of 530 nM in minimal media, making it one of the most potent biofilm inhibitors reported to date (Junker and Clardy 2007).

The plant derived sugar 1,2,3,4,6-penta-O-galloyl-b-n-glucopyranose (PGG) **58**, which inhibits biofilm formation by *S. aureus* by preventing surface attachment of cells and reducing the production of the important matrix component polysaccharide intercellular adhesin (PIA), is a strong iron chelator and it has been shown that addition of $FeSO_4$ restores biofilm formation and PIA production, indicating that metal ion chelation is the mechanism of biofilm inhibition by PGG (Lin et al. 2012).

2.4 Natural Products and Natural Product Analogues

Natural products provide a diverse array of chemical structures and possess a plethora of biological activities. Natural products that inhibit or disperse bacterial biofilms provide a starting point for medicinal chemistry programs from which more efficacious compounds can be developed, and may even lead to the identification of new anti-biofilm targets. Much of the natural product inspiration for these programs has come from compounds isolated from plants and marine organisms.

2.4.1 Plant Natural Products

Interest in studying natural products derived from plant sources for the discovery of new biologically active compounds is not uncommon, and many traditional medicines have been rooted heavily in the use of plant extracts (Lai and Roy 2004). The antibiotic properties of garlic have long been known and can be attributed to the disulfide allicin **59** (Fig. 6.8) (Slusarenko et al. 2008). *P. aeruginosa* biofilms treated with garlic extracts in vitro exhibit enhanced susceptibility to treatment with tobramycin and to grazing by polymorphonuclear (PMN) leukocytes, and

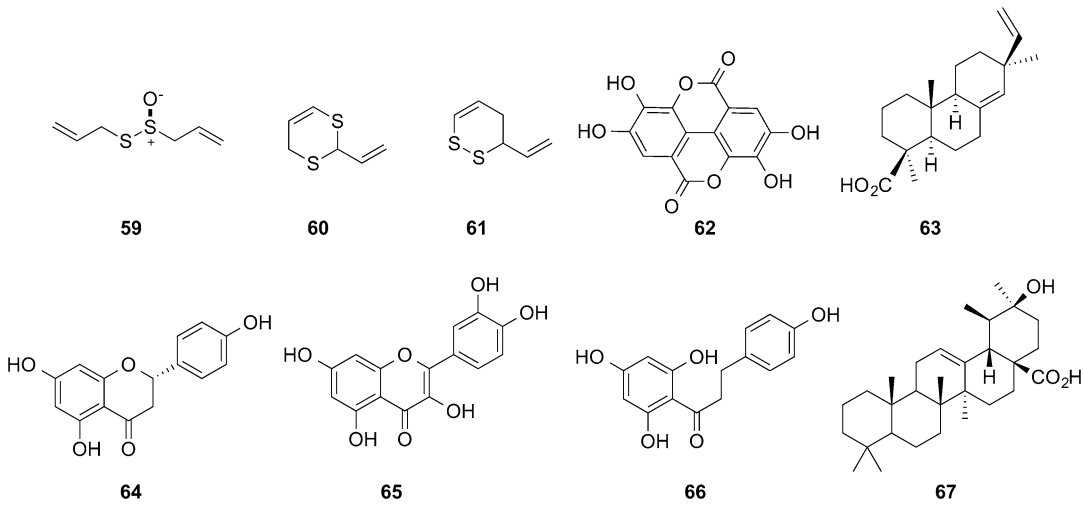


Fig. 6.8 Plant natural products that modulate biofilm formation

garlic extracts have also been shown to clear pulmonary *P. aeruginosa* infections in mouse models when dosed prophylactically (Bjarnsholt et al. 2005). Compounds identified as being responsible for these activities include the dithianes **60** and **61**, which have been shown to modulate QS in LuxR systems and do not display microbicidal activity (Persson et al. 2005).

Extracts from *Rubus ulmifolius* (Elmleaf blackberry), which are rich in the polyphenol ellagic acid **62** and its glycosylated derivatives, inhibit biofilm formation by *S. aureus* at concentrations of 50–200 μg/mL, and ellagic acid alone also possesses anti-biofilm properties (Quave et al. 2012). The resin acid 4-epi-pimaric acid **63**, which was isolated from *Aralia cachemirica* L. (Araliaceae), inhibits biofilm formation by *S. mutans* on a saliva-coated surface at sub-MIC concentrations (4 μg/mL) (Ali et al. 2012). Resveratrol, which is found in the skin of grapes and berries, inhibits biofilm formation by *V. cholerae* (Augustine et al. 2014). Several flavonoids from citrus species, including naringenin **64**, and quercetin **65**, are antagonists of AHL and AI-2-mediated cell–cell signaling in *V. harveyi*, and also inhibit biofilm formation by *V. harveyi* and *E. coli* by over 80 % at 50 μM (Vikram et al. 2010). The antioxidant phloretin **66** (found in apple tree leaves) reduces biofilm formation by EHEC O157:H7 by 89 % at 25 μg/mL without affecting the growth of planktonic cells and also without affecting commensal *E. coli* K-12 biofilms. This is significant as the eradication of commensal bacteria by conventional antibiotics is a major problem and can result in increased susceptibility to infection by opportunistic pathogens, making antibacterial strategies that selectively eliminate pathogenic bacteria a potential future therapeutic avenue for human medicine. Phloretin represses several genes including those encoding toxins, AI-2 importer genes, and curli genes, and reduces fimbria production in EHEC biofilm cells (Lee et al. 2011b). The triterpene ursolic acid **67** (Fig. 6.8), which was identified from a screen of 13,000 compounds, inhibits *E. coli* biofilm formation at 22 μM without affecting growth, upregulating genes responsible for several processes tied to biofilm formation including: chemotaxis, motility, and heat shock (Ren et al. 2005).

2.4.2 Marine Natural Products

Halogenated Furanones

Much research has centered on the biological activities and effects that halogenated furanones (HFs) have on bacterial QS (Hentzer et al. 2002; Manefield et al. 2002; Ren et al. 2001). HFs were isolated from the marine red algae *Delisea pulchra*

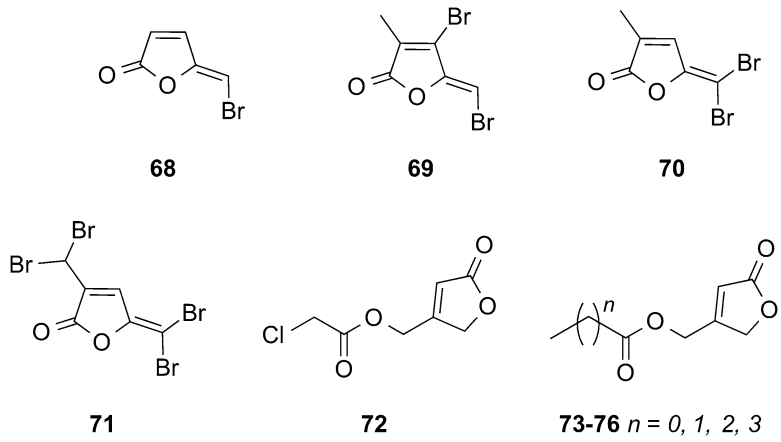


Fig. 6.9 Halogenated furanones and derivatives that modulate biofilm formation

and exhibit structural similarities to AHLs, possessing a non-polar aliphatic carbon "tail" of varying lengths attached to a relatively polar "head". Naturally occurring HFs inhibit biofilm formation and swarming in *E. coli* and *B. subtilis* at 20–60 μg/mL (Ren et al. 2001, 2002), Synthetic manipulation of the furanone scaffold has resulted in a number of HF analogues anti-biofilm activity. HFs have been shown to inhibit luminescence in *V. fischeri*, to affect the expression of numerous genes involved in virulence factor production and biofilm formation in *P. aeruginosa* (Hentzer et al. 2003; Rasmussen et al. 2000) and to penetrate *P. aeruginosa* biofilm matrices and interfere with bacterial QS, without any associated microbicidal properties (Hentzer et al. 2002). HF **68**, which lacks a side chain and possesses a vinyl bromide, is one analogue that exhibits such activity. Biofilms grown in the presence of HF **68**, display noticeable changes in biofilm maturation and structure, similar to those observed in *P. aeruginosa* mutants lacking the *lasI* system. The furanone causes rapid bacterial detachment, resulting in a loss of biomass in comparison to untreated biofilms, while other experiments have shown that furanone treated biofilms are sensitized to tobramycin (Hentzer et al. 2003). HF **68** also exhibits activity against Gram-positive biofilms, effecting a 63 and 76 % reduction in growth of *S. mutans* and *Streptococcus intermedius* biofilms respectively at a concentration of 60 μM (Lonn-Stensrud et al. 2007). HFs **69**, **70**, and **71** inhibit *E. coli* biofilm formation by up to 80 % at concentrations of 224 μM (**69** and **70**) and 141 μM (**71**) (Han et al. 2008), while furanones **72–76**, which contain an exocyclic ester functionality and are non-brominated, are effective inhibitors of *P. aeruginosa* biofilm formation (Kim et al. 2008) (Fig. 6.9).

Despite this promising activity, there are some issues with halogenated furanones that currently limit their therapeutic application, including toxicity, carcinogenic properties, and instability under aqueous conditions (Hentzer and Givskov 2003) Future medicinal chemistry efforts will be necessary to overcome some of these limitations.

2-Aminoimidazoles

The marine alkaloids oroidin **77** and bromoageliferin **78** (Fig. 6.10) are nitrogen-dense small molecules characterized by the incorporation of one or more 2-aminoimidazole (2-AI) sub-units that are produced by marine sponges of the family Agelasidae (Braekman et al. 1992). They are believed to a play a role as a chemical anti-feeding defense mechanism against predators, and were first reported to inhibit biofilm formation by the Gram-negative marine α-proteobacterium *Rhodospirillum salexigens* in 1997, exhibiting IC_{50} values of 169 μM and 2.43 nM respectively (Yamada et al. 1997).

These alkaloids have been used as structural inspiration for the development of simplified, synthetically accessible small molecules with anti-biofilm activity. Two simplified analogues of bromoageliferin: TAGE (*trans*-bromoageliferin) **79** and CAGE (*cis*-bromoageliferin) **80** (Fig. 6.10) inhibit biofilm formation by *P. aeruginosa* via a non-microbicidal mechanism, while TAGE also disperses established *P. aeruginosa* biofilms (Huigens et al. 2007). Installation of dibrominated acylpyrrole moieties similar to those present in bromoageliferin onto the TAGE scaffold results in compound **81**, which exhibits increased biofilm inhibitory activity against *P. aeruginosa*, with low micromolar IC_{50} values; however **81** does not disperse pre-formed biofilms with the same efficiency as TAGE (Huigens et al. 2008).

Oroidin **77** exhibits a similar level of activity to TAGE and CAGE for inhibition of biofilm formation by *P. aeruginosa* (Richards et al. 2008a), and a structure-activity-relationship (SAR) study of the oroidin scaffold was subsequently conducted thorough the construction of a 50-member library of analogues. Any modifications to the 2-AI head of the molecule completely abolished activity establishing the importance of this moiety for anti-biofilm activity. The most active molecule discovered was dihydrosventrin (DHS) **82** (Fig. 6.10), a reduced analogue of the oroidin family alkaloid sventrin, which has also been isolated from marine sponges. DHS inhibits the formation of biofilms by *P. aeruginosa*, *A. baumannii,* and *Bordatella bronchiseptica* with mid-micromolar IC_{50} values, and also disperses preformed biofilms of the same three species (Richards et al. 2008c). The construction of a library of second-generation analogues led to the identification of the *para*-bromo phenyl derivative **83**, which exhibits more potent non-microbicidal anti-biofilm activity against *A. baumannii* than DHS ($IC_{50} = 27$ μM, $EC_{50} = 41$ μM) (Richards et al. 2008d).

Other oroidin analogues with potent anti-biofilm activity include those in which the native amide bond directionality is reversed (Richards et al. 2008b). Several such analogues that possess an aliphatic tail group (**84**–**86**), are low micromolar inhibitors of *P. aeruginosa* biofilm development, exhibiting at least four times more potent activity than oroidin. The most active reverse amide 2-AI (**85**) is also an extremely effective dispersion agent, eradicating pre-formed *P. aeruginosa* biofilms with mid-micromolar EC_{50} values. Several of the reverse amide class of 2-AIs, including compound **86**, also inhibit biofilm formation by *A. baumannii* and a biotinylated analogue of compound **86** was used to identify the molecular target of this class of molecules in *A. baumannii* as BfmR (Thompson et al. 2012), the response regulator component of the BfmRS TCS. BfmR plays an important role in a biofilm formation and *bfmR* mutants exhibit significantly reduced biofilm-forming ability and altered planktonic cellular morphology (Tomaras et al. 2008), thus making BfmR an attractive anti-biofilm target.

The incorporation of a triazole moiety into the oroidin analogues has resulted in a number of highly active compounds (Rogers and Melander 2008), with the most active compounds possessing a substituted unsaturated aryl pendant group. Compound **87** (Fig. 6.10), which possesses broad-spectrum biofilm inhibition and dispersion properties, exhibiting low micromolar-high nanomolar IC_{50} values against *P. aeruginosa*, *A. baumannii,* and *S. aureus,* also exhibits synergism with several antibiotics for the dispersion of biofilms of several bacterial strains, and re-sensitizes planktonic bacteria of drug-resistant strains of *S. aureus* and *A. baumannii* to the effects of conventional antibiotics (Rogers et al. 2010a).

Aryl-2-AI derivatives (Fig. 6.10) have also proven to be active anti-biofilm compounds, with compound **88** effectively inhibiting *E. coli* biofilm formation (Bunders et al. 2010). Steenackers and co-workers subsequently reported a related series of aryl 2-AI compounds, the parent compound of which, compound **89**, displays moderate biofilm inhibitory activity against *S. typhimurium* and *P. aeruginosa*. Incorporating substituents on the phenyl ring of **89** resulted in increased biofilm inhibition activity, and introduction of substituents at the 5-position of the 2-AI ring resulted in a further slight increase in activity, with the most active analogues **90**–**96**

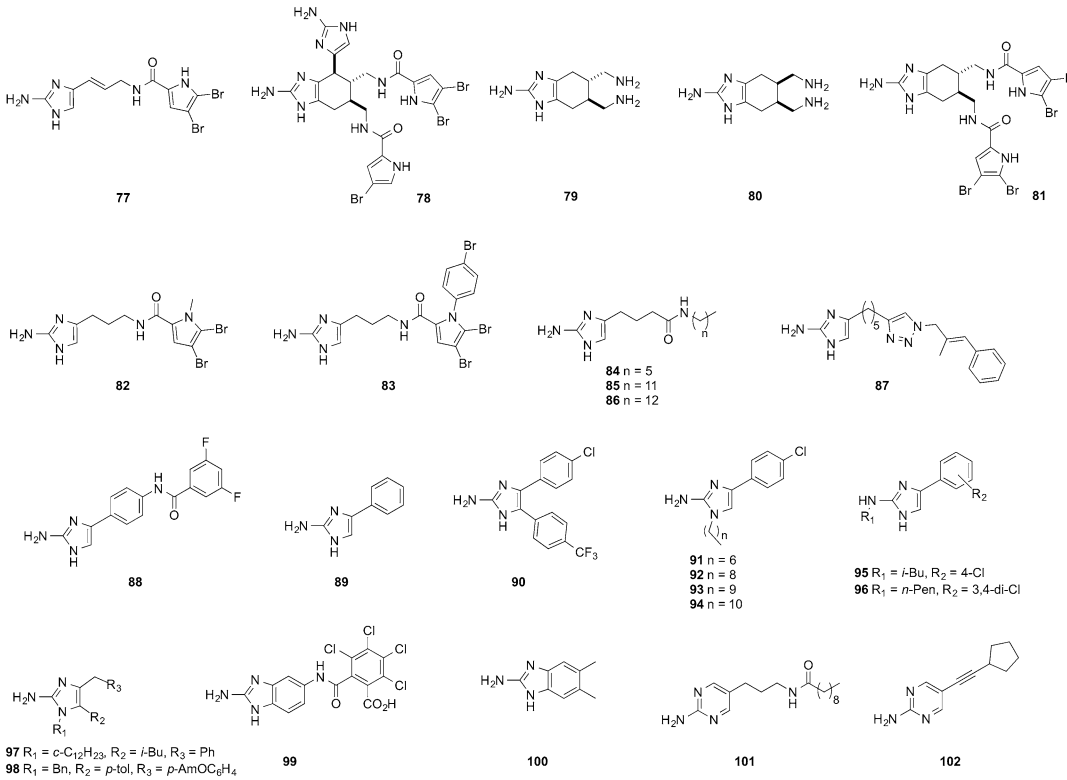


Fig. 6.10 2-AI containing marine natural products oroidin **77** and bromoageliferin **78**, synthetic 2-AI derivatives **79**–**98**, 2-ABI derivatives **99** and **100**, and 2-AP derivatives **101** and **102**

exhibiting low micromolar IC_{50} values for the inhibition of biofilm formation by *S. typhimurium* and *P. aeruginosa* (Steenackers et al. 2011a, b). A related series of 1,4,5-trisubstituted naamine alkaloids, including compounds **97** and **98**, also inhibit biofilm formation by *S. typhimurium and P. aeruginosa* with micromolar IC_{50} values (Ermolat'ev et al. 2010).

Scaffolds related to the 2-AI heterocycle that have been investigated for anti-biofilm properties include the 2-aminobenzimidazole (2-ABI) and 2-aminopyrimidine scaffolds (2-AP) (Fig. 6.10). The 2-ABI **99** is highly active against Gram-positive bacteria, both inhibiting and dispersing biofilms via a mechanism that was inhibited by the presence of Zn (II), and the compound was subsequently shown to bind Zn (II) (Rogers et al. 2009). A series of related 2-ABIs was later studied against *P. aeruginosa*, leading to the identification of compound **100**, which inhibits biofilm formation with an IC_{50} value of 4 μM and disperses pre-formed biofilms with an EC_{50} value of 92 μM (Frei et al. 2012). 2-APs are less active inhibitors of biofilm formation than their 2-AI counterparts, particularly against Gram-negative bacteria and none reported thus far disperse pre-formed biofilms. However compounds **101** and **102** do inhibit biofilm formation by *S. aureus* (Lindsey et al. 2012).

Other Marine Natural Products

Other natural product scaffolds of marine origin that possess anti-biofilm activity include: the diterpene alkaloid (−)-ageloxime D **103** (Fig. 6.11), which was isolated from the marine sponge *Agelas nakamurai,* and inhibits biofilm formation by *S. epidermidis* without affecting bacterial growth (Hertiani et al. 2010), and the structurally simple bacterial metabolite ethyl *N*-(2-phenethyl) carbamate **104** (Fig. 6.11),

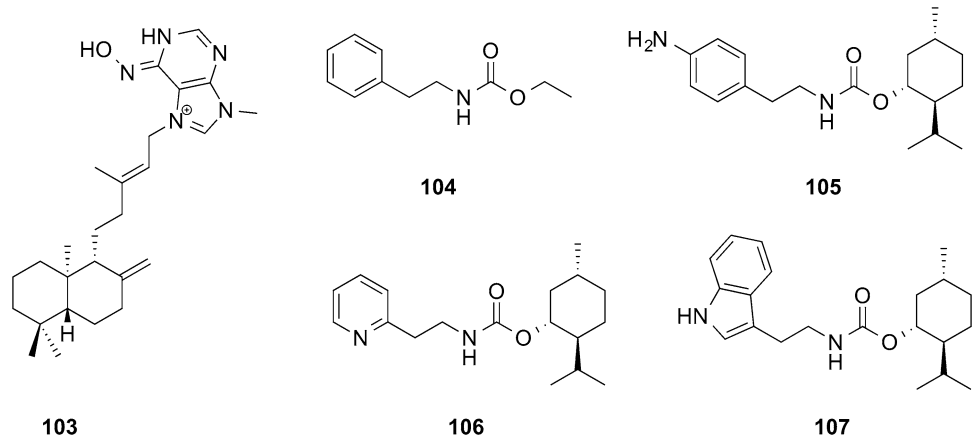


Fig. 6.11 Other marine natural products that modulate biofilm formation; (−)-ageloxime D **103**, naturally occurring carbamate **104** and synthetic derivatives **105**–**107**

which was isolated from the culture medium of the marine bacterium SCRC3P79 (Cytophaga sp.), and possesses moderate anti-biofilm activity against *R. salexigens* (Yamada et al. 1997). Compound **104** was subsequently shown to be moderately active in inhibiting biofilm formation by several medically relevant strains of bacteria, and construction of a library of readily accessible analogues based upon compound **104** led to the identification of a number of compounds with increased activity, including the menthyl derived compounds **105**–**107**, which inhibit biofilm formation by several strains of *S. aureus* with IC_{50} values in the mid to low micromolar range (Rogers et al. 2010b).

3 Macromolecule Approaches

3.1 Enzymatic Degradation of Matrix Components

Given that the matrix typically accounts for over 90 % of the dry mass of a biofilm and forms the basis of the three-dimensional structure of the biofilm, immobilizing the cells and allowing for cell-cell communication, degradation of the matrix is an attractive approach to eradicating biofilms. As mentioned earlier, bacteria within a biofilm produce EPS that constitute the matrix. Enzymatic degradation of the biomolecules that constitute the EPS is an innate phenomenon employed by several diverse bacterial species, and involves the secretion of enzymes such as glycosidases, proteases, and DNases that degrade various components of the EPS (Kaplan 2010). Examples of endogenously produced matrix degrading enzymes include the *S. aureus* DNase thermonuclease, the glycoside hydrolase dispersin B, and alginate lyase, which is produced by *P. aeruginosa*. These enzymes, and many others, are used by the bacteria to initiate dispersion of the biofilm, which contributes to bacterial survival and disease transmission (Kaplan 2010), and several of these enzymes have been investigated as potential therapeutic agents.

Dispersin B inhibits the formation of biofilms by several medically relevant bacterial species including *S. aureus*, *S. epidermidis*, and *E. coli*, and disperses *S. epidermidis* and *E. coli* biofilms (Izano et al. 2008; Kaplan et al. 2004; Itoh et al. 2005). Dispersin B also sensitizes *S. epidermidis* biofilm cells to the action of antimicrobials (Izano et al. 2008; Donelli et al. 2007) and is active in vivo, lowering the rate of catheter colonization by *S. aureus* in combination with triclosan in a rabbit model of infection (Darouiche et al. 2009). Alginate lyase degrades the polysaccharide alginate and enhances the microbicidal activity of aminoglycosides against *P. aeruginosa* biofilms in vitro (Alkawash et al. 2006; Alipour et al. 2009; Lamppa and Griswold 2013).

Alginate lyase has demonstrated in vivo efficacy, enhancing the clearance of mucoid *P. aeruginosa* when coadministered with amikacin in a rabbit model of endocarditis (Bayer et al. 1992).

Biofilms formed in the presence of DNase exhibit reduced biomass and decreased antibiotic tolerance (Tetz and Tetz 2010), and the use of nucleases to combat biofilms has been explored against a number of bacterial strains. An extracellular DNase (NucB), produced by *Bacillus licheniformis,* induces rapid biofilm dispersal against several species of bacteria including *B. subtilis, E. coli,* and *Micrococcus luteus* (Bayer et al. 1992), while *S. aureus* also produces a nuclease, known as Nuc, that exhibits biofilm inhibitory activity (Kiedrowski et al. 2011) Recombinant human DNase I (rhDNase) inhibits biofilm formation by both *S. aureus* and *S. epidermidis,* disperses *S. aureus* biofilms, and increases the susceptibility of both *S. aureus* biofilm cells to chlorhexidine gluconate and povidone iodine, and *P. aeruginosa* biofilm cells to aminoglycosides in vitro (Alipour et al. 2009; Kaplan et al. 2012). rhDNase also displays activity in vivo, increasing the survival of *S. aureus*-infected *C. elegans* when administered in combination with tobramycin (Kaplan et al. 2012). rhDNase I (also known as dornase alfa and marketed as Pulmozyme by Genentech) is used in the clinic for the treatment of pulmonary disease in cystic fibrosis (CF) patients (Parsiegla et al. 2012), in which biofilm mediated *P. aeruginosa* infections are a major contributing factor to lung tissue damage (Hoiby et al. 2010b). Administration of Pulmozyme leads to reduced demand for antibiotics and improved lung function in CF patients (Frederiksen et al. 2006).

Endogenous proteases also play a role in biofilm dispersal (Boles and Horswill 2008) and exogenous addition has been investigated for the dispersal of established biofilms. For example, the *S. epidermidis* serine protease Esp inhibits *S. aureus* biofilm formation and eradicates preformed *S. aureus* biofilms. Esp also enhances the susceptibility of *S. aureus* biofilms to the antimicrobial peptide human beta-defensin 2 (hBD2), and exhibits activity in vivo, eliminating human nasal colonization by *S. aureus* (Iwase et al. 2010). Proteinase K exhibits anti-biofilm activity against *S. aureus*, believed to be by effecting an increase in proteolytic activity (Park et al. 2012). Finally, the metalloprotease serratopeptidase (SPEP) from *Serratia marcescens*, a widely used an anti-inflammatory therapeutic, inhibits biofilm formation by *P. aeruginosa* and *S. epidermidis,* enhances the activity of ofloxacin against biofilms of these bacteria (Selan et al. 1993), and inhibits biofilm formation by *L. monocytogenes* (Longhi et al. 2008).

3.2 The Use of Antibodies to Combat Biofilms

Another non-small molecule approach to eradicating biofilms is the use of antibodies. Monoclonal antibodies (mAbs) that bind the *P. aeruginosa* cell surface anchored exopolysaccharide Psl, which plays a role in the formation and maintenance of biofilms by acting as a scaffold for other biofilm initiating components (Jackson et al. 2004; Digiandomenico et al. 2012), were identified from a screen of an M13 phage-based human antibody library. Lead mAbs inhibit host cell attachment by *P. aeruginosa* and impart significant protection in multiple animal models of *P. aeruginosa* infection including a mouse acute lethal pneumonia model and a thermal injury model (Digiandomenico et al. 2012). Antibodies to the partially de-*N*-acetylated form of the staphylococcal surface polymer poly-N-acetylglucosamine (PNAG), which promotes biofilm formation, increase killing of *S. aureus* by human neutrophils, while passive immunization of mice with anti-dPNAG-DT rabbit sera results in increased clearance of *S. aureus* from the blood compared to mice treated with normal rabbit sera (Maira-Litran et al. 2005).

4 Conclusion

The contribution of the biofilm phenotype to the pathogenesis and host/antibiotic resistance of numerous bacterial infections, combined with the ineffectiveness of conventional antibiotics in eradicating such infections, has led to a serious

need for alternative strategies to target bacteria residing within a biofilm. Numerous different approaches to this problem have been investigated, including the design or identification of small molecules that interfere with bacterial communication and signaling pathways and inhibit biofilm formation or cause biofilm dispersion, and the use of enzymes to degrade specific components of the matrix thereby causing the biofilm to disperse. In combination with conventional antibiotics these strategies have the potential to treat biofilm-based infections and have a significant impact in the fight against infectious disease.

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