

Medicinal Chemistry as a Conduit for the Modulation of Quorum Sensing

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Introduction

The evolutionary development of bacterial cell-to-cell communication, a process termed “quorum sensing” (QS^a), enabled coordinated behavioral efforts of bacterial populations, thus providing for microbial interaction and competition with higher organisms. QS signaling defines the process of bacteria secreting and responding to small diffusible chemical signals, or autoinducers, in a cell density-dependent process.¹ As the number of cells, and thus autoinducer concentrations, increases, bacteria synchronize their gene expression to behave as a unified group. These concerted efforts are beneficial to the bacterial population but often come at the expense of human health, as QS has been shown to regulate processes such as biofilm formation and the expression of virulence factors. Consequently, the modulation of QS has emerged as a prophylactic and therapeutic antivirulence target of considerable interest.^{2,3}

QS signaling is mediated by autoinducing molecules that can be categorized into three major classes:^{4–7} (i) *N*-acyl homoserine lactones (AHLs), (ii) oligopeptides, and (iii) autoinducer-2 (AI-2).^{8,9} AHLs are produced by many Gram-negative bacterial species, and signals are differentiated by acyl chain length and oxidation patterns. Likewise, oligopeptides, such as staphylococcal autoinducing peptides (AIPs), are the signals employed by Gram-positive bacteria and are often chemically posttranslationally modified. Lastly, the AI-2 class of signals is derived from the precursor 4,5-dihydroxy-2,3-pentanedione (DPD) and has been shown to signal in both Gram-positive and -negative bacteria. Because of the shared DPD core of the

known AI-2 signaling molecules and the ubiquity of the DPD synthase in the bacterial kingdom, AI-2 has been suggested as a language of interspecies communication. In addition to these three classes, other small molecules including the *Pseudomonas* quinolone signal (PQS),¹⁰ 3-hydroxypalmitic acid methyl ester,¹¹ bradyoxetin,¹² and (*S*)-3-hydroxytridecan-4-one¹³ have also been identified. In general, individual species rely on chemically distinct signals to avoid interspecies cross-talk or interference. However, as discussed in this Perspective, the signals of one species often exert agonistic or antagonistic effects on the QS systems of other species.

Three main paradigms have been explored for the development of QS modulators as potential therapeutics: (i) interference with the signal synthase, (ii) sequestration of the autoinducer, and (iii) antagonism of the receptor, with the latter having received the most attention to date for the discovery of QS modulators. Additionally, other strategies, such as prevention of signal secretion or inhibition of downstream signaling events, have also been examined. In this Perspective, we will focus on the receptor antagonism approach toward QS modulators and review their development from a medicinal chemical perspective with a focus on the methods and rationale used for their discovery and/or design and synthesis. We will discuss agonists as well as antagonists of QS systems and have included relative potencies (EC₅₀ values for agonists and IC₅₀ values for antagonists) where given in the original literature. In consideration of these values, it is important to state that strict comparisons may only be applied within a set of analogues examined in a particular assay, largely because of variations in reporter assays used for analogue evaluation. Finally, we will discuss studies that have strived to establish QS as a viable target for the development of antimicrobial therapies.

AHLs

The best-studied QS circuits are the AHL-based systems employed by Gram-negative bacteria, and consequently, a variety of autoinducer analogues have been developed for this system. Historically, QS was first discovered in the marine bacterium *Vibrio fischeri*, which uses 3-oxo-C₆-HSL to regulate the generation of its bioluminescence.¹⁴ In general, AHLs are synthesized from two metabolic intermediates, *S*-adenosylmethionine (SAM) and a fatty acid from the acyl–acyl carrier protein complex, in a reaction that is catalyzed by an AHL-synthase, also known as a LuxI-type protein, named after LuxI in *V. fischeri*. Most AHLs diffuse freely across the bacterial cell membranes,

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^a Abbreviations: QS, quorum sensing; AHL, acyl homoserine lactone; AIP, autoinducing peptide; AI-2, autoinducer 2; DPD, 4,5-dihydroxy-2,3-pentanedione; PQS, *Pseudomonas* quinolone signal; 3-oxo-C₆-HSL, 3-oxo-C₆-homoserine lactone; 3-oxo-C₁₂-HSL, 3-oxo-C₁₂-homoserine lactone; 3-oxo-AHL, 3-oxoacyl homoserine lactone; 3-oxo-C₈-HSL, 3-oxo-C₈-homoserine lactone; C₁₀-HSL, C₁₀-homoserine lactone; SAM, *S*-adenosylmethionine; CF, cystic fibrosis; IC₅₀, half maximal inhibitory concentration; EC₅₀, half maximal effective concentration; gfp, green fluorescent protein; H-bond, hydrogen bond; SAR, structure–activity relationship; *p*C-HSL, *p*-coumaryl homoserine lactone; *P. aeruginosa*, *Pseudomonas aeruginosa*; *D. pulchra*, *Delisea pulchra*; yfp, yellow fluorescent protein; AI-1, autoinducer 1; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; HK, histidine kinase; RR, response regulator; *agr*, accessory gene regulator; TSST-1, toxic shock syndrome toxin 1; TRAP, targeting of RNAIII-activating peptide; RAP, RNAIII-activating peptide; RIP, RNAIII-inhibiting peptide; S-THMF-borate, (2*S*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate; R-THMF, (2*R*,4*S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran; AI-3, autoinducer 3; MHF, 4-hydroxy-5-methyl-3-(2*H*)-furanone; CAI-1, (*S*)-3-hydroxytridecan-4-one.

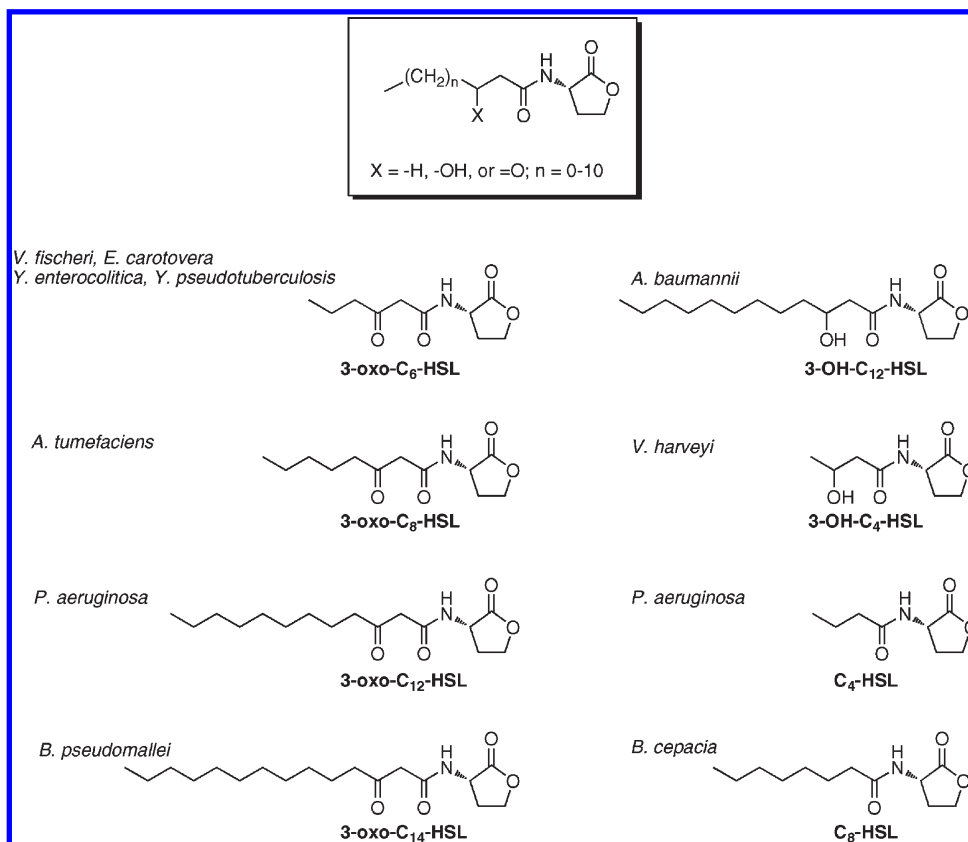


Figure 1. General structure of the AHL signal and representative examples of naturally occurring signals.

but AHLs with extended acyl chains, such as the 3-oxo-C₁₂-HSL signal of *Pseudomonas aeruginosa*, might require active secretion into the environment.¹⁵ When a critical threshold concentration is reached, the AHL signal binds its target receptor protein, (LuxR-type proteins), and this binding event in turn serves to activate the expression of target genes. In the case of *V. fischeri*, these genes, located on the *lux* operon, are responsible for bioluminescence generation. Interestingly, the initial discovery of QS-dependent bioluminescence regulation in *V. fischeri* was the first description of a group of bacteria acting in concert to achieve a common goal. Since this seminal discovery, several homologues of the LuxI- and LuxR-type proteins have been identified in other Gram-negative bacteria, such as the LasI/LasR proteins in *Pseudomonas aeruginosa* and the TraI/TraR proteins in *Agrobacterium tumefaciens*.¹⁶

A number of 3-oxo-AHLs have been shown to play an important role in processes involved in bacterial pathogenicity, particularly in the case of *P. aeruginosa* (Figure 1). This common environmental microorganism has acquired the ability to take advantage of weaknesses in the host defenses to become an opportunistic pathogen in humans.¹⁷ Most prominent is the role of *P. aeruginosa* in patients suffering from cystic fibrosis (CF), the most common inherited lethal genetic disorder, which follows an autosomal recessive inheritance pattern in Caucasian people. Approximately 30 000 people in the United States suffer from CF, and because of impaired lung defense function, CF patients are vulnerable targets for *P. aeruginosa*.¹⁸ The QS machinery of *P. aeruginosa* coordinates the regulation of virulence factors, including elastase, rhamnolipids, and phenazines as well as controls biofilm formation, which often has dire ramifications on human health, especially in the lungs of CF patients.

The importance of QS to the virulence of *P. aeruginosa* was demonstrated in a study of *P. aeruginosa* infections in burn

wounds by Abdul Hamood et al.¹⁹ A mouse model was used to monitor the spread of the bacteria in burned skin as well as bacterial dissemination throughout the body, and *P. aeruginosa* mutants lacking QS machinery exhibited reduced spread. QS is also involved in the regulation of pathogenicity in many other human pathogens, such as clumping and motility in *Yersinia pseudotuberculosis*²⁰ and *Yersinia pestis*,²¹ swimming and swarming motility in *Yersinia enterocolitica*,²² biofilm formation in *Acinetobacter baumannii*,^{23,24} virulence factor production in *Burkholderia mallei*,²⁵ pathogenicity in *Burkholderia pseudomallei*,²⁶ and protease activity and biofilm formation in the *Burkholderia cepacia* complex.^{27,28} Indeed, in several models of *B. cenocepacia* infection, including mammalian models, AHL-mediated QS was shown to play a central role in the control of pathogenic traits and was required for full pathogenicity in mouse and rat models.^{29,30} In this light, the potential for therapeutic intervention by targeting QS is evident; however, initial studies aimed toward the development of QS modulators mainly focused on the elucidation of the molecular mechanisms of the QS response.

The earliest example of the development of QS modulators was reported over 20 years ago by Anatol Eberhard and co-workers in a study aimed at deciphering the mechanism of luminescence induction in *V. fischeri*.³¹ A panel of 19 compounds was synthesized, with variations in chain length and saturation, oxidation pattern at the 3 position, and composition of the lactone ring (Figure 2). From this panel of compounds both agonists and antagonists were identified, and surprisingly only slight variations from the structure of the parent autoinducer, 3-oxo-C₆-HSL, produced profound changes in the observed bioluminescence. For example, extension of the carbon chain by two methylene units (3-oxo-C₈-HSL) still produces 50% bioluminescence, whereas movement of

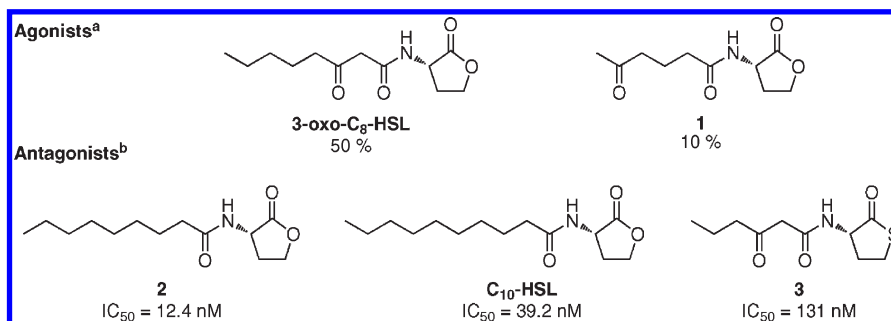


Figure 2. Representative examples of first AHL analogues designed around 3-oxo- C_{12} -HSL developed by Eberhard et al.³¹ (a) Percentages represent light output induced by analogue compared to the natural autoinducer. (b) Concentration of analogue needed to reduce the activity of 46.8 nM of autoinducer to that of 23.4 nM.

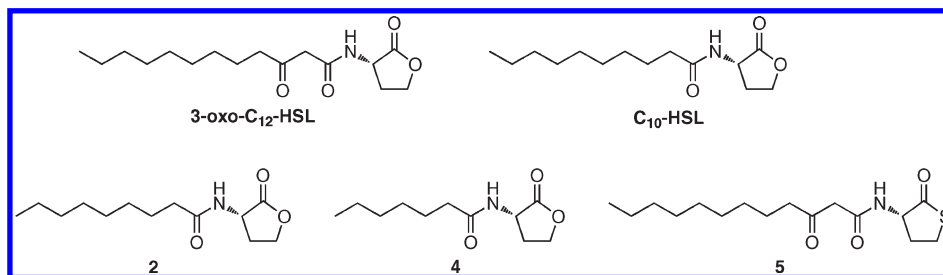


Figure 3. Examples of antagonists developed by Schaefer et al. against 3-oxo- C_{12} -HSL activity.³³

the oxo functionality from the 3 to 5 position (**1**) results in only 10% bioluminescence. Interestingly, compound **2** and C_{10} -HSL exhibited minimal agonist activity but powerful antagonist activity. The conversion of the lactone ring to thiolactone **3** also resulted in antagonistic activity (Figure 2). A similar set of compounds was evaluated as AHL analogues in *Erwinia carotovora*, which also uses 3-oxo- C_6 -HSL-dependent QS to regulate carbapenem biosynthesis.³² Again, similar trends were observed, as analogues with slight variations in acyl chain length or rings structure acted as agonists, albeit with diminished potency from the natural signal. From these trends, it was apparent that distinct chemical structures are necessary to induce full QS activity in different bacterial strains; however, it also became clear that small structural changes to the chemical scaffold of the natural autoinducers provide for widely variable biological activities.

The interactions between AHL analogues and the LuxR receptor were later studied in greater detail using an expanded repertoire of analogues and a recombinant *E. coli* strain containing the QS-regulon of *V. fischeri*.³³ In this work, the authors used a radiolabeled 3-oxo- C_6 -HSL analogue to investigate the competition for LuxR between the analogues and parent AHL. Analogues that were found to compete for LuxR binding were subsequently examined for either agonist or antagonist activity. Similar trends were observed with this panel of analogues as described previously by Eberhard et al.,³¹ in that the 3-oxo and lactone functionalities were required for productive LuxR activation, whereas acyl chains longer than five carbons resulted in antagonists of LuxR (Figure 3). These competition experiments, coupled with the QS activity of each analogue, strongly supported the now-accepted hypothesis of a binding event between the AHL signal and the LuxR protein. Furthermore, the activity of these analogues was indicative of a certain degree of flexibility within the binding site of the LuxR receptor, thus providing a foundation for the development of more elaborate analogues.

Around the same time, studies were underway in the laboratory of Barbara Iglewski to probe the binding of the *P. aeruginosa* AHL signal, 3-oxo- C_{12} -HSL, to its cognate receptor LasR.³⁴ A series of structural analogues with varying chain lengths, oxidation patterns, and heterocycle substitutions were synthesized via EDC-coupling of the sodium or lithium salt of the appropriate fatty acid chain with either the 2-amino lactone or amino thiolactone (Figure 4). These analogues were evaluated for agonist activity by monitoring the expression of a *lacZ* reporter fused to the *las* operon of *P. aeruginosa*. The trends observed with these analogues were similar to those observed with the LuxR-AHL interactions in that conservative variations of the 3-oxo- C_{12} -HSL parent signal resulted in diminished receptor binding. Interestingly, C_{10} -HSL, **2**, and **5**, which were found to be inhibitors of QS in *V. fischeri* (Figure 3), were discovered as agonists of QS in *P. aeruginosa*. Several antagonists against binding were uncovered in a competitive assay with radiolabeled 3-oxo- C_{12} -HSL; however, no true inhibitors of QS were found, as all analogues that compete for LasR binding were also able to induce still activity.

These early studies relied on simple analogues that focused on variations in acyl chain length, oxidation pattern, and substitution of the heteroatom in the ring to evaluate the critical functionalities of the AHL signals. Although these efforts did not reveal potent agonists or antagonists of QS, they were crucial in establishing, in principle, that bacterial communication may be modulated with synthetic analogues. Furthermore, these studies were also pivotal in instituting now-accepted concepts concerning signal–receptor interactions, namely, the sensitivity of the cognate receptor to structural deviations of the signal. On the other hand, these changes, although rationally designed from a chemical point of view, still provided for unpredictable biological effects.

A departure from the standard development of AHL analogues described above was pursued via the synthesis of conformationally constrained analogues reported by Kline et al.³⁵ Whereas previous analogues had mostly centered on

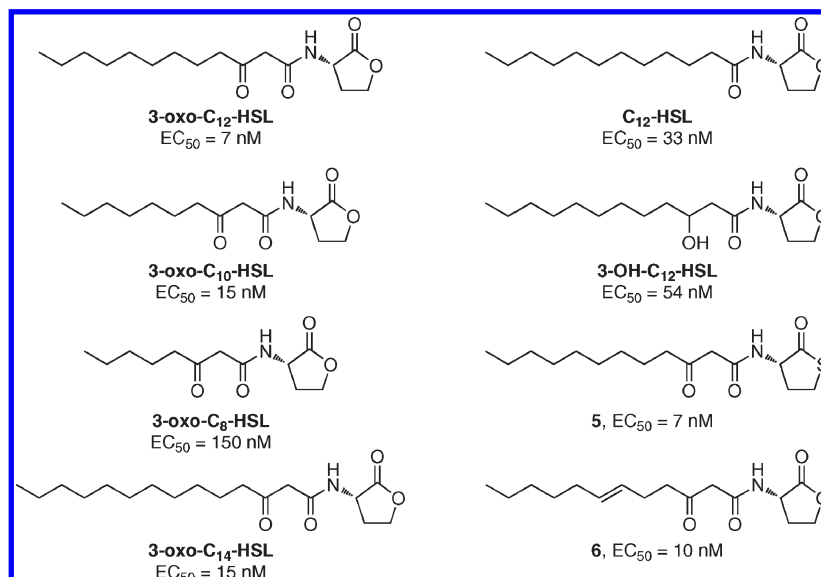


Figure 4. Analogues of 3-oxo-C₁₂-HSL designed to probe the binding interactions of LasR. EC₅₀ values represent concentration needed for 50% activation of *lasB*.³⁴

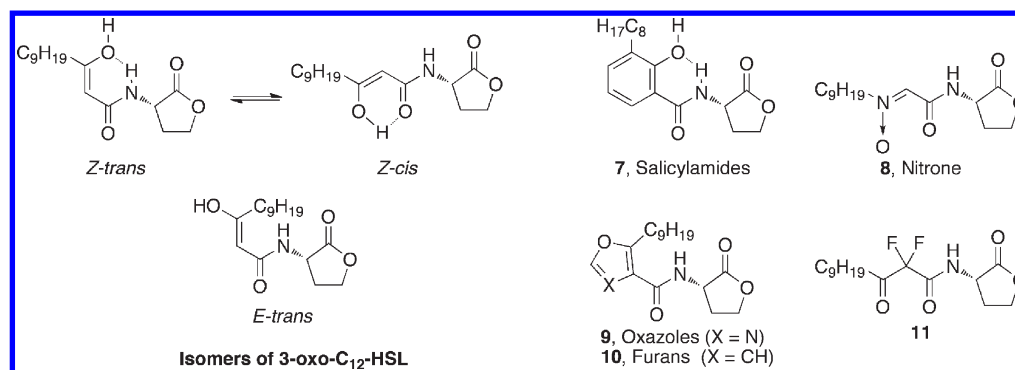


Figure 5. Isomers of 3-oxo-C₁₂-HSL and the constrained analogues developed by Kline et al.³⁵

modifying acyl tail length while keeping the analogues themselves flexible, the authors of this study sought to explore the role of the various tautomers of the β -ketoamide moiety in the QS of *P. aeruginosa*. This was achieved using four series of compounds to evaluate the biological activity of the *E* and *Z* enols, as well as the extended form of the AHL signal. To mimic the *Z* form, salicylamide **7** and nitron **8** frameworks were employed (Figure 5). On the other hand, simple heterocycles such as oxazoles **9** and furans **10** were introduced to emulate the *E* isomer. For the extended forms, gem-difluoro-substituted analogue **11** was constructed to effectively prevent enolization and subsequent tautomerization. Compound **11**, which displayed agonist activity, was the only active compound identified in this work, implying the necessity of proper acyl-tail geometry for LasR receptor binding. Despite the failure to uncover any analogues with potent activities, this work served to progress the development of AHL analogues past the previous reliance on minute, systematic changes to the AHL signal to incorporate novel chemical motifs.

The group of Hiroaki Suga introduced another new paradigm for the discovery of AHL analogues with the synthesis of a library of analogues constructed via solid phase organic synthesis. From this, several agonists and antagonists of QS in *P. aeruginosa* were uncovered from a library of compounds that replaced the homoserine lactone functionality of the

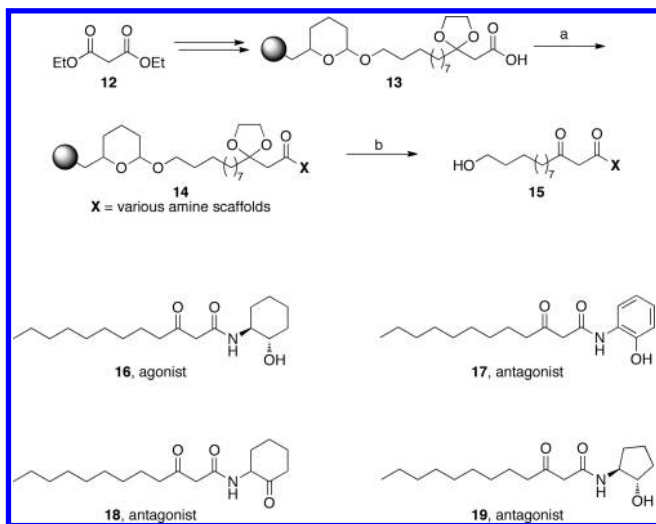
3-oxo-C₁₂-HSL signal with a variety of amines and alcohols. To rapidly generate a large panel of compounds, a solid phase approach was used in which the acyl chain was immobilized on a 3,4-dihydro-2*H*-pyran resin.^{36,37} To facilitate immobilization, the terminus of the acyl chain was modified to include a hydroxyl group. Previous work detailing the capacity of the LasR receptor to accommodate longer chains led the authors to reason that the increased length would have little effect on the biological activity. Once the acyl chain was immobilized on the resin, the ester was cleaved to reveal the carboxylic acid group (**13**), which then underwent reaction with a variety of amino alcohols or amino ketones (**14**, Scheme 1). With compounds of type **14**, treatment with acid provided for both deprotection of the ketone at the 3 position and cleavage from the resin.

With their library, compounds were evaluated for their activity against the QS of *P. aeruginosa* using a *lasI*-dependent GFP reporter assay. From the screening process, five agonists and eight antagonists were discovered, and importantly, resynthesis of these lead compounds without the terminal -OH group revealed that this modification did not interfere with the assay results. The series of (ant)agonists were initially identified and characterized using a gene-reporter assay; however, to evaluate the physiological effects of these new compounds, their effects on biofilm formation and the expression of the

virulence factors elastase and pyocyanin were evaluated. Treatment of *P. aeruginosa* with the most potent antagonist (**17**) resulted in the reduction of elastase production and alterations in the biofilm architecture although actual biofilm levels were not reduced. A few interesting observations can be made from the activity of these analogues: (1) an aminocycloalkane with an H-bond donor at the ortho-position is a shared structural feature of the antagonists, and (2) a cyclohexanol-derived agonist **16** may be converted to an antagonist by simply replacing the cyclohexanol ring with a phenol **17**, cyclohexanone **18**, or cyclopentanol **19** (Scheme 1). These observations speak to a key advancement put forth by this work: the efficiency of a solid phase approach for the production of a large panel of analogues.

Two years later, Helen Blackwell and co-workers also exploited the power of solid phase synthesis with a series of investigations aimed at the systematic evaluation of nonnatural AHL ligands. As a first step, a microwave-assisted solid phase synthesis was devised to provide rapid access to both natural and unnatural AHL analogues (Scheme 2).³⁸ This newly developed synthetic route allows for the introduction of different

Scheme 1. Solid Phase Synthesis and Representative Examples of 3-oxo-C₁₂-HSL Analogues Developed by the Suga Group^a

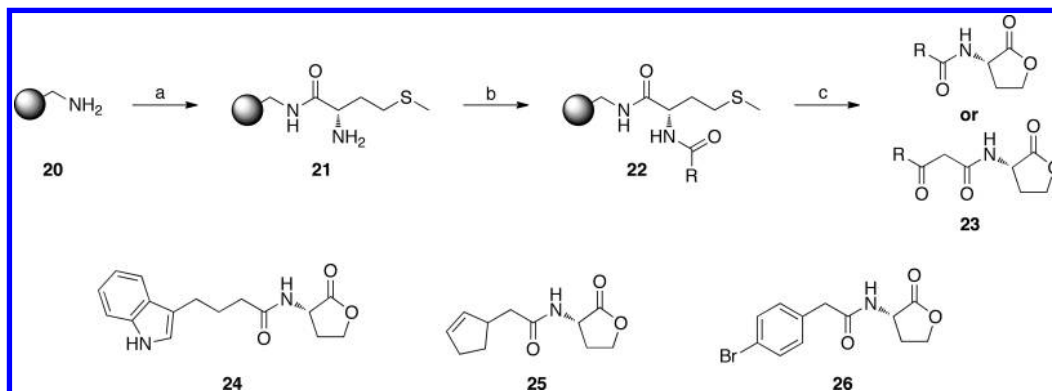


^a Compounds were initially synthesized with a terminal -OH group, and lead compounds were resynthesized as shown in **16–19**.^{36,37} Reagents: (a) various amines, EDC, DMAP, DIPEA, DMF, room temp, 72 h; (b) 95% TFA, room temp, 30 min.

acyl substituents, whereas the route developed by Suga and co-workers provided rapid access to diversity at the homoserine lactone functionality. The synthesis begins with the addition of methionine to polystyrene resin **20** to yield **21**, followed by the introduction of various carboxylic or protected β -keto acids for the generation of diversity in the acyl chain (**22**). The analogues were then subjected to treatment with cyanogen bromide in a microwave assisted cyclization/resin cleavage reaction to provide the target analogues of type **23**, which comprised both 3-oxo and non-3-oxo analogues. This new route was used to rapidly produce a variety of natural AHL signals in high purity, along with a panel of ~15 nonnatural AHL analogues, which were subsequently tested against QS-dependent reporter strains for both agonist and antagonist activity by measuring β -galactosidase activity in *Agrobacterium tumefaciens* and GFP fluorescence in *P. aeruginosa*. The screening revealed three compounds (**24–26**) that were 1–2 orders of magnitude more potent than the known *A. tumefaciens* QS inhibitors and about 2-fold more active than known *P. aeruginosa* QS inhibitors (Scheme 2). Importantly, the inhibition of QS-dependent gene expression in *P. aeruginosa* by **24–26** also translated to significant activity against biofilm formation. The structural similarities of these new QS modulators suggested similar modes of binding by the receptor proteins of the two organisms studied and provided the Blackwell laboratory with a structural guide for the construction of a larger set of compounds for the study of QS across a range of bacterial species.

In a recent series of publications, Blackwell and co-workers continued to build upon their previous findings and synthesized another library (~90) of compounds and examined the activity across three bacterial species: *A. tumefaciens*, *P. aeruginosa*, *V. fischeri*.^{39,40} Rather than focus exclusively on bulky substitutions at the terminus of the acyl chain as before, this library also featured an expanded repertoire of compounds to probe the effects of both chain length and ring stereochemistry in addition to acyl chain substitution. As predicted, the screening of a large focused library revealed both antagonists and agonists against the three strains. One particularly interesting observation came in the discovery of 3-oxo-C₈-HSL, the natural ligand for the *A. tumefaciens* TraR receptor, as a potent inhibitor against the QS of *P. aeruginosa* and a modest inhibitor against *V. fischeri* (although no IC₅₀ value was reported), speaking to the potential for QS as a form of interspecies competition as well as cooperation. However, what sets this study apart is the discovery of antagonists

Scheme 2. Solid Phase Synthesis and Representative Examples of AHL Analogues Developed by the Blackwell Group^{38,a}



^a Reagents: (a) (i) Fmoc-Met-OH, DIC, HOBT, CHCl₃/DMF, microwave 50 °C (2 × 10 min); (ii) DMF, microwave 150 °C, 7 min; (b) various carboxylic/protected β -keto acids, DIC, HOBT, CHCl₃/DMF, microwave 50 °C (2 × 10 min); (c) CNBr, TFA, CHCl₃/H₂O, microwave 60 °C, 30 min.

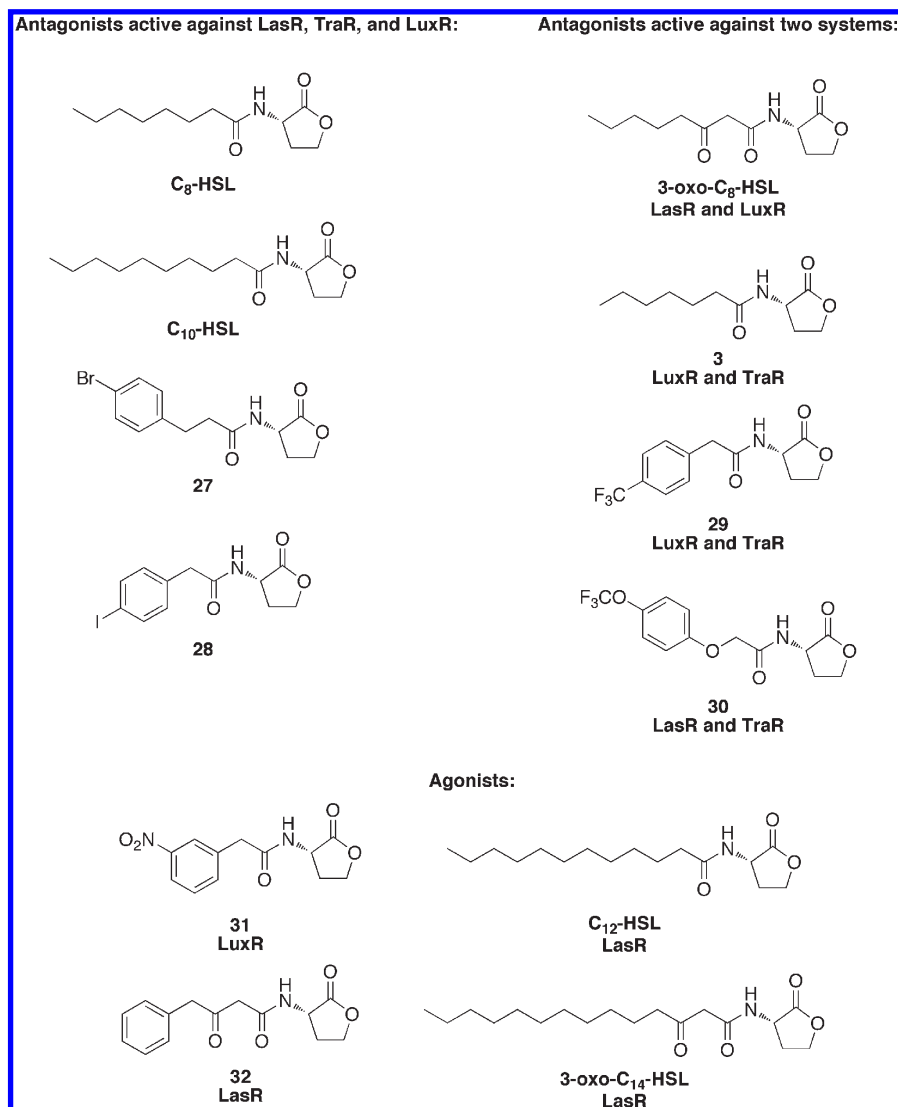


Figure 6. Representative examples of AHL analogues active across multiple species.

(Figure 6 and Table 1) that are active against all three different bacterial species examined, thus allowing the authors to establish a detailed SAR of the active ligands. It should be noted that in the development of all AHL analogues to date, small structural changes to the parent AHL signal resulted in vastly different biological activities. Thus, the pioneering work of Suga and later Blackwell serves to demonstrate the power of efficient synthetic routes to introduce small structural changes to conduct SAR studies as well as the discovery of AHL-based QS modulators. Additionally, the design and synthesis of focused libraries have provided for the rational design and synthesis of haptens by our laboratory, which have been used to generate antibodies against the autoinducers of several bacteria.^{41,42}

The work from the Blackwell laboratory also confirmed previous findings that the QS signals of one species may act as inhibitors against the QS of other species. Recently, a naturally occurring autoinducer was discovered in the soil bacterium *Rhodopseudomonas palustris* with structural similarities to the aryl-derived antagonists developed by Blackwell and co-workers. This new homoserine lactone signal, *pC*-HSL, is derived from *p*-coumaric acid rather than cellular fatty acids and reveals that natural homoserine lactone derived signals are not limited to straight chain acyl chains but may also comprise aryl chains (Figure 7).⁴³ Furthermore, two other

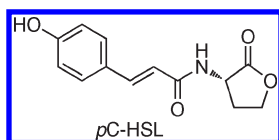
species of bacteria, the plant symbiont *Bradyrhizobium* and the marine bacterium *Silicibacter pomeroyi*, were found to be capable of producing *pC*-HSL type signals, implying *pC*-HSL signaling across a wide variety of bacterial species. Structural similarities between *pC*-HSL and many of the aryl-based antagonists discovered by the Blackwell laboratory suggest a possible role for *pC*-HSL as an antagonist of the QS of other species; however, to date, *pC*-HSL has not been tested for its activity against the QS of any other species. Such a finding would also speak to a deeper source of QS inhibitors, as the discovery of new QS signals in one species may also result in the discovery of new QS inhibitors against others species in the same manner that the discovery of new bacterial metabolites has produced a vast array of antibiotic compounds.

In a different approach, Michael Meijler and co-workers investigated a series of autoinducer analogues designed with electrophilic functionality for the specific covalent modification of the target protein, with the goal of rendering the receptor protein unable to initiate the QS signaling cascade.⁴⁴ For the design of the covalent probes, the authors took advantage of the high affinity and specificity of LasR for the natural ligand 3-oxo-C₁₂-HSL and constructed structurally related compounds but containing a diminutive electrophilic group. The authors reasoned that a nucleophilic cysteine residue (Cys79)

Table 1. IC₅₀ (Antagonists) or EC₅₀ (Agonists) Values for Compounds in Figure 6 against Three Bacterial Strains^a

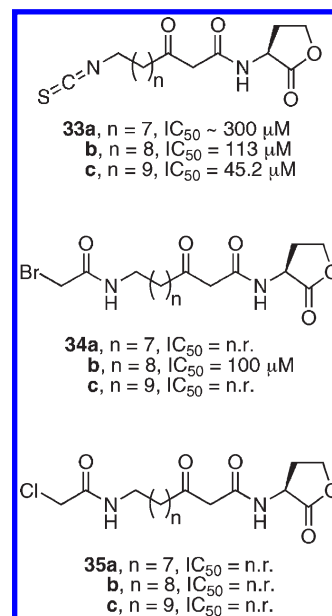
| compd | <i>P. aeruginosa</i> LasR ^{b,e} | <i>A. tumefaciens</i> TraR ^c | <i>V. fischeri</i> LuxR ^{d,e} |
|----------------------------|---------------------------------------------|--------------------------------------------|-------------------------------------------|
| Antagonists | | | |
| 3-oxo-C ₈ -HSL | 0.11 | | |
| C ₁₀ -HSL | 0.25 | 1.05 | 0.40 |
| 27 | 0.34 | 0.92 | 1.35 |
| 28 | 1.72 | 1.25 | 0.86 |
| C ₈ -HSL | 1.75 | 0.77 | 0.77 |
| 30 | 4.67 | 0.46 | |
| 4 | | 0.69 | 1.36 |
| 29 | | 0.81 | 0.61 |
| Agonists | | | |
| 3-oxo-C ₁₄ -HSL | 0.01 | | |
| C ₁₂ -HSL | 0.04 | | |
| 32 | 0.54 | | |
| 31 | | | 0.35 |

^a All values reported in μM . A blank entry indicates that the value was not determined. ^b Antagonism assay performed using an *E. coli* LasR reporter strain in the presence of 7.5 nM 3-oxo-C₁₂-HSL. ^c Antagonism assay performed using *A. tumefaciens* reporter strain in the presence of 100 nM 3-oxo-C₈-HSL. ^d Antagonism assay performed using *V. fischeri* reporter strain in the presence of 5 μM 3-oxo-C₆-HSL. ^e Agonism assays performed using the appropriate reporter strain in the absence of natural autoinducer.

**Figure 7.** Structure of pC-HSL.

in the LasR binding pocket would serve as a reactive partner and thus constructed a series of compounds with three electrophilic moieties: isothiocyanates **33**, bromoacetamides **34**, and chloroacetamides **35** (Figure 8). The capacity of the AHL-based probes to covalently modify LasR was first examined using purified LasR protein, and indeed, the isothiocyanate probes were found to react with Cys79. Importantly, these same compounds were found to inhibit QS in *P. aeruginosa* reporter strains, as well as decrease biofilm formation and pyocyanin production in wild-type strains, serving to establish these compounds as new motifs for the development of QS inhibitors. One concern that may arise from the use of electrophiles, including the brominated furanones (vide infra), to target QS stems from potential nonspecific alkylation and resulting toxicity; however, in the case of these electrophilic AHL analogues, the authors examined only those concentrations for which no growth inhibition was observed.

Natural products derived from eukaryotic sources have served as another source of QS disrupting compounds, and perhaps the most studied of these is the secretion of brominated furanones by the red algae *Delisea pulchra*. Although marine dwelling plants often suffer from macrofouling by higher organisms, usually caused by the initial formation of bacterial biofilms, *D. pulchra* was found to be relatively free from fouling organisms. Further microscopic analysis revealed bacterial populations to be reduced on the surface of *D. pulchra* relative to neighboring plants. A rationale for this observation has been provided by the laboratory of Staffan Kjelleberg, who demonstrated that the algae was releasing a series of brominated furanone metabolites that inhibit several AHL-dependent processes including swarming motility in

**Figure 8.** Analogs of AHL signals designed for the covalent modification of the LasR receptor protein. IC₅₀ values represent activity against a luminescent *P. aeruginosa* reporter strain (n.r. = not reported).

Serratia liquefaciens and bioluminescence in *V. harveyi* and *V. fischeri* (**36–39**, Figure 9).⁴⁵

Since their discovery, the activity of brominated furanones has been explored extensively, and naturally occurring furanones have also been found to inhibit AI-2-based QS in *Vibrio* sp. Because of structural similarities, the furanones were initially believed to compete with the natural AHL and AI-2 autoinducers for binding to their cognate receptors,⁴⁶ but subsequent reports demonstrated that the halogenated furanones destabilize and accelerate turnover of the LuxR receptor in *V. fischeri*⁴⁷ and inactivate the *V. harveyi* QS master regulator protein LuxR_{vh}.⁴⁶ Despite a common name, these two LuxR proteins have completely different functions, though it is likely that a similar mode of inhibition is at work based on the reactivity of the brominated furanones. These compounds possess multiple electrophilic centers capable of Michael addition at the α,β -unsaturated ketone, nucleophilic addition to the lactone carbonyl, or halogen substitution. Indeed, recent studies have indicated that the halogenated furanones undergo reaction with the DPD synthase, LuxS, likely through a Michael addition (vide infra).⁴⁸ A common mode of action would be encouraging for the development of antimicrobial strategies against human pathogens, as homologues of LuxR_{vh} from *V. harveyi* have been identified in the human pathogens *V. cholerae*, *V. parahemolyticus*, and *V. vulnificus*. Thus, the prevalence of a common target implicates a potential for halogenated furanones as a broad spectrum inhibitor for the treatment of disease.⁴⁶ However, it is the same broad reactivity, particularly toward alkylation, that is likely responsible for the toxic effects against mammalian cells that have been observed with this class of compounds.^{49,50}

The reported utility of the furanone compounds also extends to the inhibition of QS in *P. aeruginosa*, and furanones have been shown to affect the siderophore biosynthesis of *P. aeruginosa* via interaction with the QS system,⁵¹ although minimal effects have been observed on the other QS phenotypes of *P. aeruginosa*.⁵² To overcome this limitation, screening of combinatorial libraries derived from the natural furanone structure has revealed furanones **40** and **41**, both of

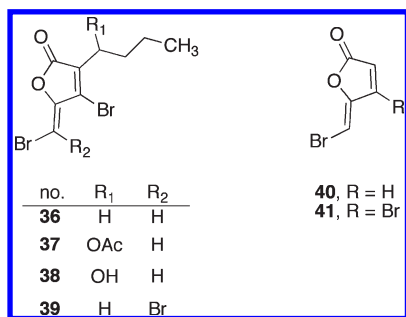


Figure 9. Structures of brominated furanones.

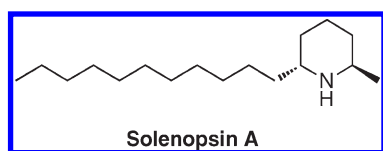


Figure 10. Structure of solenopsin A.

which lack a hydrophobic side chain, with activity against the QS of *P. aeruginosa* (Figure 9).^{47,52,53} Using a GFP-expressing strain of *P. aeruginosa*, compound **40** was shown to specifically inhibit the *las* system, as well as the production of the virulence factors chitinase and elastase. Importantly, compound **40** was also capable of disrupting intercellular communication within *P. aeruginosa* biofilms, ultimately resulting in the loss of biofilm integrity and overall biomass.⁵² The utility of these QS inhibitors has also been extended to the study of unidentified QS regulated process, and toward this end furanone **41** was used in a series of GeneChip microarray based studies to delineate both the QS regulatory network of *P. aeruginosa* and the effects of the furanone compound.⁵³ Perhaps the most significant finding is the efficacy of compounds **40** and **41** against QS in mouse infection models. Mice with lungs infected by a QS-dependent *gfp*-expressing strain of *P. aeruginosa* were treated with these compounds, and in 4–6 h a significant reduction in GFP fluorescence was observed relative to untreated controls. Inhibition of QS by **40** also corresponded to increased bacterial clearance from the lungs, as well as a prolonged survival time of 45% of mice with lethal infections.⁵⁴

Another natural product that has shown promise against the QS of *P. aeruginosa* is solenopsin A, a venom alkaloid from the fire ant *Solenopsis invicta* (Figure 10).⁵⁵ Initially investigated because of structural similarities with the 3-oxo-C₁₂-HSL signal and the antagonists developed by Suga et al. (Scheme 1),^{36,37} solenopsin A was found to inhibit the production of the virulence factors pyocyanin and elastase B. When competition experiments were performed between the natural autoinducers of *P. aeruginosa* and solenopsin A, C₄-HSL, rather than 3-oxo-C₁₂-HSL, restored QS signaling. While other compounds such as compound **18** developed by Suga et al. possess activity against both *las* and *rhl* systems,³⁶ this represents a significant finding based on the relative lack of specific inhibitors against C₄-HSL-dependent QS, and as such, solenopsin A may represent a valuable basis for the development of QS inhibitors against the *rhl* system of *P. aeruginosa*.

While natural products and focused libraries designed around the natural AHL signal have received the most attention for the development of QS modulators, E. Peter Greenberg and co-workers have developed a cell-based, ultrahigh-throughput screening platform to search for inhibitors of LasR-based QS.⁵⁶ The screening process relies on a strain of

P. aeruginosa expressing yellow fluorescent protein (YFP) under the control of the LasR-dependent promoter *prsaL*; thus, agonism or antagonism is detected by changes in the fluorescent signal. A 200 000 member library was screened, and 20 inhibitory compounds were discovered. The two most potent inhibitors, **42** and **43** (Figure 11A), were structurally similar to the native *P. aeruginosa* signal in that both contain an extended acyl chain attached to a 5- or 6-member ring.

The nature of the ultrahigh-throughput screen also allowed for the identification of agonists, and it was this aspect of the assay that allowed for the identification of the first agonist, TP-1 (**44**), that is structurally unrelated to the natural signal (Figure 11B).⁵⁷ Interestingly, **44** exhibited excellent specificity for the LasR receptor and is in fact a stronger activator (EC₅₀ = 0.014 μM) of LasR than 3-oxo-C₁₂-HSL itself (EC₅₀ = 0.140 μM). Indeed, this potent biological activity of **44**, along with its enhanced stability to pH and enzymatic degradation relative to 3-oxo-C₁₂-HSL, has rendered the triphenyl scaffold a promising lead for the study of *las*-dependent QS and the development of novel QS modulators. Inspired by these findings, Greenberg and co-workers also performed a series of in silico docking experiments between triphenyl-derived compounds and the LasR homologue TraR to identify a set of similar compounds, and interestingly, biological evaluation of this new set of analogues revealed both agonists (e.g., **46** and **47**) and one antagonist (**48**). Subsequent docking studies of the active compounds predicted that the generic triphenyl motif is capable of binding at the LasR active site, and unique hydrogen bonding patterns are observed in the models of agonists and antagonists. Even without explicit knowledge of the mechanism of action of the TP compounds, this class of molecules nevertheless represents a potential new class of QS modulators without the pitfalls posed by pH or enzymatic degradation that may plague the lactone-derived analogues.

The interaction between the triphenyl compounds and LasR has been further studied on the molecular level with the recent publication of the crystal structure of LasR in complex with the parent 3-oxo-C₁₂-HSL, **44**, **46**, and **47**.⁵⁸ Here, the authors demonstrate that productive binding requires that the AHL signal fulfill certain H-bonding requirements and the side chain be of sufficient length and hydrophobicity for proper receptor configuration. Despite significant structural differences, TP agonists **44**, **46**, and **47** are nevertheless capable of fulfilling these requirements, accounting for the observed activity. A crystal structure of the only inhibitory TP compound, **48**, was not obtained because of the instability of the **48**–LasR complex. However, docking experiments with **48** and LasR demonstrated unfavorable steric interactions between one of the chlorine atoms and a nearby leucine residue, likely disrupting the conformation of LasR. Such a destabilization of LasR by **48** is also reminiscent of the destabilization of the *V. fischeri* receptor LuxR by the brominated furanone compounds; thus, despite differences in the target proteins and discovery of these two structurally unrelated inhibitors, it may be that they both operate via a similar mechanism of action.

A critical finding arising from the crystallography study was the revised structure of the TP-1 (**45**) structure from the original publication by Greenberg and co-workers.⁵⁷ High resolution electron density maps of the crystal structure revealed an electron-rich atom, indicative of the chlorine, at the position originally thought to be occupied by the nitro group (Figure 11B). This revised structure is seemingly more in line with the previous findings by Müh et al., wherein TP-1 is remarkably stable to base.⁵⁷ However, neither rigorous

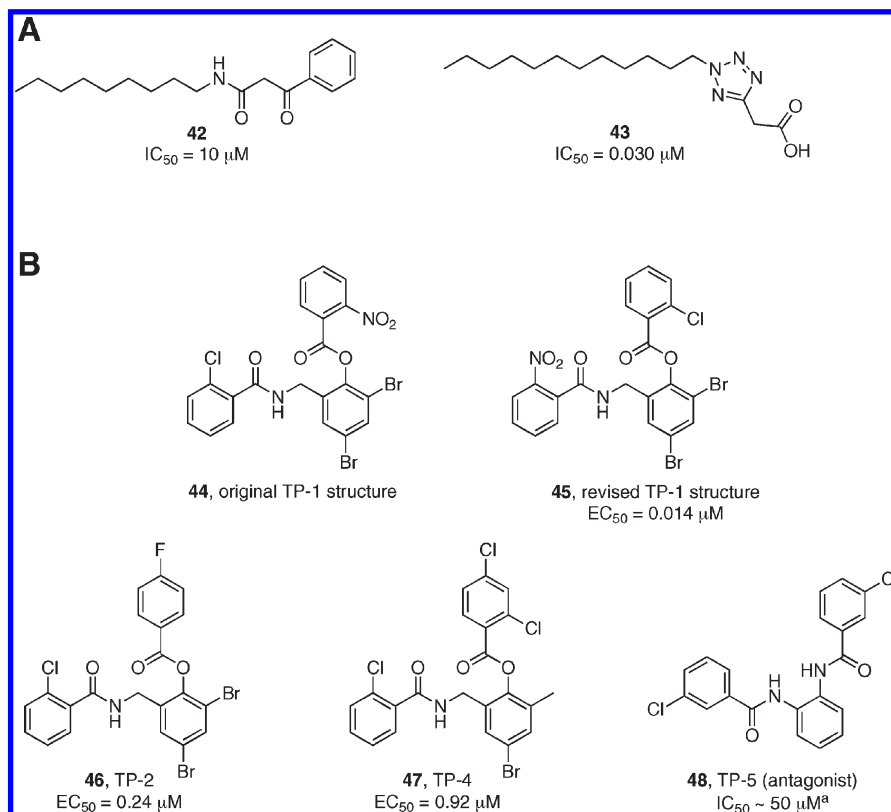


Figure 11. (A) QS inhibitors identified via high-throughput screening by Müh et al. IC_{50} values were determined using a LasR-dependent *yfp P. aeruginosa* reporter strain.⁵⁶ (B) Structures of triphenyl compounds. EC_{50} values represent concentration needed for half-maximal induction of *yfp* fluorescence. (a) IC_{50} value for QS inhibition.

chemical characterization of TP-1 (**44/45**) nor the stability of any of these agonists has been published to date.

While most of the above work has focused on the investigation of ligand binding and development of antagonists against LuxR-type transcriptional activator proteins, details are sparse concerning the interactions of AHLs with transmembrane LuxN receptors. In many Gram-negative bacteria, the receptor protein LuxR binds intracellular AHL, which then initiates QS through direct interaction with DNA. In the case of the model QS organism *V. harveyi*, LuxN binds extracellular AHL and initiates a phosphorylation cascade to begin the activation of QS. Toward a better understanding of this phenomenon, Bonnie Bassler and co-workers have recently published a series of studies, the first of which involved the screening of a large library of compounds with the Broad Institute to find a LuxN inhibitor.⁵⁹ In this screen, 35 000 small molecules were tested for bioluminescence inhibition, and after elimination of compounds found to be toxic or nonspecific for AHL-based QS, 15 compounds were identified as potent antagonists of LuxN. Pointing toward the utility of high-throughput screens for the identification of QS modulators, the most potent identified antagonists did not bear structural similarity to the natural 3-OH- C_4 -HSL signal (**49–53**, Figure 12A). These antagonists, in combination with a large panel of LuxN mutants, were also used to decipher the necessary interactions of the LuxN receptor both with the natural signal and with the phosphorelay system required for signal transduction.

With these compounds, the authors also questioned whether similar structures could inhibit both the transmembrane LuxN and cytoplasmic LuxR-type receptors.⁶⁰ Thus, the same set of 15 compounds was evaluated for antagonist activity against CviR, the LuxR-type receptor of the human pathogen

Chromobacterium violaceum. The authors first examined the activity of their compounds, as well as several AHL analogues with varying acyl tail lengths, in a strain of *E. coli* that expresses CviR and CviR-dependent *gfp*. Interestingly, CviR bound intermediate chain AHL analogues (e.g., C_8 - and C_{10} -HSL) with similar affinities as natural C_6 -HSL, and although these complexes still bound the target DNA, transcription of QS genes was inhibited. On the other hand, longer chain AHL analogues (e.g., C_{12} - and C_{14} -HSL) and one potent antagonist (**54**) were found to interact with CviR but prevent DNA binding of the complex, indicating two distinct modes of action for these QS inhibitors. To further explore the scaffold of lead compound **54**, analogues were constructed and chlorolactone **56** (Figure 12B) was found to inhibit CviR-dependent GFP fluorescence in *E. coli* with an IC_{50} of $0.377 \mu M$ and violacein production in vivo by *C. violaceum* with an IC_{50} of $0.295 \mu M$. Finally and perhaps most significantly, the authors studied an infection model using *C. elegans* to demonstrate that both **54** and **56** were able to extend the lifespan of the nematode upon infection with lethal levels of *C. violaceum*. In doing so, the authors demonstrate that the disruption of bacterial communication does have prophylactic benefits.

The natural QS signals of bacteria, specifically the 3-oxo- C_{12} -HSL molecule of *P. aeruginosa*, have been shown to exert potent biochemical effects on mammalian host cells. For example, 3-oxo- C_{12} -HSL induces apoptosis in human breast cancer cells,⁶¹ as well as in macrophages and neutrophils,⁶² and in research stemming from our laboratory and others, modulates transcriptional activity of NF- κ B, the central regulator of gene expression in the innate immune system during infection and inflammation.^{63–66} Furthermore, 3-oxo- C_{12} -HSL has exhibited inhibition of proliferation and cytokine secretion

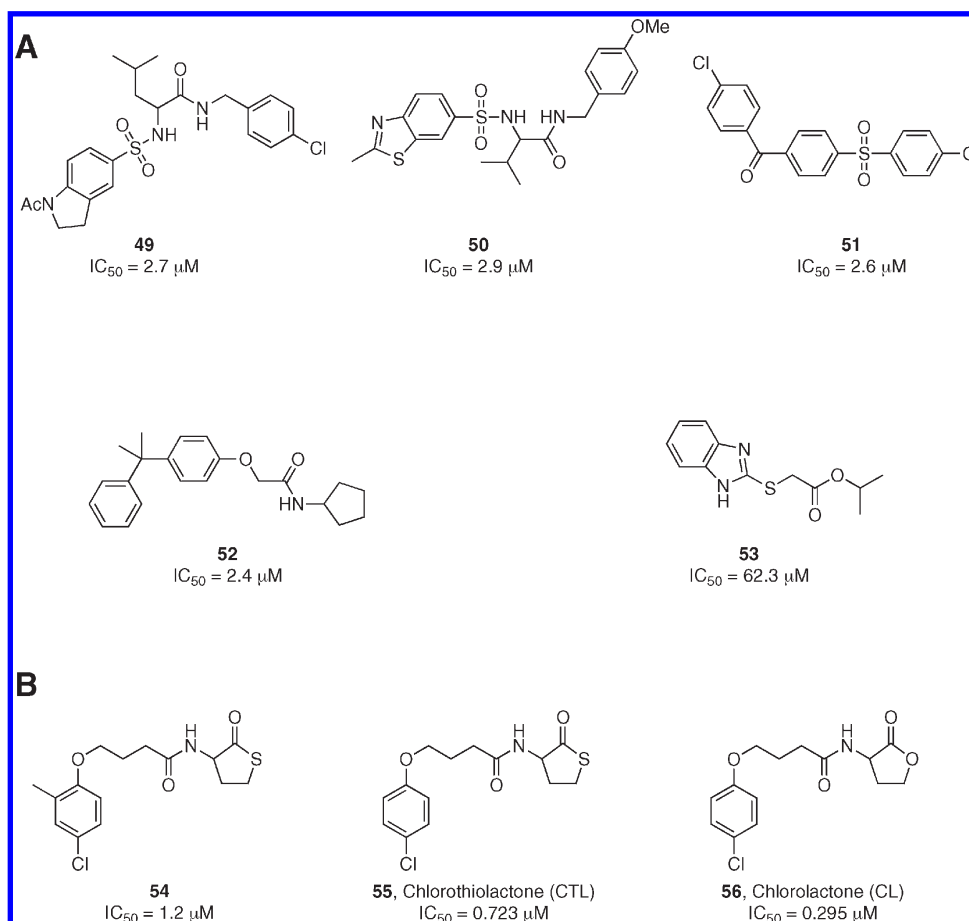


Figure 12. (A) LuxN inhibitors identified by the screening of a 35 000 member library from the Broad Institute. IC₅₀ values represent the concentration needed for 50% reduction of bioluminescence in *V. harveyi*. (B) LuxR inhibitor **54** identified from the panel of LuxN inhibitors and its synthetic derivatives **55** and **56**. IC₅₀ values represent the concentration needed to inhibit violacein production by 50% in *C. violaceum*.

in human leucocytes, an aspect of AHLs that has been further investigated by capitalizing on the 3-oxo-C₁₂-HSL scaffold as a lead for the development of analogues to enhance the immunosuppressive activity, with the potential for new treatments of autoimmune diseases.⁶⁷

Analogues of 3-oxo-C₁₂-HSL have also been examined for activity against cancer cells; however, in light of the potential negative effects of *P. aeruginosa* QS on cancer patients, the analogues were designed to minimize QS activity and optimize anticancer activity.⁶⁸ In this study, a series of phenylacetylhomoserine lactone analogues were constructed by microwave-assisted synthesis and assayed for both activity against cancer cell growth and lack of agonism toward *P. aeruginosa* QS. The 3-oxo functionality was required for inhibition of cancer cell growth, but many analogues, including **58**, also exhibited excellent QS activation (Figure 13). One promising lead was identified (**60**), which incidentally shares the structural feature of the C₁₂ acyl chain with the natural 3-oxo-C₁₂-HSL signal, suggesting therapeutic avenues for the development of QS analogues outside the realm of antimicrobial intervention. Thus, in summary, research toward the modulation of bacterial communication not only may provide new vistas into the control of bacterial infection but also may signal new directions in the development of agents against other human diseases and conditions.

AIPs

Gram-positive bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus pneumoniae*, in contrast

to the small molecule signals of Gram-negative bacteria, use linear or post-translationally modified peptides as their auto-inducing signals.⁶⁹ Specifically, the human pathogens *S. aureus* and *Staphylococcus epidermidis* utilize cyclic peptides, referred to as autoinducing peptides (AIPs).^{70–72} Most of the work concerning the development of QS modulators in Gram-positive bacteria has focused on the AIP systems of *Staphylococci*, specifically *S. aureus*, and as such will be the focus of this section. These oligopeptides are secreted into the environment by transport proteins and detected by a receptor histidine kinase (HK).^{72,73} As part of a classical two-component system, the HK receptor is coupled to a response regulator (RR) protein and, upon ligand binding, activates the RR protein to induce expression of the target genes. This system plays a crucial role in the pathogenesis of *S. aureus*, as the expression of a number of genes encoding virulence factors is regulated by a QS-dependent global regulatory locus known as accessory gene regulator (*agr*).⁷⁴ The *agr* system contributes to pathogenesis by orchestrating the temporal, cell-density-dependent expression of virulence genes encoding cell surface proteins, such as protein A, coagulase, and fibronectin-binding proteins, as well as secreted proteins including proteases, hemolysins, toxic shock syndrome toxin 1 (TSST-1), and enterotoxin B.^{75,76} It is important to note that, in contrast to most other bacterial QS systems, in staphylococci, biofilm formation is inversely controlled by QS; i.e., inhibition of QS signaling enhances the formation of biofilm, a fact that might make the development of prophylactic or therapeutic QS antagonists particularly challenging.^{77,78}

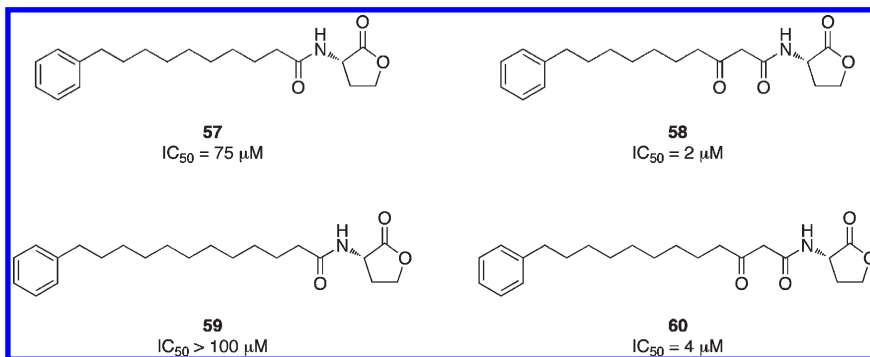


Figure 13. Analogues of 3-oxo- C_{12} -HSL with activity against cancer cell growth. IC_{50} values represent the concentration required to inhibit PC3 growth by 50%.

The *agr* operon is composed of four genes, *agrA–D*, which encode the complete AIP-dependent QS signaling circuit.⁷² The AIP, which is synthesized as a 46 amino acid (AgrD) propeptide, is processed by the membrane bound protein AgrB and subsequently secreted. The extracellular AIP binds to its cognate AgrC transmembrane receptor, causing autophosphorylation of AgrC, which in turn phosphorylates AgrA to activate promoters P2 and P3. The P2 promoter initiates transcription of the *agr* operon, resulting in increased synthesis of the AIP, while the P3 promoter is responsible for the up-regulation of a large number of virulence factors.⁷³

The AIPs are composed of seven to nine amino acids, including a highly conserved cysteine residue, which forms a thioester linkage with the C-terminal carboxyl group to generate the cyclic peptide (Figure 14).⁶⁹ The cyclic structure of the peptide is required for binding to its cognate AgrC receptor, while the other half of the AIP is known as the “tail” and is a linear sequence of two to four amino acids.⁷⁹ The tail sequence is important for activation of the AgrC receptor.⁷⁹ All *S. aureus* strains can be classified into four different *agr* groups, labeled I–IV, producing four different AIPs that bind to their respective AgrC receptors.⁸⁰ Interestingly, no cross-activation is observed between AIPs and AgrC receptors of different *agr* groups, but rather the binding of an AIP to a non-native AgrC receptor results in inhibition of *agr* functions.^{73,80,81} The significance of this phenomenon in vivo has yet to be delineated, but in spite of this uncertainty, the cross-inhibition of AIPs has provided a mechanism for the study of QS inhibition in *S. aureus* both in vivo and in vitro, as well as a roadmap for the development of novel AIP signaling antagonists.⁷³

The validity of the *agr* system as a target for therapeutic intervention has been established, in part, by the observation that *S. aureus agr* mutants are attenuated for virulence.⁸² Additionally, the cross-group inhibition of the AIPs was exploited to demonstrate that a QS inhibitor is indeed capable of modulating *S. aureus* pathogenesis in animal models.⁸³ In this study, a solid phase synthetic route was used to establish the structures of AIPs I and II. Subsequently, mice were infected with group I *S. aureus* and concurrently treated with AIP-II. The resulting inhibition of the *agr* system caused diminished abscess formation by group I *S. aureus*, a finding that served to establish the therapeutic potential of QS inhibition and confirm the role of QS as an indispensable process in *S. aureus* infections. Notably, synthetic efforts by our laboratory toward the AIP signals have also enabled the generation of AIP analogues to be used as haptens for the generation of anti-AIP antibodies. In particular, an anti-AIP-IV antibody suppressed

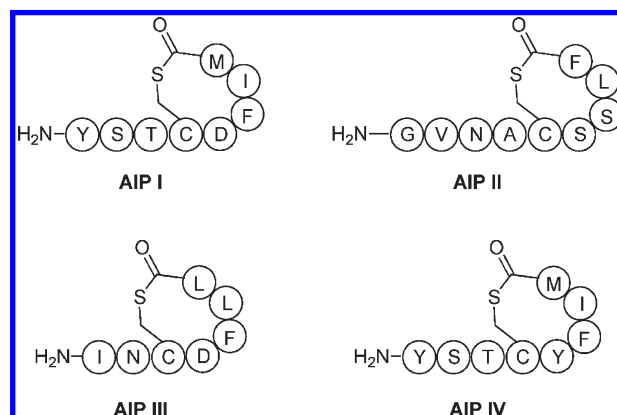


Figure 14. Structures of the AIP signals used by *S. aureus*.

abscess formation in a murine model and protected mice from a lethal *S. aureus* challenge.⁸⁴

The groups of Tom Muir and Richard Novick have jointly carried out numerous structure activity studies of the AIPs with their corresponding AgrC receptor and have shed light on important structural features required for both agonist and antagonist activity.^{79,80} This work led to the development of a global inhibitor (**61**) of the *S. aureus agr* locus in all four groups (Figure 15 and Table 2).⁸⁰ Global inhibitor **61** is composed of the AIP-II macrocycle without the linear amino acid tail region. Interestingly, **61** contains the thioester linkage, which was found to be important for antagonism of group II AgrC (self-inhibition), while the lactam or lactone linkage retained inhibitor activity for the other AgrC groups.

Furthering the initial development of a global inhibitor, derivatives of the AIP-II macrocycle were produced and evaluated for antagonism of the AgrC receptor.⁸⁵ Various alterations were made to the parent compound to explore important structural features important for activity: replacement of serine with glycine (**62–64**), N-methylation of the amides (**65–68**), substitution of the adjacent serines with a methylene linker (**69**), replacement of the thioester with a lactam (**70**), and removal of the cysteine acylated amino group (**71**, Figure 16).

The analogues were tested in a cell-based reporter assay for inhibition of the AgrC receptor of groups I and II reporter strains (Table 3). Interestingly, all the analogues are more potent antagonists to the group I AgrC receptor than the group II AgrC receptor. It has been shown that the AIP analogues are not potent antagonists for their parent AgrC receptor.^{79,86,87} Replacement of the serine residues with a glycine residue gave mixed results. Substitution of the serine closest to the N-terminal (**62**) resulted in an increased IC_{50} for

group I and a drastic IC_{50} increase for the group II AgrC receptor. However, replacement of the serine residue closest to the C-terminal (**63**) resulted in a decrease in the IC_{50} value by 2-fold for both I and II group AgrC receptors. The substitution of both serine residues with glycine (**64**) resulted in a small decrease of the IC_{50} values for both AgrC groups. N-Methylation of the amides in the macrocycle (**65**, **66**) showed a large increase in the IC_{50} , and in the case of leucine or phenylalanine, NH methylation (**67**, **68**) abolished activity. Inhibition of both groups I and II AgrC receptors was also drastically decreased by substitution of the two adjacent serine residues with a propyl linker (**69**) and replacement of the thioester

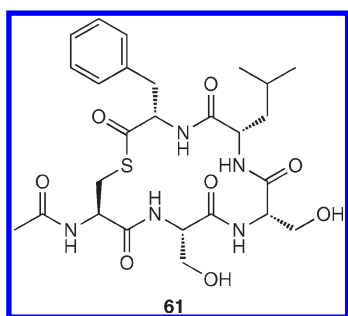


Figure 15. Structure of global inhibitor **61**.

Table 2. Inhibition of the *agr* Response by Global Inhibitor **61**

| | group I | group II | group III | group IV |
|----------------------|---------|----------|--------------------|----------|
| IC_{50} (μ M) | 0.272 | 0.209 | 0.010 ^a | 0.188 |

^a Experiment was based on HPLC analysis of the δ toxin. All other results were based on the β -lactamase reporter assay.

bond with an amide (**70**). Finally, removal of the amino group of the cysteine (**71**) again resulted in an increase in the IC_{50} . In this SAR study of the macrocycle region of AIP-II the authors demonstrated a strict requirement of amino acid sequence for tight binding to the AgrC groups I and II receptors. However, macrocycle **62** was identified as a good inhibitor of the AgrC of groups I and II, albeit with 5-fold decreased activity in group II. Thus, an inhibitor was identified from the SAR study, and the authors were able to conclude that thiolactone linkage, along with the phenylalanine and leucine residues, were the only residues essential for antagonism of both the AgrC I and parent AgrC II receptors. However, additional functionalities are required for antagonism of the parent AgrC receptor, a finding that may be rationalized by the fact that the AIP-II signal itself is already capable of inhibition of AIP-I receptors, whereas the AIP-II signal must undergo significant

Table 3. IC_{50} Values for Inhibition of the AgrC Receptor of Groups I and II^a

| analogue | group I | group II |
|-----------|---------------|---------------|
| 62 | 0.189 | 1.160 |
| 63 | 0.040 | 0.194 |
| 64 | 0.077 | 0.238 |
| 65 | 0.639 | 7.880 |
| 66 | 0.319 | 1.050 |
| 67 | no inhibition | no inhibition |
| 68 | no inhibition | no inhibition |
| 69 | 1.141 | 507 |
| 70 | 1.390 | 167 |
| 71 | 1.680 | 47.3 |

^a All values reported in μ M.

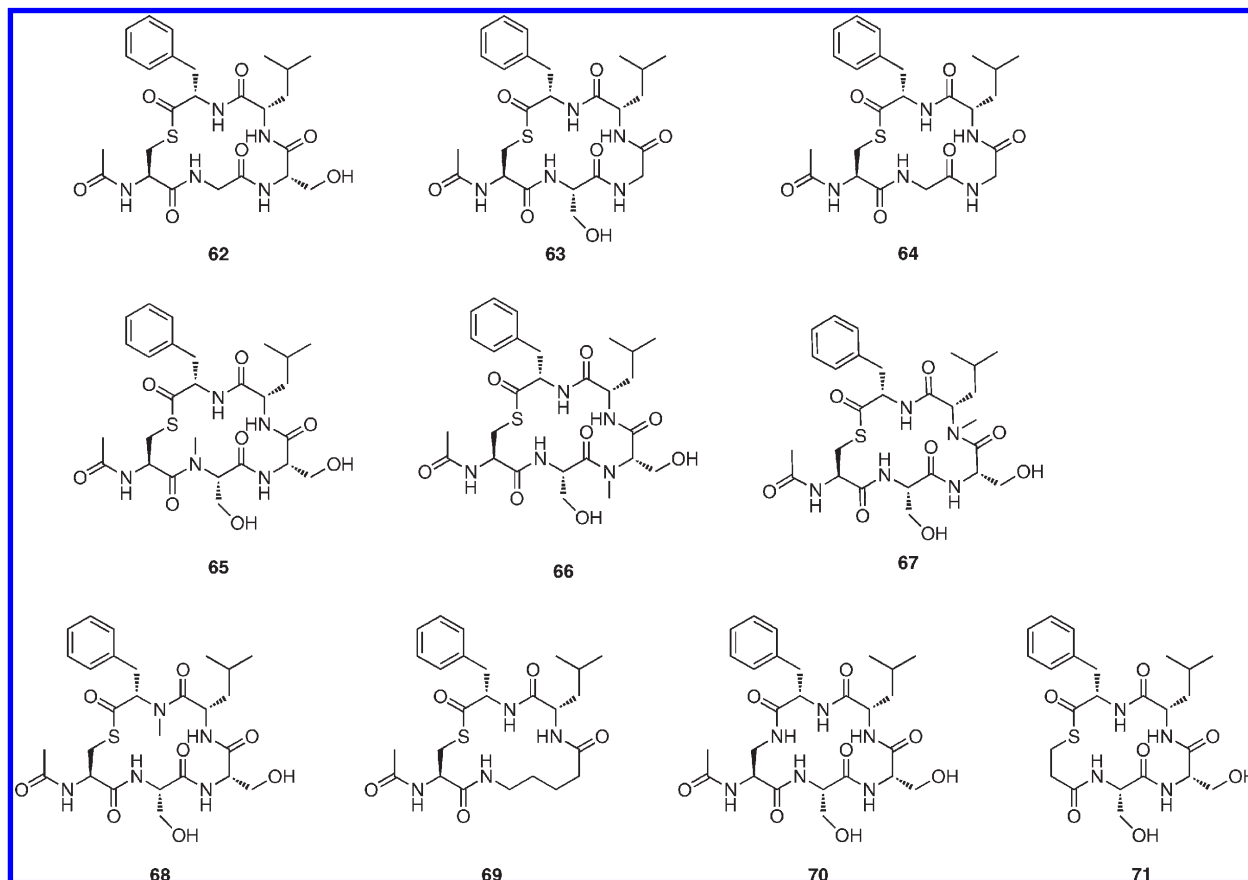


Figure 16. SAR study of AIP-II.

modification so that it is not recognized as an agonist by its cognate receptor.

In another study, Weng Chan and co-workers focused on the AIP for development as an inhibitor of groups I and II AgrC receptors.⁸⁸ Previously, the laboratory of Paul Williams identified a potent inhibitor (**72**) of the AgrC receptor of group I by replacing an aspartic acid of AIP-I with an alanine residue.⁷⁶ In a later study, **72** was demonstrated to be an even more potent inhibitor of the AgrC receptor of group II (group I $IC_{50} = 21$ nM; group II $IC_{50} = 4$ nM).⁸⁸ They sought to enhance this analogue by replacing the methyl group of the alanine side chain with an ethyl group (**73**) to increase the hydrophobic nature of that position (Figure 17). This subtle change from a methyl to an ethyl group produced an analogue more potent than the parent molecule in regard to the inhibition of the AgrC of group II (2.8 nM). However, **73** was not as potent an antagonist against the AgrC group I, resulting in a 6-fold increase in IC_{50} from the parent analogue (**72**). Along with this subtle alteration they also replaced the tyrosine residue at the N-terminus with 4-benzoylphenoxybutyric acid (**74**, **75**) to create an access point for chemical derivatization and the incorporation of the benzophenone photophore to probe the binding locations of the AIPs to the AgrC receptors. This alteration at the N-terminus of the tail region resulted in decreased potencies for the AgrC receptors of groups I and II, but nevertheless, **74** and **75** are capable of binding to the AgrC receptors and could be further developed for photolinking experiments. Although Chan and co-workers were able to produce a potent inhibitor of the AgrC receptor of group II, this work reinforces the observations that antagonists derived from an AIP have excellent cross-inhibitory activity and only modest in-group activity, demonstrating potential limitations of AIP analogues as broad-spectrum inhibitors.

Several reports by Naomi Balaban and co-workers have described another potential avenue for the inhibition of *S. aureus* QS, which lies in the prevention of *agr* activation via the TRAP (targeting of RNAIII-activating peptide) pathway. TRAP has been suggested to be activated by the autoinducing RNAIII activating protein (RAP), which in turn controls the activation of *agr*. An inhibitor of this system, discovered by Balaban and co-workers, is a naturally occurring seven amino acid peptide, coined RNAIII inhibiting peptide (RIP), with the sequence YSPWTFN-NH₂.^{89,90} This peptide was suggested to inhibit QS of *S. aureus* by competing with RAP for activation of TRAP and ultimately to inhibit *agr* QS. Indeed, RIP has been shown to inhibit toxin production and even mitigate wound infections by *S. aureus*.^{90,91} However, RIP also prevented biofilm formation, which usually does not indicate *agr* QS antagonism in *S. aureus*.^{92,93} Thus, the validity of RIP as a bona fide QS inhibitor has been questioned. Recently, a series of reports demonstrated that mutations in the gene encoding TRAP do not result in altered *agr* activation,⁹⁴ biofilm formation,⁹⁵ and overall in vivo virulence.⁹⁶ While RIP may still represent a viable lead for the discovery of both virulence and biofilm formation inhibitors of *S. aureus*, its mechanism of action has not been unequivocally delineated but likely does not involve specific *agr* QS quenching. Interestingly, the pharmacophore of RIP was determined by Balaban and colleagues using in silico techniques, screened against a library of commercially available small molecules, and a nonpeptidic RIP analogue, namely, hamamelitannin, with similar biological activity was discovered.⁹⁷

Most of the work described in this section thus far has focused on cyclic peptide analogues of natural AIPs, but

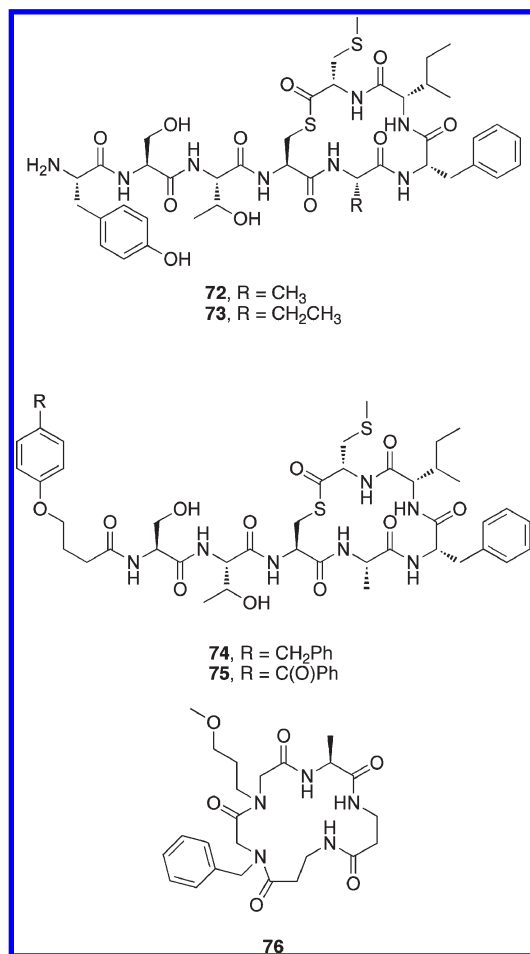


Figure 17. Structures of AIP analogues.

another class of molecules that could be developed into antagonists of the AgrC receptor is peptoids. Peptoids are oligomers composed of N-substituted glycine units and have exhibited high biostability and can be easily synthesized by combinatorial approaches to produce large compound libraries.^{98,99} Indeed, peptoids are currently being investigated for biological activity in a number of areas including the development of antimicrobial therapies.¹⁰⁰ In one study, a series of peptoid-peptide hybrids, or peptomers, derived from the AIP-I signal were synthesized, and one of the peptomers (**76**) was shown to stimulate biofilm formation in *S. aureus* (Figure 17).¹⁰¹ As mentioned above, it had previously been shown that inhibition of the AIP QS signaling resulted in increased biofilm formation,⁷⁸ leading to the hypothesis that **76** was an antagonist of the AgrC receptor of group I *S. aureus*. However, a direct comparison of this peptoid with other known AIP antagonists is difficult, as the antagonist activity of **76** was deduced from the formation of biofilm rather than a direct inhibition of the AIP-regulated QS system. Nevertheless, the peptoid scaffold does show promise as an inhibitor of the AgrC receptor and may provide a new avenue for the development of novel analogues.

AI-2 and DPD

In addition to the two QS systems described above that are exclusive to either Gram-negative or -positive bacteria, AI-2 QS systems have been proposed to exist in both Gram-positive and Gram-negative strains, and indeed, the synthase for the AI-2 precursor 4,5-dihydroxy-2,3-pentanedione (DPD), LuxS,

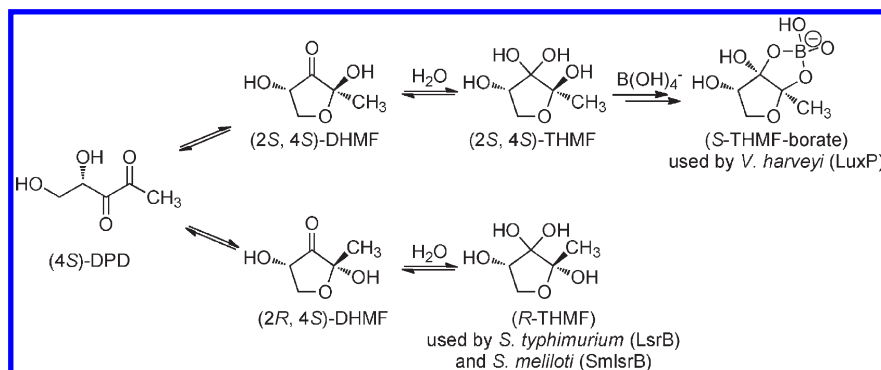


Figure 18. Equilibrium of the two known AI-2 signals.

is present in a wide variety of bacteria.¹⁰² AI-2 has also been found to regulate an array of microbial processes, including bioluminescence in *Vibrio harveyi*,¹⁰² AI-2 uptake and destruction in *Salmonella typhimurium* and *Sinorhizobium meliloti*,^{103,104} virulence factor production in *Vibrio cholerae* and *Vibrio vulnificus*,^{105,106} and the formation of mixed biofilms by *Streptococcus oralis* and *Actinomyces naeslundii*.¹⁰⁷

The first structure of AI-2 was solved, using X-ray crystallography, in complex with the *V. harveyi* receptor protein LuxP.¹⁰⁸ In this crystal structure, AI-2 was found to exist as a furanosyl borate diester (*S*-THMF-borate, Figure 18), likely arising from the complexation of boronic acid by the cyclized form of DPD. Although initially puzzling, this finding was later rationalized by the high concentrations of boric acid in marine environments (0.4 mM) inhabited by *V. harveyi*. Subsequent studies revealed that the enteric bacterium *S. typhimurium* responds to the furanosyl form of *R*-DPD without boron ((*2R,4S*)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran, *R*-THMF), leading to the redefinition of AI-2 autoinducers as a group of signals derived from DPD.¹⁰⁹ Recently, the third crystal structure of an AI-2 binding protein was solved, this time of the SmlsB (LsrB of *Sinorhizobium meliloti*) protein in complex with the *R*-THMF form of DPD.¹⁰⁴ These findings support the hypothesis of AI-2 as an interspecies signal in that the three identified AI-2 structures are formed from DPD, implying that DPD released by one species may be detected and recognized by several different species as various isomers. Interestingly, *S. typhimurium* and *S. meliloti* respond to the *2R* isomer, whereas *V. harveyi* recognizes a borate diester form of AI-2 derived from the *2S* isomer (Figure 18). As such, the reactivity, variable stereochemistry, and capacity for metal chelation of cyclic DPD highlight its potential for interspecies signaling and argue that modified DPD-based signals may be recognized in the extremely diverse environments inhabited by bacteria. The discovery of the AI-2 system in *S. meliloti* is particularly intriguing, as this soil-dwelling bacterium does not produce its own DPD. This has led to the hypothesis that the AI-2 response system of *S. meliloti* may allow for the survey of the bacterium's surroundings and also to interfere with the communication of its competitors via internalization of AI-2 signals.¹⁰⁴

Since the initial discovery of *luxS*,¹¹⁰ it has been correlated to a variety of phenotypes, including bioluminescence, expression of virulence factors, and biofilm formation. However, the discovery of a large proportion of AI-2 regulated processes has relied on the creation of *luxS* mutants; this technique has been the target of criticism, as LuxS plays an important role in the removal of toxic intermediates from the activated methyl cycle.¹¹¹ As such, the observed phenotypes of *luxS* mutants

may be a result of either the loss of communication or the changes in metabolic fitness. In spite of this criticism, several reports have provided evidence in support of AI-2 as an interspecies signal.^{104,107,112,113}

An interspecies and interkingdom signaling system, defined by the bacterial receptor protein QseC, has been reported by Vanessa Sperandio and coworkers. The QseC receptor recognizes the uncharacterized bacterially derived signal AI-3, as well as adrenergic signals produced by mammalian hosts, to regulate the production of virulence factors in a variety of bacteria.^{114,115} This system was first characterized in enterohemorrhagic *E. coli*, but homologues of QseC are present in a variety of pathogenic bacteria, including *Salmonella* sp., *Haemophilus influenzae*, and *Shigella* sp.¹¹⁴ Mutation of *luxS* was initially found to effect the production of AI-3 as well as AI-2,¹¹⁶ but subsequent studies have demonstrated that the LuxS enzyme is not directly responsible for AI-3 synthesis.¹¹⁷ The therapeutic utility of disrupting this interspecies and interkingdom communication was established with the discovery of a compound that specifically inhibits QseC regulated signaling without toxicity toward the bacteria and consequently prevents lesion formation on cultured epithelial cells by enterohemorrhagic *Escherichia coli* and protects mice from death by *Francisella tularensis* and *Salmonella typhimurium* infections.¹¹⁸

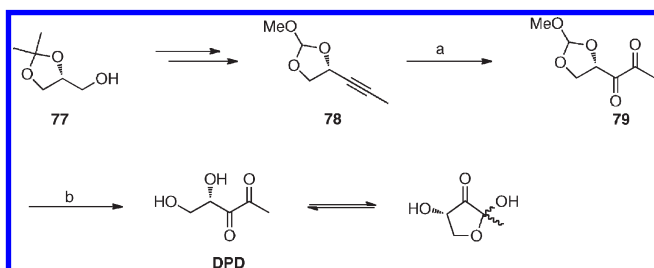
Chemical confirmation of DPD as the core signaling molecule of AI-2-based QS was provided through chemical synthesis and biological evaluation of DPD by our laboratory (Scheme 3).¹¹⁹ In spite of the small size of the DPD molecule, it presented a synthetic challenge because of its highly oxygenated carbon framework as well as its reactivity and propensity for polymerization. Synthetic efforts were further hampered by harsh oxidation conditions to generate the diketone moiety as well as the lability and volatility introduced by the methoxy methylene acetal protecting group (**79**). An alternative synthesis was later reported by Martin Semmelhack and co-workers for multigram quantities (Scheme 4A),¹²⁰ while an additional synthetic route was published using O₃-mediated oxidative cleavage to form the diketone (Scheme 4B).¹²¹

The establishment of DPD as the core signaling molecule of AI-2 based QS provided an opportunity for the development of both structure-based and computationally designed agonists and antagonists. As a first step, several studies sought to establish the role of boron in the AI-2 signal. With an established route to synthetically pure DPD, ¹H NMR was used to monitor the spontaneous complexation of boron to DPD and the DPD analogue laurencione.¹²⁰ The natural products laurencione and 4-hydroxy-5-methyl-3-(2*H*)-furanone (MHF) were also shown to form a boron complex, but both of these

analogues exhibit lower activity than DPD.^{122,123} Additionally, during efforts to evaluate the potential for different metal species to serve as a boron mimic, McKenzie et al. deduced that the putative carbonate analogue, **88**, of the borate diester AI-2 species effectively mimics the biological activity of borate-DPD (Figure 19).¹²⁴

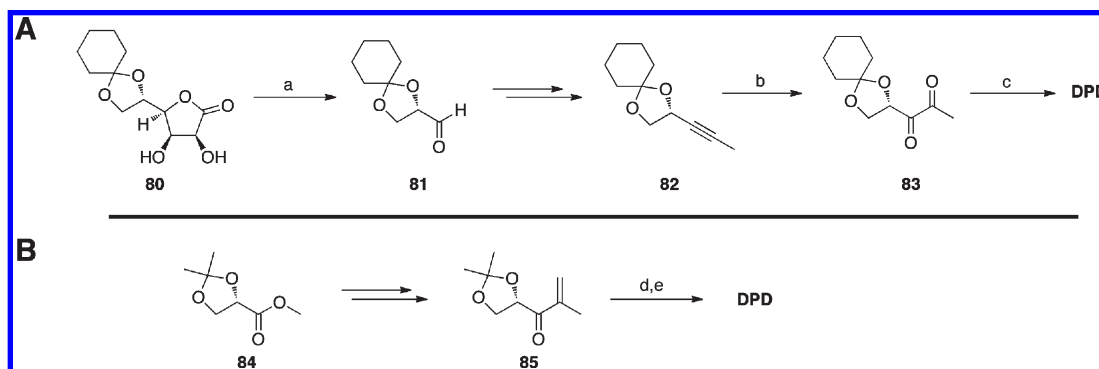
Because of the instability of DPD at high concentrations and the relatively compact framework, the DPD molecule itself presented a challenge for organic chemists. As such, many studies, both initial and current, focused on the synthesis of one or two simple analogues. This is exemplified in a report from the research group of Alain Doutheau, in which bis-acetyl DPD was constructed using the Baylis–Hillman/ozonolysis methodology developed by the same laboratory (Scheme 5A).^{125,126} Because the final product of the previous syntheses of DPD is always a dilute aqueous solution, it was reasoned that protection of the diol as the bis-acetate **91** would allow for the isolation of this pure DPD precursor, which would then be cleaved *in vivo* to reveal the active signal. This was indeed the case, as **91** displayed equal activity to DPD itself.¹²⁵ This same synthetic route was also used for the construction of C5-alkylated and 5-O-acylated derivatives **95**, although only the racemic compounds were obtained (Scheme 5B).¹²⁶ Another compound, the trifluoromethyl analogue **96** (Figure 20), was reported as a chiral form using the camphor-derived Oppolzer's sultam reagent in the Baylis–Hillman reaction, and the compound demonstrated the most potent agonistic activity observed to date, although it was less active than DPD in the *V. harveyi* bioluminescence assay ($EC_{50} = 30 \mu\text{M}$ vs $EC_{50} = 3 \mu\text{M}$ for DPD).¹²⁷ The expected rationale for this observed activity was that the electron withdrawing effects of the trifluoromethyl group would render the adjacent carbonyl more electrophilic and promote

Scheme 3. Synthesis of DPD as Described by Meijler et al.^{119,a}



^a Reagents: (a) KMnO_4 , acetone, aqueous buffer, room temp, 20 min; (b) H_2O , pH 6.5 ($\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (0.1 M), NaCl (0.15 M)), 24 h.

Scheme 4. Synthesis of DPD Described by (A) Semmelhack et al.¹²⁰ and (B) De Keersmaecker et al.^{121,a}



^a Reagents: (a) KHCO_3 , KIO_4 , $\text{H}_2\text{O}/\text{CH}_2\text{Cl}_2$, room temp, 18 h; (b) RuO_2 , NaIO_4 , MeCN , CCl_4 , H_2O , room temp, 15 min; (c) H_2SO_4 , 2 h; (d) DOWEX 50X8-100, MeOH , reflux, 1.5 h (e) O_3 , MeOH , Me_2S , 24 h.

formation of the active cyclic species; however, modeling studies of this compound revealed that the trifluoromethyl group also caused the two adjacent oxygen atoms (in the hydrated form) to be less available to form the requisite H-bonds with the LuxP receptor, possibly accounting for the diminished activity relative to DPD.

As a first step toward the discovery of new modulators of AI-2 based QS, our laboratory created a panel of alkyl-DPD analogues to systemically probe the effects of the substitution pattern at C1 (Scheme 6).¹²⁸ The synthesis is founded upon previously reported routes for the construction of DPD but modified for the introduction of alkyl groups at the C1 position (**100**).^{119,120} Modulation of bioluminescence was examined using *V. harveyi* MM32, a cell line incapable of producing luminescence through either the AHL pathway or AI-2 pathways in the absence of exogenous DPD.^{119,120,129} When the analogues ($25 \mu\text{M}$) were incubated with *V. harveyi* and $1 \mu\text{M}$ DPD to monitor antagonism, a synergistic effect was observed. Interestingly, these compounds were inactive in the absence of DPD, leading to the hypothesis that these analogues are interacting with the AI-2 receptor LuxP in a manner productive only in the presence of natural DPD, possibly via interaction with an allosteric binding site and increased hydrophobic interactions.

The panel of C1-substituted alkyl-DPD analogues was also evaluated for (ant)agonist activity in *S. typhimurium*, as measured by a β -galactosidase transcription assay.¹²¹ Assays were conducted using a ΔluxS strain with a *lacZ-lsr* fusion, and assays were performed in the presence of DPD (antagonist assay) or in the absence (agonist assay) of DPD. No agonists were identified from the screening, but in the presence of $50 \mu\text{M}$ DPD, all compounds acted as antagonists of AI-2 based QS. Notably, the propyl- and butyl-substituted analogues were found to be potent inhibitors with IC_{50} values 10-fold less than the concentration of the natural DPD signal (Table 4). These analogues possessed two interesting and unique properties; first, they had strikingly different biological effects in the two different species *V. harveyi* and *S. typhimurium*.

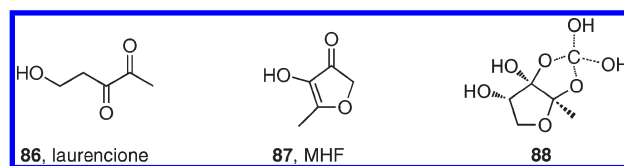
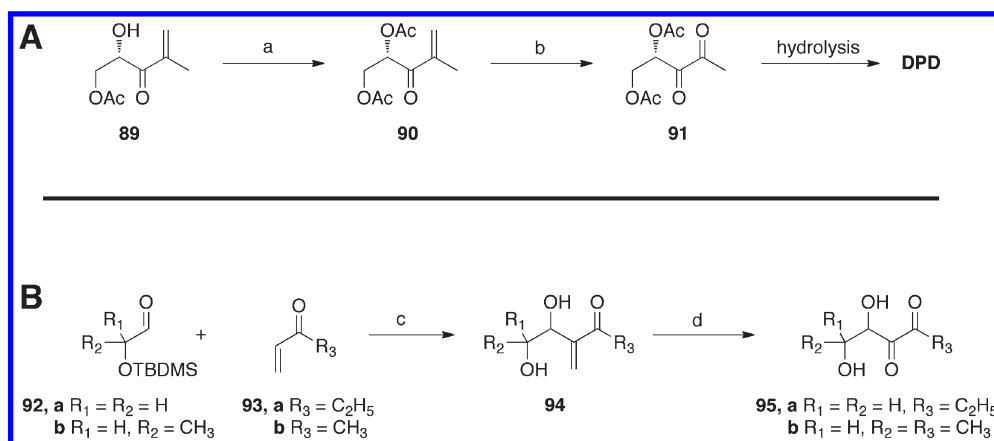
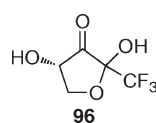
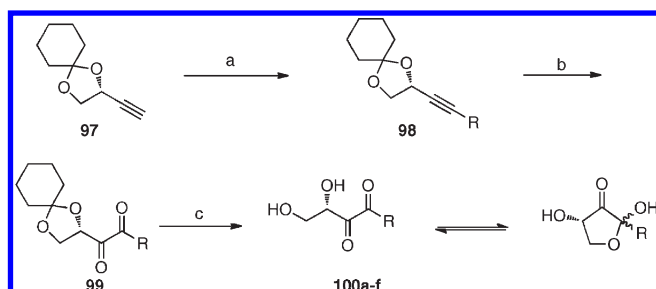


Figure 19. Structures of laurencone, MHF, and the putative carbonate-DPD species.

Scheme 5. Synthesis of (A) Ac₂-DPD **91** and (B) C5-Alkyl DPD Analogues **95**^a

^a Reagents: (a) Ac₂O, pyr, Et₃N, CH₂Cl₂, 0 °C, 18 h; (b) O₃, MeOH, Me₂S, -78 °C to room temp, 24 h; (c) (i) THF, DABCO, 0 °C; (ii) TBAF, THF, room temp; (d) O₃, MeOH, Me₂S, -78 °C to room temp.

**Figure 20.** Structure of trifluoro-DPD.**Scheme 6.** Synthesis of C1-Alkyl DPD Analogues^a

^a Reagents: (a) *n*-BuLi, R-I, -78 °C to rt, 12 h; (b) RuO₂, NaIO₄, MeCN, CCl₄, H₂O, 15 min; (c) pH 1.5, 6 h.

Second, the difference of the length of the alkyl chain brought a dramatic change to the biological activity ranging from the natural substrate to no activity to a potent inhibitor. Interestingly, these alkyl-DPD analogues were also found to inhibit the bioluminescence of *V. harveyi* when added to cultures that had already initiated QS.⁵⁰ This finding may speak to the difference in QS signal transduction between low and high cell density cultures of *V. harveyi* or potential differences in activity of QS modulators under different assay conditions.

A later report from Meijer and co-workers observed a similar trend of synergistic agonist effects between the alkyl-DPD analogues and the natural DPD signal, with a decrease in activity correlating to increasing alkyl chain length.¹³⁰ Another study, this time by the laboratory of Herman Sintim, observed the same synergistic activity between alkylated DPD analogues, although in this study there was not a clear trend between size and shape of the alkyl groups and the observed synergistic activity, thus suggesting a promiscuous mechanism of signal recognition.¹³¹ The alkyl-DPD analogues of the former study were also shown to have an effect on the QS of *P. aeruginosa*. Although *P. aeruginosa* does not possess a known LuxS enzyme, it has been shown to respond to the AI-2 signal to regulate pathogenicity.¹³² Thus, the alkyl-DPD

Table 4. Activity of Alkyl-DPD Analogues against the QS of *S. typhimurium*^a

| compd | R | IC ₅₀ in <i>S. typhimurium</i> ^b |
|------------------------------|------------------------------------------------------------------|--------------------------------------------------------|
| 100a , ethyl-DPD | CH ₂ CH ₃ | >50 |
| 100b , propyl-DPD | CH ₂ CH ₂ CH ₃ | 5.30 |
| 100c , butyl-DPD | CH ₂ (CH ₂) ₂ CH ₃ | 5.04 |
| 100d , hexyl-DPD | CH ₂ (CH ₂) ₄ CH ₃ | 24.9 |
| 100e , phenyl-DPD | CH ₂ CH ₂ (C ₆ H ₅) | >50 |
| 100f , azidobutyl-DPD | CH ₂ (CH ₂) ₃ N ₃ | 20.3 |

^a All values reported in μM. ^b Assay performed in the presence of 50 μM DPD.

analogues inhibited *lasI*-dependent luminescence in a modified PAO1 reporter strain and pyocyanin production in wild type *P. aeruginosa*.¹³⁰ Although the mechanism of QS inhibition in *P. aeruginosa* was not determined, the authors hypothesize that the alkyl-DPD analogues act on the *las*-regulated system, as these analogues bear structural resemblance to known inhibitors of LasR (e.g., **2**, Figure 2), including the brominated furanones (**36–39**, Figure 9).³⁹

As described in the AHLs section, the halogenated furanone natural products have shown excellent activity against both AHL and AI-2 dependent QS systems. In particular, the natural product (*SZ*)-4-bromo-5-(bromomethylene)-3-butyl-2(*5H*)-furanone **36** possesses excellent inhibitory activity against the AI-2-regulated bioluminescence system of *V. harveyi*. The activity of this compound against AI-2 based QS was assessed using a double mutant of *V. harveyi* that lacks the receptors for the AHL and CAI-1 signals; thus, the only luminescence produced must be a result of the AI-2 system. Both the natural product **36** and the synthetic furanone **41** (Figure 9) were shown to inhibit bioluminescence in this strain, with no effects on cell growth. Willy Verstraete and co-workers have shown that treatment with either the natural **36** or synthetic furanone **41** imparts protection of the shrimp *Artemia nauplii* from lethal infections of *V. harveyi* and *V. campbellii*.¹³³ However, the furanones become toxic at concentrations slightly higher than those needed to significantly increase survival of the shrimp, possibly limiting the utility of these compounds in vivo. Nevertheless, these data suggest a possibility for therapeutic intervention via AI-2 QS inhibition in animals. Furthermore, treatment of bacterial infection in aquaculture has seen heavy dependence on the use of antibiotics and, consequently, the rise of antibiotic resistance; as such, it is for this same reason that QS represents an attractive target in the treatment of human disease.

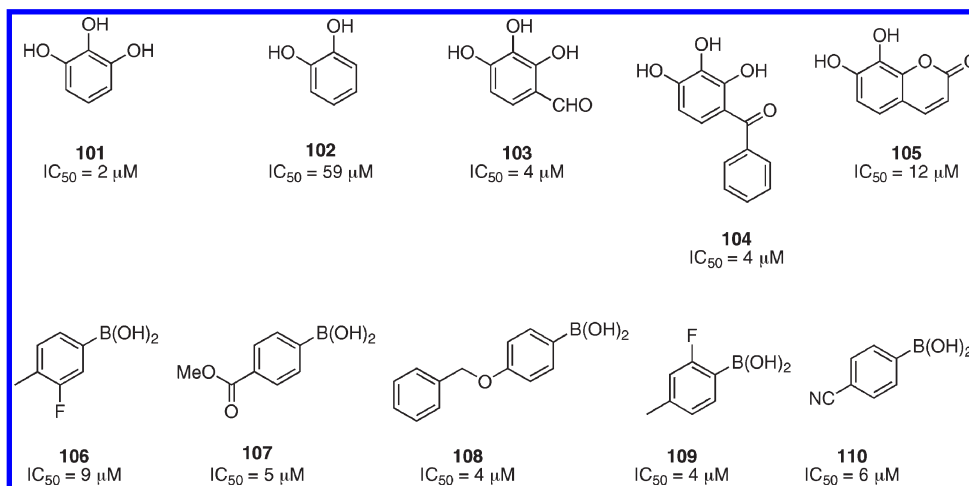


Figure 21. Representative structures of polyphenols and boronic acid AI-2 inhibitors. IC₅₀ values represent the concentration required for 50% inhibition of *V. harveyi* bioluminescence.

In addition to the effects on *Vibrio* sp. and *P. aeruginosa*, the brominated furanones have exhibited QS inhibitory activity against a variety of bacteria, including both Gram-positive and -negative strains. Notably, the brominated furanones inhibit the swarming behavior, biofilm formation, and percentage of live cells in biofilms of *E. coli*. However, colony growth on agar plates revealed no cytotoxicity of the furanone against *E. coli*, indicating specific QS inhibition.¹³⁴ A subsequent study by Thomas Wood and co-workers employed DNA microarrays to show that the furanone inhibited 80% of the same genes that were activated by AI-2.^{135,136} Additionally, therapeutic and industrial applications may be found for the brominated furanone compounds outside of specific QS inhibition, as they have exhibited activity against biofilm formation by *S. typhimurium*,¹³⁷ virulence gene expression of *Bacillus anthracis*,¹³⁸ swarming and biofilm formation of *Bacillus subtilis*,¹³⁹ and steel corrosion caused by *Desulfotomaculum orientis*.¹⁴⁰ Thus, the brominated furanones have therapeutic and industrial value beyond that of specific QS inhibition, although it should be noted that the brominated furanones also significantly inhibit bacterial growth in many of the above-mentioned examples, raising concerns about the specificity of the QS antagonism.

Two possible mechanisms of action have been reported for the QS inhibition of the brominated furanones: interaction with the LuxR_{Vh} regulatory protein (vide supra) and covalent modification of the DPD synthase LuxS.⁴⁸ A recent report by Zhaohui Zhou and co-workers details the inhibition of the LuxS enzyme by the brominated furanones, which the authors propose occurs via two possible mechanisms both of which occur through conjugate addition by a nucleophilic residue in the active site of LuxS.⁴⁸ In support of this, the enzyme–inhibitor complex was subjected to mass spectrometry, and a LuxS adduct was found that corresponds to the addition of one furanone molecule and loss of a bromine atom. Furthermore, by use of protein digestion and MS analysis, binding of the furanone was found to occur at a cysteine residue in the active site, whereas other more accessible and reactive cysteine residues were not modified. The modification of either LuxR_{Vh} or LuxS proteins by the brominated furanones represents an attractive target for the development of antimicrobial therapies, as neither enzyme is present in humans. However, the brominated furanones have also exhibited toxicity against mammalian cells, including human cell lines,^{50,141} but nevertheless

may represent a viable scaffold for the development of less toxic analogues.

Binghe Wang and co-workers have published a series of screening efforts for the discovery of AI-2 inhibitors, encompassing both the evaluation of small focused libraries and virtual screening techniques. Their first two studies focused on libraries centered around two key structural features of the *V. harveyi* AI-2 signal: the vicinal diol and the borate diester.^{142,143} Several cyclic alcohols, including laurencione (**86**) and MHF (**87**), have previously been evaluated as AI-2 antagonists, but these efforts had only yielded “hits” with agonist activity.^{120,122,123} Thus, in their first study, a series of aromatic and five-membered ring diols were evaluated for their ability to inhibit AI-2-based QS in *V. harveyi*.¹⁴² From this screen, five potent inhibitors were discovered, all of which were derived from the pyrogallol structure **101** (Figure 21). However, it was found that pyrogallol derivatives with ionizable groups exhibited diminished activity. A structural rationale for these findings was provided via docking studies with the LuxP protein, which revealed that the pyrogallol structure is capable of the same H-bonding interactions as the natural AI-2 signal, but the presence of an aspartic acid residue in the binding pocket of the LuxP receptor protein may preclude binding by ionized functionalities. These researchers also reported a similar screening process, this time using a series of about 50 boronic acids, and again, several low micromolar inhibitors were uncovered (**106–110**, Figure 21).¹⁴³ Similar to the pyrogallol derivatives, ionizable groups on the boronic acids diminished the biological activity. It was also determined that arylboronic and vinylboronic acids represented the best inhibitors, likely due to their increased acidity promoting the formation of the tetrahedral borate species found in the natural AI-2 signal. On the basis of these results, a recent study by Ni et al. focused on the evaluation of several arylboronic acids and again found several inhibitors with IC₅₀ values in the single digit micromolar range.¹⁴⁴ The acidity of the boronic acid again seemed to play a crucial role, as more acidic compounds exhibited increased potency.

Taking a different approach and in light of a relative dearth of information pertaining to the development of AI-2 inhibitors, Wang and co-workers later undertook a virtual screening approach to model a total of 1.7 million compounds against the *holo* form of the LuxP receptor protein;¹⁴⁵ 42 compounds were identified as potential hits, and 27 of these were evaluated

for QS inhibition. Two compounds (**111** and **112**), both containing a sulfone group, were found to be moderate inhibitors with IC_{50} values in the range of 35–55 μM (Figure 22). Interestingly, over half of the hits identified from the in silico screen possessed a sulfone group, likely because of stabilization in the binding site of LuxP imparted by interactions between the oxygen atoms of the sulfone group and active site arginine residues. Furthermore, structural comparisons between active and inactive sulfone-derived compounds

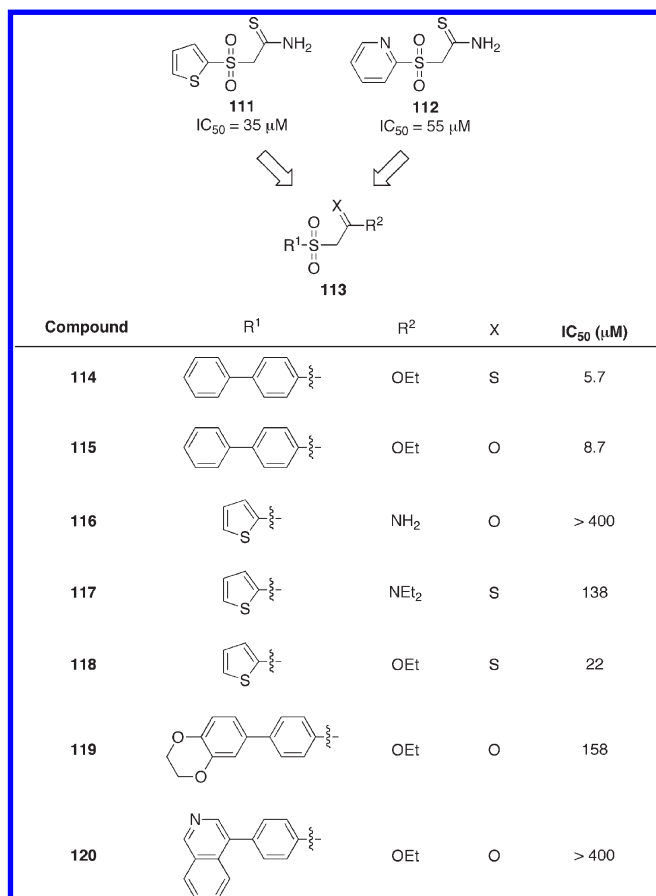


Figure 22. Sulfone based AI-2 inhibitors identified by in silico screening and elaboration of lead compounds. IC_{50} values represent the concentration required for 50% inhibition of *V. harveyi* bioluminescence.

revealed the proximity of the sulfone to the aromatic group and the presence of a methylene group between the sulfone and thioamide as critical for activity (**113**). The two active compounds identified by these in silico efforts also served as lead compounds for the development of other antagonists, and about 40 analogues were designed and synthesized based on the SAR established in the previous study (Figure 22).¹⁴⁶ Of these compounds, several displayed more potent inhibition than the original lead compounds (**114**, **115**), and the authors were able to optimize the sulfone/thioamide-derived inhibitors with the conversion of the thioamide to a slightly hydrophobic ester and the incorporation of a biphenyl system adjacent to the sulfone. The authors later combined these rules and evaluated a library of phenothiazine derivatives and again discovered potent inhibitors.¹⁴⁷

Screening of plant oils in search of compounds that modulate the biofilm formation of *P. aeruginosa* and *E. coli* has also uncovered QS inhibitors, as cinnamaldehyde **121** was found to inhibit the biofilm formation of both species as well as the swimming motility of *E. coli*.¹⁴⁸ Later studies have detailed the activity of cinnamaldehyde as an inhibitor of both AHL- and AI-2-based QS in *V. harveyi*,¹⁴⁶ and analogues of cinnamaldehyde have been synthesized and evaluated to elucidate the mechanism of QS inhibition and potentially modulate the virulence of *Vibrio* sp. (Figure 23).^{149,150} However, only 2-nitrocinnamaldehyde displayed activity approaching that of the lead compound **121**. To determine the mechanism of action, compound **121** was examined in several QS mutants of *V. harveyi* and in binding studies with the LuxR_{Vh} regulatory protein to confirm LuxR_{Vh} as the biological target.¹⁵⁰ The cinnamaldehyde compounds, specifically **121** and **122**, were also found to inhibit protease and pigment production, two virulence factors important in the virulence of *Vibrio anguillarum* during infection of marine animals. Finally, **121** was demonstrated to protect *Artemia* shrimp from *V. harveyi* infection. As such, there are clear parallels between the observed activity of cinnamaldehyde derivatives and that of the brominated furanones (vide supra). Additionally, both of these inhibitors are Michael acceptors, and thus, it is unclear whether these cinnamaldehyde derivatives act through specific QS inhibition or via nonspecific protein alkylation;¹⁴⁵ nevertheless, the cinnamaldehyde derivatives may still serve as viable candidates for the treatment of infection by various *Vibrio* species.

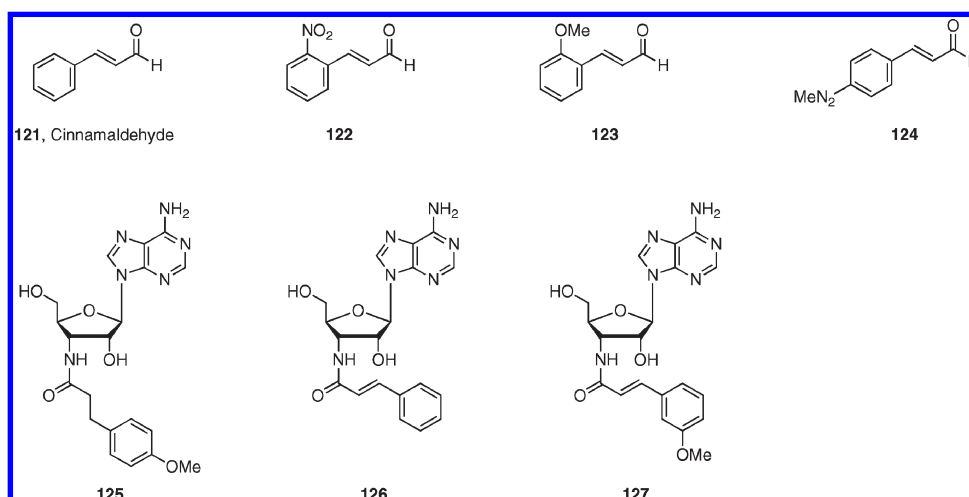


Figure 23. Structures of cinnamaldehyde and nucleoside analogues with activity against AI-2-based QS.

In a more recent study, Tom Coenye and co-workers tested a set of known QS inhibitors, along with a series of nucleoside analogues against the QS of various *Vibrio* spp.¹⁵¹ Nucleoside **125** was found to have potent effects against *V. harveyi*, and again through the use of QS mutants, the target was narrowed to the LuxPQ receptor protein. This was unanticipated, as initial reasoning led the authors to expect interference with DPD production based on structural similarities between **125** and the DPD precursor *S*-adenosylmethionine. Nucleoside **125** also exhibited activity against protease production and biofilm formation by *V. anguillarum*, biofilm formation by the human pathogen *V. vulnificus*, and mortality of *Artemia* shrimp caused by *V. harveyi* infection. Importantly, no significant cytotoxic effects were observed with **125** against the shrimp or mammalian and human cells. On the basis of these findings, a small panel of analogues was constructed to determine the functional groups required for activity, but each modification resulted in decreased activity (Figure 23). Notably, the introduction of a double bond in **126** and **127** did not result in increased activity. Thus, although this structural modification does render these two compounds to be Michael acceptors, a characteristic shared by other reported AI-2 inhibitors, no enhanced potency is achieved. In addition to establishing **125** as a candidate for development as a QS inhibitor, these studies also serve to support the role of AI-2 based QS in the pathogenesis of *V. anguillarum*, *V. vulnificus*, and *V. cholerae* and the control of QS as a means of treating infection in animals.

Conclusion

Since the discovery of the first QS systems, microbiologists have been intrigued by the prospect of controlling bacterial behavior with the use of man-made molecules. At the same time, chemists have been captivated by the synthetic challenges posed by the signals employed by bacteria for communication. As such, several sources for the discovery of new signaling molecules and signaling antagonists have been explored ranging from natural products to the high-throughput screening of diverse chemical libraries. Although many of these challenges have been overcome, the ongoing discovery of new chemical signals will continue to challenge both organic and biological chemists. One significant obstacle on the road to anti-QS strategies as viable antimicrobial treatments is definitive evidence that the modulation of QS will indeed result in changes in clinical outcomes. While this has been demonstrated in animal models, the examples are limited in mammalian models. Furthermore, some of the known QS inhibitors have shown cytotoxic effects against mammalian cells; as such, the onus falls to medicinal chemists to develop compounds capable of the inhibition of QS and the resulting virulence patterns while avoiding undesired toxic side effects. Despite these challenges, chemists should be encouraged at this prospect, as sufficient *in vitro* and *in vivo* evidence suggests that QS is a suitable target for antimicrobial therapies and discoveries in this area may turn a new page in the ongoing struggle against bacterial infections.

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Biographies

Colin A. Lowery received his B.Sc. degree in chemistry from the University of Virginia (2004) and conducted research with Prof. Milton Brown. In 2004, he joined the laboratory of Prof.

Janda as a graduate student of the Kellogg School of Science and Technology at The Scripps Research Institute where he was the recipient of a Sanofi-Aventis graduate fellowship. After completing his Ph. D. studies in 2010, he has continued to work in the Janda laboratory and his current research is focused on quorum sensing, particularly the synthesis of autoinducer analogues and the activity of autoinducers in biofilms of *P. aeruginosa*.

Nicholas T. Salzameda received his B.A. in Chemistry from the University of San Diego, CA (2001). He then earned his Ph.D. from the University of Nevada, Reno (2006), in Chemistry working in Prof. David Lightner's group synthesizing dipyrrole analogues of the natural product bilirubin and studying their shape and hydrogen bonding properties. From 2006 to 2010, Nicholas was a postdoctoral fellow in the Janda laboratory in the Department of Chemistry at The Scripps Research Institute. In Professor Janda's laboratory, Nicholas was involved in the synthesis of DPD analogues and the development of inhibitors of the botulinum neurotoxin. Currently, Nicholas is an Assistant Professor in the Department of Chemistry and Biochemistry at California State University, Fullerton.

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