HHS Public Access

Author manuscript

ACS Infect Dis. Author manuscript; available in PMC 2024 April 13.

Published in final edited form as:

ACS Infect Dis. 2024 April 12; 10(4): 1212–1221. doi:10.1021/acsinfecdis.3c00593.

Abiotic small molecule inhibitors and activators of the LasR quorum sensing receptor in *Pseudomonas aeruginosa* with potencies comparable or surpassing *N*-acyl homoserine lactones

Daniel E. Manson[1,†], **Gene E. Ananiev**[2], **Song Guo**[2], **Spencer S. Ericksen**[2], **Emma E. Santa**[1], **Helen E. Blackwell**[1]

[1]Department of Chemistry, University of Wisconsin–Madison, 1101 University Ave., Madison, WI 53706, USA

[2]Small Molecule Screening & Synthesis Facility, University of Wisconsin Carbone Cancer Center, 600 Highland Ave., Madison, WI 53792, USA

Abstract

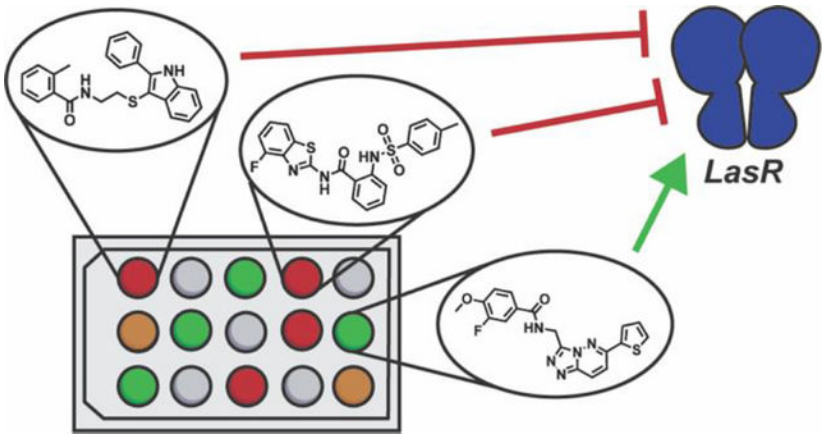
The opportunistic pathogen *Pseudomonas aeruginosa* controls almost 10% of its genome, including myriad virulence genes, via a cell-to-cell chemical communication system called quorum sensing (QS). Small molecules that either inhibit or activate QS in *P. aeruginosa* represent useful research tools to study the role of this signaling pathway in infection and interrogate its viability of as an anti-virulence approach. However, despite active research in this area over the past 20+ years, there are relatively few synthetic compounds known to strongly inhibit or activate QS in *P. aeruginosa*. Most reported QS modulators in this pathogen are of low potency or have structural liabilities that limit their application in biologically relevant environments, such as mimics of the native *N*-acyl homoserine lactone (AHL) signals. Here, we report the results of a high-throughput screen for abiotic small molecules that target LasR, a key QS regulator in *P. aeruginosa*. We screened a 25,000-compound library and discovered four new structural classes of abiotic LasR modulators. These compounds include antagonists that surpass the potency of all known AHL-type compounds and mimetics thereof, along with an agonist with potency approaching that of LasR's native ligand. The novel structures of this compound set, along with their anticipated robust physicochemical profiles, underscore their potential value as probe molecules to interrogate the roles of QS in this formidable pathogen.

Graphical Abstract

Correspondence: (H.E.B.) blackwell@chem.wisc.edu.
†*Current address:* Cooley LLP, New York, NY 10001

ASSOCIATED CONTENT

Supplemental information (details of strains and plasmids, expanded experimental protocols, computational docking data, and full dose-response curves) is available free online.



Keywords

N-acyl L-homoserine lactone; bacterial communication; Gram-negative bacteria; intercellular signaling; LuxR-type receptor; *Pseudomonas aeruginosa* ; virulence

Many common bacteria communicate using low molecular weight molecules, or "autoinducers," in a phenomenon known as quorum sensing (QS).[1] This cell-to-cell signaling process allows populations of bacteria to gauge their local densities and at high cell number engage in group-beneficial behaviors, some of which are relevant to human health, agriculture, and industrial processes.[2, 3] The chemical nature of QS, dependent on the concentration of a signal in the local bacterial environment, has attracted many researchers to develop chemical strategies to divert or alter the sensing of these signals.[4, 5] Our research group and others have been actively involved in the development of non-native small molecules and peptides capable of competing with native QS signals pathways.[4, 6–10] Such compounds have utility as tools to delineate the roles of this complex signaling mechanism in infection and host-microbe interactions.[2, 11]

The archetypal QS circuit in Gram-negative bacteria is the LuxI/LuxR-type receptor/synthase pair, first characterized in the bioluminescent marine symbiont *Vibrio fischeri.*[12] LuxI-type synthase enzymes catalyze the formation of *N*-acyl L-homoserine lactone (AHLs), which are produced at low basal levels at low cell density. At a sufficient bacterial cell number (and thus AHL concentration), these low molecular weight ligands bind to and activate their target intracellular receptors, i.e., LuxR-type proteins. Once complexed with their AHL signal, LuxR-type proteins act as transcription factors that most commonly dimerize and bind to DNA (in an associative mechanism), altering QS-controlled gene expression.[1, 2]

P. aeruginosa is a common Gram-negative pathogen that regulates many of its virulent behaviors (i.e., ability to infect) via QS.[13] It has a high rate of antibiotic resistance and is dangerous to immunocompromised populations, particularly cystic fibrosis (CF) patients. The intimate relationship between virulence and QS in *P. aeruginosa* has motivated considerable research toward the discovery of chemical and biological strategies capable of blocking QS in this pathogen, and thereby, potential infections.[8–10, 14–19]

The QS circuity in *P. aeruginosa* is comprised of at least two complete LuxI/LuxR-type pairs (LasI/LasR and RhlI/RhlR), QscR (an "orphan" LuxR type receptor without an associated LuxI-type synthase), and PqsR (a LysR-type receptor, unrelated to the LuxR-type family).[13] A simple schematic of these circuits is shown in Figure 1. LasI produces *N*-(3-oxo)-dodecanoyl L-homoserine lactone (OdDHL), which is recognized by both LasR and QscR. RhlI produces *N*-butyryl L-homoserine lactone (BHL), which is recognized by RhlR. PqsR binds the Pseudomonas Quinolone Signal (PQS), 2-heptyl-3-hydroxy-4-(1*H*)-quinolone, which is synthesized by a dedicated biosynthetic gene cluster (via the HHQ signal intermediate).[20] These four receptors and signals work together to finely tune *P. aeruginosa* QS in response to different environmental cues.[21]

Among these interconnected QS systems, LasR plays a prominent role in *P. aeruginosa*.[22] This receptor regulates the production of virulence factors such as elastase B and alkaline protease.[23] It also upregulates production of rhamnolipid, HCN, and pyocyanin via control of the RhlI/RhlR and PqsR systems. Accordingly, significant effort has been devoted to the identification of small molecules that can attenuate the activity of LasR, the most common mechanism blocking its native AHL signal, OdDHL, from binding (although few studies have demonstrated this pathway definitively).[13] To find such ligands, the OdDHL scaffold, along with other native AHLs, have been studied and derivatized extensively.[4, 19, 24] However, these efforts focused on AHL-type ligands have failed to produce a LasR antagonist with an IC_{50} value under 10 μM in *P. aeruginosa*, as based on in-cell transcriptional reporter gene assays.[15] This failure is at least in part due to chemical and biological liabilities inherent to the AHL scaffold. AHLs, both naturally occurring and non-native, are actively effluxed from *P. aeruginosa* by the MexAB-OprM multidrug efflux pump.[25] Additionally, the lactone ring is prone to hydrolysis in aqueous media,[26, 27] and *P. aeruginosa* (along with other bacteria and hosts) contains enzymes that can hydrolyze either the lactone or amide bonds, rendering the compounds inactive.[28] These liabilities have motivated our laboratory[29, 30] and others[31, 32] to develop QS modulators of *P. aeruginosa* and other Gram-negative bacteria that are not based on the AHL scaffold.[29]

Past work has shown that high-throughput screening (HTS) of small molecule libraries can provide a strategy to discover non-AHL-type QS modulators. Selected compounds uncovered in these screens are shown in Figure 2. LasR has been the focus of past HTS efforts to uncover antagonists and agonists of LuxR-type receptors in *P. aeruginosa*. The Greenberg lab made notable contributions in this area using a reporter gene assay strategy,[10, 33] discovering V-06–018, the most potent LasR antagonist known at the time of its discovery in 2006 (LasR IC_{50} = 5.2 μM; all potency values reported here from Moore et al., 2015),[15] PD-12 (later shown to inhibit LasR indirectly),[15] and the triphenyl (TP) series of ligands. This latter series includes the agonist TP-1, which surpasses LasR's native ligand OdDHL in terms of potency (EC_{50} = 71 nM vs. 139 nM), and TP-5, a LasR antagonist with modest potency (IC_{50} = 69 μM).[10, 34] Rahme and co-workers have applied HTS very successfully to develop small molecule inhibitors of PqsR, such as M64.[16] Virtual screening also has been applied to identify QS modulators in *P. aeruginosa*. For example, 8-azaguanine (Figure 2), identified *in silico* to bind LasR, was found to antagonize LasR in cell-based gene reporter assays and abrogate QS-controlled phenotypes in *P. aeruginosa*, albeit at high μM concentrations.[35] Compound C1 was identified in a similar manner by Vetrivel *et al.* and,

along with two other related compounds, could inhibit LasR activity in an *E. coli* reporter strain at high nM concentrations;[36] the effects of these molecules on cell growth, however, was not reported. Many other compounds have been predicted *in silico* to bind LasR, but very few have been validated experimentally.[37, 38] While not arising from HTS efforts per se, smaller scale screening efforts have identified plant-derived natural products (e.g., phenols and flavonoids) and extracts that modulate LasR; these compounds/extracts have seen limited mechanistic study and many are active at numerous targets beyond LasR.[39, 40]

Synthetic modulators of LasR discovered via HTS have served as starting points for the development compounds with improved activities or altered properties, including the TP series[30, 42, 43] and V-06–018. In 2020, our laboratory reported a LasR antagonist with a submicromolar IC_{50} (V-40, Figure 2) that arose through our study of the structure-activity relationships (SARs) surrounding V-06–018's activity profile in LasR.[41] However, improved potency is not the only desired characteristic; the lead compounds from that past study are highly lipophilic (e.g., V-40) and cannot be readily diversified further, which will limit their application as probes in certain biological settings. In order to develop more versatile probe molecules, we remain interested in the development of novel non-AHL scaffolds that are (1) highly potent and selective at modulating LasR in *P. aeruginosa* based assays (IC_{50} values in the single-digit μM or lower in *P. aeruginosa*), (2) free of hydrolytically and enzymatically labile bonds, (3) readily soluble in aqueous media, and (4) comprised of a structure that is amenable to further chemical diversification. Identifying such compounds was the goal of the current study.

Herein, we report the discovery of nine small molecule LasR modulators that resulted from the screening a 25,000-compound library in a *P. aeruginosa* reporter strain. The small molecules can be divided into four broad structural classes that, to our knowledge, have not been reported previously to modulate LuxR-type proteins in bacteria. Several of these compounds were capable of strong LasR antagonism, with IC_{50} values below 1 μM, and one compound exhibited LasR agonism with potency and efficacy levels approaching LasR's native ligand, OdDHL. Moreover, these molecules are highly soluble and physically robust in biological media, and readily amendable to synthetic derivatization. Follow-on biological screens indicate that these compounds can modulate LasR via direct interactions; insights into the possible binding modes of these compounds with the LasR ligand-binding site were provided by molecular docking experiments. These compounds represent robust new chemical tools to study QS pathways in *P. aeruginosa* and their roles in infection, and should advance future probe design.

RESULTS & DISCUSSION

Implementation and execution of a high-throughput screen.

We selected the commonly used *P. aeruginosa*-based LasR reporter strain PAO-JP2 (Δ*lasIrhlI*)[23] for our HTS experiments. This *P. aeruginosa* mutant lacks the ability to synthesize its OdDHL and BHL signals, but retains the LasR and RhlR receptors, and LasR activity is reported via a plasmid encoding GFP (p*lasI*-LVAGFP). The strain contains the MexAB-OprM efflux pump mentioned above;[25] we reasoned its presence would facilitate the identification of chemical scaffolds that are not readily effluxed from *P. aeruginosa*. To

start, we miniaturized our standard reporter gene assay protocol[41] for LasR from a 96-well microtiter plate format to a 384-well plate format. The principal challenge in this process was the accumulation of *P. aeruginosa* biofilm in the low volume wells, which was overcome by starting the assay at a low cell density (see Methods for full details).

Using the miniaturized assay, we screened a 25,000-compound library (from Life Chemicals Inc.) at a concentration of 10 μM. These compounds are structurally diverse and satisfy Lipinski's Rule-of-5, Veber conditions, and Lilly Med Chem Rules.[44] The library was pre-filtered to remove pan-assay interference compounds (PAINS),[45, 46] known reagents, and potentially chemically unstable molecules. As a positive control for LasR agonism, we used the native agonist OdDHL (at 300 nM), and as a control for LasR antagonism, we used the known inhibitor V-06–018 (at 10 μM). We also used a known antibiotic, ciprofloxacin, as a control for cell death. Each screening well constituted a competitive inhibition experiment and contained OdDHL at 300 nM (to activate LasR) and a screening candidate molecule. We identified 172 compounds (~0.7% hit rate) from the library that either inhibited or increased LasR activity ≥15% relative to our controls. These compounds were selected for secondary screening in the *P. aeruginosa* reporter strain over a range of concentrations to obtain dose-response activity profiles. We removed any suspect PAINS and compounds with enzymatic liabilities/chemical instabilities not caught in the initial pre-filtering as we triaged the hit compounds. Ultimately, we focused on a set of the most potent compounds, along with several of their commercially available analogues; we purchased samples of these nine compounds and characterized their LasR activity profiles in more detail, as described below.

Structures and activities of hit compounds.

The structures of the nine compounds and related analogs uncovered in the HTS study are shown in Figure 3, and their antagonism and agonism activity profiles in LasR are listed in Table 1 (see Figures S1 and S2 for dose response curves). None effected bacterial cell growth over the concentrations tested (Figure S3) and all were readily soluble in aqueous solutions (with 1% DMSO). To the best of our knowledge, none of these compounds have been previously reported to modulate LuxR-type proteins or any other biological targets.

We found three classes (I–III) of LasR antagonists and one class (IV) of LasR agonist in the set of HTS hits (Figure 3). The clustering of the hits into a small set of structural classes underscores the fidelity of the HTS assay and indicates that various pharmacophores capable of inhibiting LasR may be discovered via HTS. Each of these four classes presented a chemical scaffold that was modular in nature (e.g., a plurality of cyclic moieties connected by linker(s)), a feature that should enable further synthetic modifications. Amongst the eight LasR antagonists identified, seven (**1**–**7**) have higher potencies in the *P. aeruginosa* reporter assay relative to all known AHL-based antagonists,[47] and two compounds (**3** and **7**) have potencies comparable to the most potent known non-AHL antagonist. For comparison, we have included *P. aeruginosa* reporter assay data for the most potent known AHL-based LasR agonist and antagonist (OdDHL and CL [chlorolactone],[8] respectively), the most potent known non-AHL LasR agonist and antagonist (TP-1 and V-40, respectively),[41] and the non-AHL LasR antagonist related to TP-1 (TP-5) in Table 1.

The compounds in class I (**1** and **2**), comprised of a benzamide joined to a 2-phenyl-indole moiety by way of a two-carbon thioether moiety, had indistinguishable efficacies (~50%) and potencies (approximately 3 μM), suggesting a variety of *ortho* substituents on the benzamide moiety can be tolerated (Table 1). Class II (**3**–**6**), which we term "tris-aryl" based on their moderate resemblance to the TP series of ligands, had indistinguishable IC_{50} values between 1 and 3 μM. These compounds differed, however, in maximum LasR inhibition; compound **6** had a higher efficacy than compounds **3** and **4**. We note the structural similarity between compounds **3**–**6** and TP-5 (Figures 2 and 3).[10] As in compounds **3**–**6**, the central ring of TP-5 is unsubstituted apart from the linkers to the other rings. In contrast, the central ring of TP-1 (and related analogs TP-2–4),[10] all of which are LasR agonists, are brominated at positions 2 and 4. Additionally, the terminal rings of compounds **3**–**6**, like TP-5, are linked to the central ring by two-atom functional groups (an amide and a sulfonamide for **3**–**6** vs. two amides for TP-5). The agonists TP-1–4, however, have an extra methylene unit in the linker between the first and second ring, and an ester linking the second and third ring. Perhaps most notably, the tris-aryl compounds we report here (**3**–**6**) are approximately 30 times more potent than TP-5 in *P. aeruginosa* (TP-5 IC_{50} = 69 μM, Table 1). Indeed, despite its lower efficacy (38%), compound **3** has a sub-micromolar potency (0.8 μM) comparable to V-40, which is the most potent and efficacious LasR non-AHL type antagonist known, underscoring the potential utility of this new tris-aryl ligand scaffold for LasR modulation. Further synthetic studies on this scaffold can clarify whether this improvement in potency is a function of the different ring identity (phenyl vs. benzothiazole), substituents on the rings, the functional groups that link them (i.e., ester vs. sulfonamide), or a combination thereof.

The class III compound set (Figure 3), comprised of an alpha-benzisoxazole-acetamide linked at the 3' position of the benzisoxazole to a phenol derivative by way of an internal alkyne, contained the second of the two most potent LasR antagonists (**7**) and the most efficacious LasR antagonist in this study (**8**, Table 1). Compound **7**, like **3**, has a potency (IC_{50} = 0.4 μM) that is comparable to V-40, yet is two-fold more efficacious than **3**. In turn, compound **8** has similar efficacy (84%) as V-40, yet was the least potent antagonist of the hit compounds we evaluated (IC_{50} = 18 μM). These results for **7** and **8** indicate that substitution on the aryl ether moiety plays a large role in determining the activity profile of this compound class in LasR.

Class IV contained a single compound, **9** (Figure 3), which shares the benzamide group with the class I compounds yet has an alternate one-atom linkage to a thiophene-substituted triazolopyridazine moiety. Interestingly, when **9** was screened for competitive LasR antagonism assay in the HTS format, it was found to *increase* GFP production relative to controls (Figure S1). We therefore characterized its LasR agonism profile, along with those of compounds **1**–**8** to complete their activity assessment (Figure S2). Only compound **9** was active in the LasR agonism assay, and it was found to be a relatively potent LasR agonist (EC_{50} = 0.7 μM). To our knowledge, only OdDHL, very closely related AHL analogues, and TP-1 have sub-micromolar agonism potencies in *P. aeruginosa* reporter systems.[15] Compound **9** therefore represents a new and abiotic LasR agonist scaffold.

Screening in *E. coli* supports compounds can target LasR.

We conducted our HTS and follow-up assays in *P. aeruginosa* because we are most interested in the application of these compounds as probes to study LasR in its native organism. To investigate whether these compounds can target LasR directly, however, and not some other part of the *P. aeruginosa* QS circuitry (or another non-QS target), we used a heterologous reporter system (i.e., the *E. coli* JLD271 strain; see Methods), which produces LasR and contains a reporter plasmid (β-gal) to measure LasR activity.[48] Our compounds retained activity in this reporter strain, supporting that they can target LasR directly (see Figures S4 and S5, Table S2).[49] However, we found that the potencies and efficacies of antagonists **1**–**8** were reduced in the *E. coli* LasR reporter relative to in the *P. aeruginosa* LasR reporter system; for example, we could not determine IC_{50} values for compounds **3** and **6** in the *E. coli* system. We ascribe this phenomenon of reduced apparent IC_{50} in *E. coli* (which we have observed previously)[15, 41] to the increased amount of LasR produced in the heterologous system relative to native production from the bacterial genome.[41, 50] In turn, receptor overexpression has been shown previously to *increase* the apparent potency of agonists.[50] Accordingly, agonist **9** was two orders of magnitude more potent in *E. coli* relative to *P. aeruginosa* (EC_{50} values of 8 vs. 700 nM, respectively). Additionally, compounds **6**–**8**, which were incapable of agonizing LasR in the *P. aeruginosa* system, were found to moderately agonize LasR in the *E. coli* system (Figure S5). These compounds are therefore best characterized as partial LasR agonists, with agonism profiles too weak to be measured in the *P. aeruginosa* background. This latter activity profile does not impact the viability of **6**–**8** as probes to block LasR activity in *P. aeruginosa*, however, because the agonist activity was so minor as to not be measurable in experiments conducted in the native organism.

Molecular docking provides insights into protein-ligand interactions.

To investigate potential interactions of these compounds with LasR, we performed computational docking studies on selected hit compounds from each structural class (**1**, **6**, **7**, and **9**) into the OdDHL-binding site of LasR (selected 3D views shown in Figure 4). We used a composite LasR ligand-binding domain (LBD):agonist complex structure based on those reported by the Bottomley, Bassler, Nair, and our own groups (for AHL and TP-type agonists; see Methods).[42, 51–53] Given that all reported structures of LuxR-type proteins complexed with either AHL and non-AHL ligands show the small molecule bound to the AHL-binding site,[51, 54, 55] we believed it was reasonable to consider our new modulators binding in this site as well. For comparison, views of OdDHL and TP-1 bound to the composite LasR structure (Figures 4A and 4B) and overlays of these ligands with each composite docked structure for **1**, **6**, **7**, and **9** are provided (Figures 4C–F).

Prior structural studies have shown the LasR LBD:OdDHL interaction to be mediated in part by a network of hydrogen bonds to the lactone headgroup, the most critical of which is between the OdDHL amide N-H and Asp 73 (Figure 4A).[56, 57] Our docking experiments indicate that hydrogen bonds could exist between the amide N-H protons in compounds **1**, **7**, and **9** (and the sulfonamide N-H proton in **6**) and the LasR residues Asp 73, Thr 115, and Ser 129 (Figures 4C–F, *left panels)*. At least Asp 73 and Ser 129 are also known to

form hydrogen bonds to other heteroatoms on OdDHL in LasR LBD:OdDHL structures.[51, 53] This result suggests that the aryl amide groups in **1**, **7**, and **9** and aryl sulfonamide group in **6** mimic an AHL head group. The other portions of compounds **1**, **6**, **7**, and **9** then extend into the binding cavity of LasR filled by the aliphatic acyl tail of OdDHL and abut the hydrophobic residues that seal the binding site (i.e., Leu 40, Leu 125, and Ala 50), and can be considered AHL tail group mimics.

The reported crystallographic data for the LasR LBD:TP-1 complex indicate that the nitro-substituted benzamide moiety of TP-1 (docked view in Figure 4B) occupies a position in the LasR binding pocket analogous to that of the homoserine lactone and amide moiety in OdDHL and forms hydrogen bonds with at least Asp 73, Tyr 56, Trp 60, and Ser 129 (albeit the Ser 129 hydrogen bond is water-mediated),[53] and the ester-linked chloro-substituted aryl group in TP-1 fills the space in LasR "natively" occupied by the OdDHL acyl tail. Collectively, our docking data indicate that LasR can bind **1**, **6**, **7**, and **9** in a manner that is broadly similar to TP-1 and OdDHL (i.e., mediated by the same residues and analogous hydrogen bonds), and that these compounds are comprised of structural motifs that correspond to AHL head and tail groups. This hypothesis is supported by overlaying the docked poses of **1**, **6**, **7**, and **9** with either OdDHL or TP-1 (Figures 4C–F, *center panels*) in LasR; the compounds occupy the same space and are readily accommodated in the ligand-binding pocket. These overlaid structures do not reveal, however, obvious differences between the antagonists (**1**, **6**, and **7**) and the agonists (**9**, OdDHL, and TP-1) that could explain their opposite activities on LasR. As the structures used for docking originate from LasR LBD:agonist structures (no LasR:small molecule antagonist structure has been reported), neither the contacts necessary for LasR antagonism nor interactions between the LasR LBD and DNA binding domain could be considered in these docking experiments. Further characterization of these ligand:receptor binding interactions, including structural experiments, LasR mutagenesis, and new ligand design, are necessary to delineate the agonism and antagonism mechanisms of **1**, **6**, **7**, and **9** and are ongoing in our laboratory.

SUMMARY & CONCLUSIONS

This study was motivated by the need for potent and efficacious tool compounds that modulate the key QS regulator LasR in the opportunistic pathogen *P. aeruginosa*. AHL-based antagonists are poorly active in *P. aeruginosa*-based assays, and few non-AHL antagonists are known. Here, we report the discovery of eight new LasR antagonists and one new LasR agonist that are each structurally distinct from the AHL compound class. Each of the antagonists exceeds the potency of known AHL-based LasR antagonists in a *P. aeruginosa* cell-based gene reporter experiment, and two have potencies comparable to the most potent known non-AHL LasR antagonist. These new compounds should have physicochemical profiles superior to AHLs and close analogs (e.g., these compounds lack readily hydrolysable groups or a multi-carbon acyl "tail", and have high aqueous solubility), underscoring their potential value as probe scaffolds. Further, the modular structures of these compounds provide a straightforward path to the synthesis of analogs and structure-function studies. As such, we believe that the activity profiles of these molecules can likely be enhanced further by focused synthetic chemistry efforts.

The similarity between the Class II compounds reported here and the previously reported TP series of ligands is striking;[10] the reoccurrence of this chemotype in two different high-throughput screens provides compelling evidence of its utility as a chemical modulator of LasR activity. Our computational docking data indicate that the ligands from classes I–IV likely can adopt binding poses that are analogous to AHLs and the TP series, with a carbocyclic or heterocyclic “head group” packed into the same residues that surround the native homoserine lactone, an amide bond (or sulfonamide) making polar contacts with polar residues that mimic the hydrogen bond of the AHL amide (i.e., Asp 73, Ser 129, and Thr 115), and the remaining portion of the molecule occupying the hydrophobic acyl binding pocket. Additional experiments are required to elucidate specific ligand:LasR interactions and the mechanisms by which these may engender LasR antagonism or agonism. LasR mutagenesis experiments, in concert with probing additional structural analogs of these compound classes, will be particularly revealing in this context. Our results of course do not discount the possibility for additional pathways or targets for these compounds that result in LasR modulation.

To close, the discovery of the LasR probes reported herein represents an opportunity for researchers in the QS field to pivot from the historic use of AHL-based ligands to new, chemically robust modulator scaffolds for application as probes to study signaling in Gram-negative bacteria. Experiments to this end in *P. aeruginosa* and other related pathogens are ongoing and will be reported in due course.

MATERIALS & METHODS

Chemistry

The chemical library used in the HTS assay was a 25,000-compound subset of the LifeChem Pre-Plated Diversity library purchased from Life Chemicals Inc. The native ligand for LasR, OdDHL, was purchased from Sigma Aldrich. Compounds V-40 and CL were obtained from our in-house stocks and synthesized according to reported methods.[18, 41] Samples of **1**–**9** were obtained from Life Chemicals Inc. Compound stocks solution were prepared at appropriate concentrations in DMSO and stored at −20 °C until use.

Bacteriology

A list of bacterial strains and plasmids used in this study is provided in Table S1. All antibiotics (ampicillin, carbenicillin, and gentamicin), arabinose, and Luria-Bertani (LB) medium were purchased from Goldbio and used as received. Bacteria were cultured in LB medium and grown at 37 °C (with shaking at 200 RPM). Cell growth was measured by absorbance at 600 nm (OD_{600}). Absorbance and fluorescence measurements were made on a ThermoFisher Nanodrop 2000, a CLARIOstar® plate reader running MARS data analysis software, or a PerkinElmer EnVision plate reader running Envision Manger software. Dose response curves were prepared using GraphPad Prism software (version 8.3.1).

P. aeruginosa assay protocol

A 2-mL volume of LB medium with 300 μg/mL carbenicillin was inoculated with a single colony of *P. aeruginosa* reporter strain PAO-JP2 and grown overnight. The next morning,

the overnight culture was diluted 1:100 into fresh LB medium and grown until OD_{600} = 0.3 (measured on a Nanodrop). While the subculture grew, 2 μL of compound stocks at appropriate concentrations were manually dispensed into the interior wells of a black 96-well microtiter plate. Once the subculture reached the appropriate density, 198 μL of culture were dispensed into the interior wells. Plates were incubated for 6 h at 37 °C, at which point fluorescence and absorbance were read. See SI for modified HTS assay protocol using this strain and additional experimental details.

E. coli assay protocol

A 10-mL volume of LB medium with 100 μg/mL ampicillin and 10 μg/mL gentamicin was inoculated with a single colony of *E. coli* reporter strain JLD271 (pJN105-L, pSC11-L) and grown overnight. The next morning, the overnight culture was diluted 1:10 into fresh LB medium and supplemented with fresh antibiotics. Subculture was grown until OD_{600} = 0.5 (measured on a Nanodrop), at which point 0.4% w/v arabinose was added. While the subculture grew, 2 μL of compound stocks in DMSO at the appropriate concentration was added to the interior wells of a clear 96-well microtiter plate. Once the subculture reached the appropriate density, 198 μL of culture were dispensed into the interior wells, and plate was incubated for 4 h. After incubation, a Miller-type absorbance assay was performed as described previously.[41]

Ensemble and pose consensus docking methods

Target preparation.—Sixteen LasR LBD crystal structure representations were obtained from RCSB (PDB accession codes: 2UV0, 3IX3, 3IX4, 3IX8, 3JPU, 6D6A, 6D6B, 6D6C, 6D6D, 6D6L, 6D6M, 6D6N, 6D6O, 6D6P, 6MWH, 6MWL, and 6MWW). For structural comparison, given that the LasR crystal structures were typically in homooligomeric arrangements, all individual LasR LBD chains (with accompanying ligands) were isolated and aligned on an arbitrary reference domain, chain A from PDB accession 2UV0, using the built-in cealign function in PyMOL (the PyMOL Molecular Graphics System, Version 1.7.6.0 Schrödinger, LLC). Crystallographic waters and ions were removed. A single chain was selected from each crystal structure to comprise 16 target representations for docking based on the first chain identifier order in alphabetic sequence. After alignment, all crystallographic ligands in the orthosteric ligand-binding pocket were extracted and saved in SD format, and the apo protein chains were saved in PDB format. The 16 apo protein structure files were then processed in Chimera[58] using the Dock Prep function to protonate, assign MMFF partial charges, and output in mol2 format.

Ligand preparation.—Crystallographic ligands were protonated and assigned partial charges using pkatyper and molcharge (AM1BCC) utilities from OpenEye suite (QUACPAC 2.0.2.2: OpenEye Scientific Software, Santa Fe, NM). The nine compounds (**1**–**9**) uncovered in the current study were exported from ChemDraw in 2D SD format. The SD files were converted to SMILES using babel-3.3 (OpenEye Scientific), converted to 3D conformations in SD format with Omega2 (OMEGA 3.1.2.2: OpenEye Scientific), and converted to mol2 format with partial charges assigned by molcharge (AM1BCC).

Ensemble docking.—Each of the nine compounds (**1**–**9**) was docked to each of the 16 LasR target representations using Smina,[59] a fork of AutoDock Vina v1.1.2[60] in a typical ensemble approach.[61, 62] Smina's autobox_ligand feature was used to specify the docking search space based on the co-crystallized ligand identified for each LasR structure using 5 Angstrom padding.

Pose consensus analysis.—Output poses in SD format were processed using RDKit (RDKit: Open-source cheminformatics) to include docking scores, pose rank, compound ID, and target ID appended as molecular property fields. For each compound, only the top 3 scoring poses were saved when docking to each LasR target representation. This provided 16 targets * 3 poses/target = 48 total poses for each compound. Using a pose consensus approach,[63] we selected the most likely binding configurations for each compound based on the largest pose cluster observed among the compound's 48 total poses. The poses were clustered by the Butina-Taylor method[64] using pairwise RMSD as distance matrix and 2.5 Angstrom cutoff. A medoid pose is defined as that most central within a cluster based on lowest mean RMSD with respect to cluster cohorts. The individual docking scores for the top 3 poses for each compound:target pair are included in the SI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Financial support for this work was provided by the NIH (R35 GM131817). The authors thank the University of Wisconsin Carbone Cancer Center Small Molecule Screening Facility, supported by the NIH (P30 CA014520), for use of its facilities and services. The authors also thank OpenEye Scientific (Cadence Molecular Sciences) for use of the OpenEye Application Suite under free academic license.

REFERENCES

[1]. Whiteley M, Diggle SP, and Greenberg EP (2017) Progress in and promise of bacterial quorum sensing research, Nature 551, 313–320. [PubMed: 29144467]

[2]. Mukherjee S, and Bassler BL (2019) Bacterial quorum sensing in complex and dynamically changing environments, Nat. Rev. Microbiol. 17, 371–382. [PubMed: 30944413]

[3]. Wu S, Liu J, Liu C, Yang A, and Qiao J (2020) Quorum sensing for population-level control of bacteria and potential therapeutic applications, Cell Mol. Life Sci. 77, 1319–1343. [PubMed: 31612240]

[4]. Welsh MA, and Blackwell HE (2016) Chemical probes of quorum sensing: from compound development to biological discovery, FEMS Microbiol. Rev. 40, 774–794. [PubMed: 27268906]

[5]. Piewngam P, Chiou J, Chatterjee P, and Otto M (2020) Alternative approaches to treat bacterial infections: targeting quorum-sensing, Expert Rev. Anti-Infect. Ther. 18, 499–510. [PubMed: 32243194]

[6]. Majik MS, Gawas UB, and Mandrekar VK (2020) Next generation quorum sensing inhibitors: Accounts on structure activity relationship studies and biological activities, Bioorg. Med. Chem. 28, 115728. [PubMed: 33065436]

[7]. Polaske TJ, West KHJ, Zhao K, Widner DL, York JT, and Blackwell HE (2023) Chemical and Biomolecular Insights into the Staphylococcus aureus Agr Quorum Sensing System: Current Progress and Ongoing Challenges, Isr. J. Chem. 63, e202200096.

[8]. O'Loughlin CT, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, and Bassler BL (2013) A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation, Proc. Natl. Acad. Sci. U. S. A. 110, 17981–17986. [PubMed: 24143808]

[9]. Amara N, Mashiach R, Amar D, Krief P, Spieser SA, Bottomley MJ, Aharoni A, and Meijler MM (2009) Covalent inhibition of bacterial quorum sensing, J. Am. Chem. Soc. 131, 10610–10619. [PubMed: 19585989]

[10]. Muh U, Hare BJ, Duerkop BA, Schuster M, Hanzelka BL, Heim R, Olson ER, and Greenberg EP (2006) A structurally unrelated mimic of a *Pseudomonas aeruginosa* acyl-homoserine lactone quorum-sensing signal, Proc. Natl. Acad. Sci. U. S. A. 103, 16948–16952. [PubMed: 17075036]

[11]. Azimi S, Klementiev AD, Whiteley M, and Diggle SP (2020) Bacterial Quorum Sensing During Infection, Annu. Rev. Microbiol. 74, 201–219. [PubMed: 32660382]

[12]. Fuqua WC, Winans SC, and Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators, J. Bacteriol. 176, 269–275. [PubMed: 8288518]

[13]. Miranda SW, Asfahl KL, Dandekar AA, and Greenberg EP (2022) *Pseudomonas aeruginosa* Quorum Sensing, Adv. Exp. Med. Biol. 1386, 95–115. [PubMed: 36258070]

[14]. Valastyan JS, Tota MR, Taylor IR, Stergioula V, Hone GAB, Smith CD, Henke BR, Carson KG, and Bassler BL (2020) Discovery of PqsE Thioesterase Inhibitors for *Pseudomonas aeruginosa* Using DNA-Encoded Small Molecule Library Screening, ACS Chem. Biol. 15, 446–456. [PubMed: 31880429]

[15]. Moore JD, Rossi FM, Welsh MA, Nyffeler KE, and Blackwell HE (2015) A Comparative Analysis of Synthetic Quorum Sensing Modulators in *Pseudomonas aeruginosa*: New Insights into Mechanism, Active Efflux Susceptibility, Phenotypic Response, and Next-Generation Ligand Design, J. Am. Chem. Soc. 137, 14626–14639. [PubMed: 26491787]

[16]. Starkey M, Lepine F, Maura D, Bandyopadhaya A, Lesic B, He J, Kitao T, Righi V, Milot S, Tzika A, and Rahme L (2014) Identification of anti-virulence compounds that disrupt quorum-sensing regulated acute and persistent pathogenicity, PLoS Pathog. 10, e1004321. [PubMed: 25144274]

[17]. Soukarieh F, Liu R, Romero M, Roberston SN, Richardson W, Lucanto S, Oton EV, Qudus NR, Mashabi A, Grossman S, Ali S, Sou T, Kukavica-Ibrulj I, Levesque RC, Bergstrom CAS, Halliday N, Mistry SN, Emsley J, Heeb S, Williams P, Camara M, and Stocks MJ (2020) Hit Identification of New Potent PqsR Antagonists as Inhibitors of Quorum Sensing in Planktonic and Biofilm Grown *Pseudomonas aeruginosa*, Front. Chem. 8, 204. [PubMed: 32432073]

[18]. Boursier ME, Moore JD, Heitman KM, Shepardson-Fungairino SP, Combs JB, Koenig LC, Shin D, Brown EC, Nagarajan R, and Blackwell HE (2018) Structure-Function Analyses of the *N*-Butanoyl l-Homoserine Lactone Quorum-Sensing Signal Define Features Critical to Activity in RhlR, ACS Chem. Biol. 13, 2655–2662. [PubMed: 30114353]

[19]. Hossain MA, and German NA (2021) Update on Modulators of Quorum Sensing Pathways in *Pseudomonas aeruginosa*, Curr. Top. Med. Chem. 21, 2101–2108. [PubMed: 34561982]

[20]. Pesci EC, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP, and Iglewski BH (1999) Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*, Proc. Natl. Acad. Sci. U. S. A. 96, 11229–11234. [PubMed: 10500159]

[21]. Welsh MA, and Blackwell HE (2016) Chemical Genetics Reveals Environment-Specific Roles for Quorum Sensing Circuits in *Pseudomonas aeruginosa*, Cell Chem. Biol. 23, 361–369. [PubMed: 26905657]

[22]. Pesci EC, Pearson JP, Seed PC, and Iglewski BH (1997) Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*, J. Bacteriol. 179, 3127–3132. [PubMed: 9150205]

[23]. Pearson JP, Pesci EC, and Iglewski BH (1997) Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes, J. Bacteriol. 179, 5756–5767. [PubMed: 9294432]

[24]. Galloway WR, Hodgkinson JT, Bowden SD, Welch M, and Spring DR (2011) Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways, Chem. Rev. 111, 28–67. [PubMed: 21182299]

[25]. Moore JD, Gerdt JP, Eibergen NR, and Blackwell HE (2014) Active efflux influences the potency of quorum sensing inhibitors in *Pseudomonas aeruginosa*, ChemBioChem 15, 435–442. [PubMed: 24478193]

[26]. Glansdorp FG, Thomas GL, Lee JK, Dutton JM, Salmond GP, Welch M, and Spring DR (2004) Synthesis and stability of small molecule probes for *Pseudomonas aeruginosa* quorum sensing modulation, Org. Biomol. Chem. 2, 3329–3336. [PubMed: 15534711]

[27]. Boursier ME, Combs JB, and Blackwell HE (2019) *N*-Acyl L-Homocysteine Thiolactones Are Potent and Stable Synthetic Modulators of the RhlR Quorum Sensing Receptor in *Pseudomonas aeruginosa*, ACS Chem. Biol. 14, 186–191. [PubMed: 30668907]

[28]. Bokhove M, Nadal Jimenez P, Quax WJ, and Dijkstra BW (2010) The quorum-quenching *N*-acyl homoserine lactone acylase PvdQ is an Ntn-hydrolase with an unusual substrate-binding pocket, Proc. Natl. Acad. Sci. U. S. A. 107, 686–691. [PubMed: 20080736]

[29]. McInnis CE, and Blackwell HE (2011) Design, synthesis, and biological evaluation of abiotic, non-lactone modulators of LuxR-type quorum sensing, Bioorg. Med. Chem. 19, 4812–4819. [PubMed: 21798749]

[30]. O'Reilly MC, and Blackwell HE (2016) Structure-Based Design and Biological Evaluation of Triphenyl Scaffold-Based Hybrid Compounds as Hydrolytically Stable Modulators of a LuxR-Type Quorum Sensing Receptor, ACS Infect. Dis. 2, 32–38. [PubMed: 26807436]

[31]. Hodgkinson JT, Galloway WR, Wright M, Mati IK, Nicholson RL, Welch M, and Spring DR (2012) Design, synthesis and biological evaluation of non-natural modulators of quorum sensing in *Pseudomonas aeruginosa*, Org. Biomol. Chem. 10, 6032–6044. [PubMed: 22499353]

[32]. David S, Mandabi A, Uzi S, Aharoni A, and Meijler MM (2018) Mining Plants for Bacterial Quorum Sensing Modulators, ACS Chem. Biol. 13, 247–252. [PubMed: 29215859]

[33]. Muh U, Schuster M, Heim R, Singh A, Olson ER, and Greenberg EP (2006) Novel *Pseudomonas aeruginosa* quorum-sensing inhibitors identified in an ultra-high-throughput screen, Antimicrob. Agents Chemother. 50, 3674–3679. [PubMed: 16966394]

[34]. Zakhari JS, Kinoyama I, Struss AK, Pullanikat P, Lowery CA, Lardy M, and Janda KD (2011) Synthesis and molecular modeling provide insight into a *Pseudomonas aeruginosa* quorum sensing conundrum, J. Am. Chem. Soc. 133, 3840–3842. [PubMed: 21348514]

[35]. Tan SY, Chua SL, Chen Y, Rice SA, Kjelleberg S, Nielsen TE, Yang L, and Givskov M (2013) Identification of five structurally unrelated quorum-sensing inhibitors of *Pseudomonas aeruginosa* from a natural-derivative database, Antimicrob. Agents Chemother. 57, 5629–5641. [PubMed: 24002091]

[36]. Vetrivel A, Natchimuthu S, Subramanian V, and Murugesan R (2021) High-Throughput Virtual Screening for a New Class of Antagonist Targeting LasR of *Pseudomonas aeruginosa*, ACS Omega 6, 18314–18324. [PubMed: 34308062]

[37]. Nain Z, Sayed SB, Karim MM, Islam MA, and Adhikari UK (2020) Energy-optimized pharmacophore coupled virtual screening in the discovery of quorum sensing inhibitors of LasR protein of *Pseudomonas aeruginosa*, J. Biomol. Struct. Dyn. 38, 5374–5388. [PubMed: 31787031]

[38]. Magalhaes RP, Vieira TF, Melo A, and Sousa SF (2022) Identification of novel candidates for inhibition of LasR, a quorum-sensing receptor of multidrug resistant Pseudomonas aeruginosa, through a specialized multi-level in silico approach, Mol. Syst. Des. Eng. 7, 434–446.

[39]. Lima EMF, Winans SC, and Pinto UM (2023) Quorum sensing interference by phenolic compounds - A matter of bacterial misunderstanding, Heliyon 9, e17657. [PubMed: 37449109]

[40]. Xie Y, Chen J, Wang B, Peng AY, Mao ZW, and Xia W (2022) Inhibition of Quorum-Sensing Regulator from *Pseudomonas aeruginosa* Using a Flavone Derivative, Molecules 27, 2439. [PubMed: 35458637]

[41]. Manson DE, O'Reilly MC, Nyffeler KE, and Blackwell HE (2020) Design, Synthesis, and Biochemical Characterization of Non-Native Antagonists of the Pseudomonas aeruginosa Quorum Sensing Receptor LasR with Nanomolar IC(50) Values, ACS Infect. Dis. 6, 649–661. [PubMed: 32037806]

[42]. O'Reilly MC, Dong SH, Rossi FM, Karlen KM, Kumar RS, Nair SK, and Blackwell HE (2018) Structural and Biochemical Studies of Non-native Agonists of the LasR Quorum-Sensing

Receptor Reveal an L3 Loop “Out” Conformation for LasR, Cell Chem. Biol. 25, 1128–1139 e1123. [PubMed: 30033130]

[43]. O’Brien KT, Noto JG, Nichols-O’Neill L, and Perez LJ (2015) Potent Irreversible Inhibitors of LasR Quorum Sensing in *Pseudomonas aeruginosa*, ACS Med. Chem. Lett. 6, 162–167. [PubMed: 25699144]

[44]. Bruns RF, and Watson IA (2012) Rules for identifying potentially reactive or promiscuous compounds, J. Med. Chem. 55, 9763–9772. [PubMed: 23061697]

[45]. Baell JB, and Nissink JWM (2018) Seven Year Itch: Pan-Assay Interference Compounds (PAINS) in 2017-Utility and Limitations, ACS Chem. Biol. 13, 36–44. [PubMed: 29202222]

[46]. Baell J, and Walters MA (2014) Chemistry: Chemical con artists foil drug discovery, Nature 513, 481–483. [PubMed: 25254460]

[47]. We note that certain non-monotonic partial agonists have single-digit μM IC50s for the antagonism portion of their profiles; however, we do not include those in this comparison due to their agonism at high concentrations. See Moore et al. (reference #15) for additional detail.

[48]. Lindsay A, and Ahmer BM (2005) Effect of sdiA on biosensors of *N*-acylhomoserine lactones, J. Bacteriol. 187, 5054–5058. [PubMed: 15995228]

[49]. Compound 3 does not display appreciable antagonism in the E. coli system. We note that it was weakly efficacious in the P. aeruginosa system (~40% maximum inhibition), and we believe that its LasR antagonism is obscured in the E. coli system. Additionally, compound 6 displayed unusual activity in E. coli. It agonized the E. coli system, indicating it can interact with LasR, but did not antagonize when competing with a high concentration of OdDHL. Compound 6 may therefore operate via a different mechanism than the remainder of the ligands discussed here.

[50]. Wellington S, and Greenberg EP (2019) Quorum Sensing Signal Selectivity and the Potential for Interspecies Cross Talk, mBio 10, e00146–00119. [PubMed: 30837333]

[51]. Bottomley MJ, Muraglia E, Bazzo R, and Carfi A (2007) Molecular insights into quorum sensing in the human pathogen *Pseudomonas aeruginosa* from the structure of the virulence regulator LasR bound to its autoinducer, J. Biol. Chem. 282, 13592–13600. [PubMed: 17363368]

[52]. Paczkowski JE, McCready AR, Cong JP, Li Z, Jeffrey PD, Smith CD, Henke BR, Hughson FM, and Bassler BL (2019) An Autoinducer Analogue Reveals an Alternative Mode of Ligand Binding for the LasR Quorum-Sensing Receptor, ACS Chem. Biol. 14, 378–389. [PubMed: 30763066]

[53]. Zou Y, and Nair SK (2009) Molecular basis for the recognition of structurally distinct autoinducer mimics by the *Pseudomonas aeruginosa* LasR quorum-sensing signaling receptor, Chem. Biol. 16, 961–970. [PubMed: 19778724]

[54]. Churchill ME, and Chen L (2011) Structural basis of acyl-homoserine lactone-dependent signaling, Chem. Rev. 111, 68–85. [PubMed: 21125993]

[55]. Vannini A, Volpari C, Gargioli C, Muraglia E, Cortese R, De Francesco R, Neddermann P, and Marco SD (2002) The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA, EMBO J. 21, 4393–4401. [PubMed: 12198141]

[56]. Gerdt JP, McInnis CE, Schell TL, and Blackwell HE (2015) Unraveling the contributions of hydrogen-bonding interactions to the activity of native and non-native ligands in the quorum-sensing receptor LasR, Org. Biomol. Chem. 13, 1453–1462. [PubMed: 25474181]

[57]. Gerdt JP, McInnis CE, Schell TL, Rossi FM, and Blackwell HE (2014) Mutational analysis of the quorum-sensing receptor LasR reveals interactions that govern activation and inhibition by nonlactone ligands, Chem. Biol. 21, 1361–1369. [PubMed: 25242287]

[58]. Huang CC, Couch GS, Pettersen EF, and Ferrin TE (1996) Chimera: An Extensible Molecular Modeling Application Constructed Using Standard Components., In Pac. Symp. Biocomput, p 724.

[59]. Koes DR, Baumgartner MP, and Camacho CJ (2013) Lessons learned in empirical scoring with smina from the CSAR 2011 benchmarking exercise, J. Chem. Inf. Model. 53, 1893–1904. [PubMed: 23379370]

[60]. Trott O, and Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31, 455–461. [PubMed: 19499576]

[61]. Knegtel RM, Kuntz ID, and Oshiro CM (1997) Molecular Docking to Ensembles of Protein Structures, J. Mol. Biol. 266, 424–440. [PubMed: 9047373]

[62]. Lorber DM, and Schoichet BK (1997) Flexible ligand docking using conformational ensembles, Protein Sci. 7, 938–950.

[63]. Bottegoni C, Cavalli A, and Recanatini M (2006) A Comparative Study on the Application of Hierarchical-Agglomerative Clustering Approaches to Organize Outputs of Reiterated Docking Runs, J. Chem. Info. Model. 46, 852–862.

[64]. Butina D (1999) Unsupervised Data Base Clustering Based on Daylight's Fingerprint and Tanimoto Similarity: A Fast and Automated Way To Cluster Small and Large Data Sets, J. Chem. Inf. Comput. Sci. 39, 747–750.

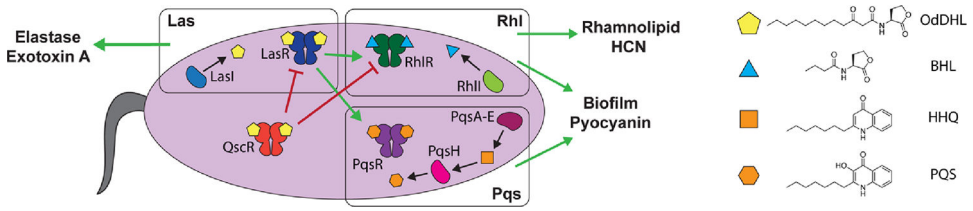


Figure 1.

Schematic of *P. aeruginosa* QS system. *P. aeruginosa* QS receptors interregulate each other and various QS controlled genes at high cell densities. Positive regulation is shown by green arrows (→) and negative regulation is shown by red blunt arrows (⊥). QS signal structures are shown on the right.

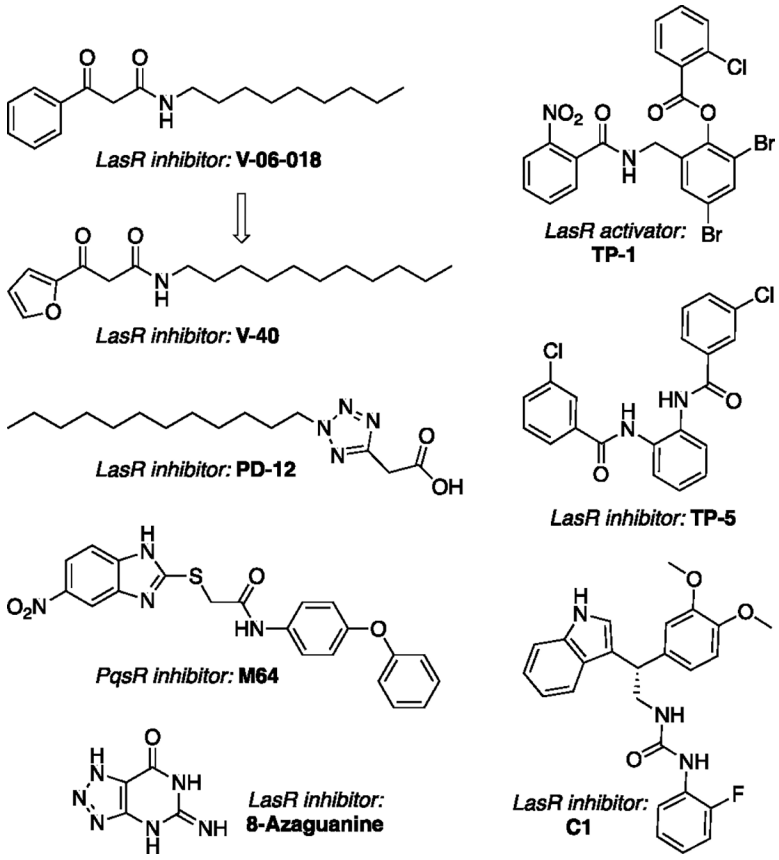


Figure 2.

Selected abiotic small molecules discovered in high throughput screens and follow-on studies and reported to modulate the activity of quorum sensing receptors in *P. aeruginosa*. V-06–018,[33] V-40,[41] PD12,[33] TP-5,[10] 8-azaguanine,[35] and C1[36] antagonize LasR; TP-1[10] agonizes LasR. M64[16] antagonizes PqsR. The revised structure of TP-1, i.e., TP-1-R,[34] is shown.

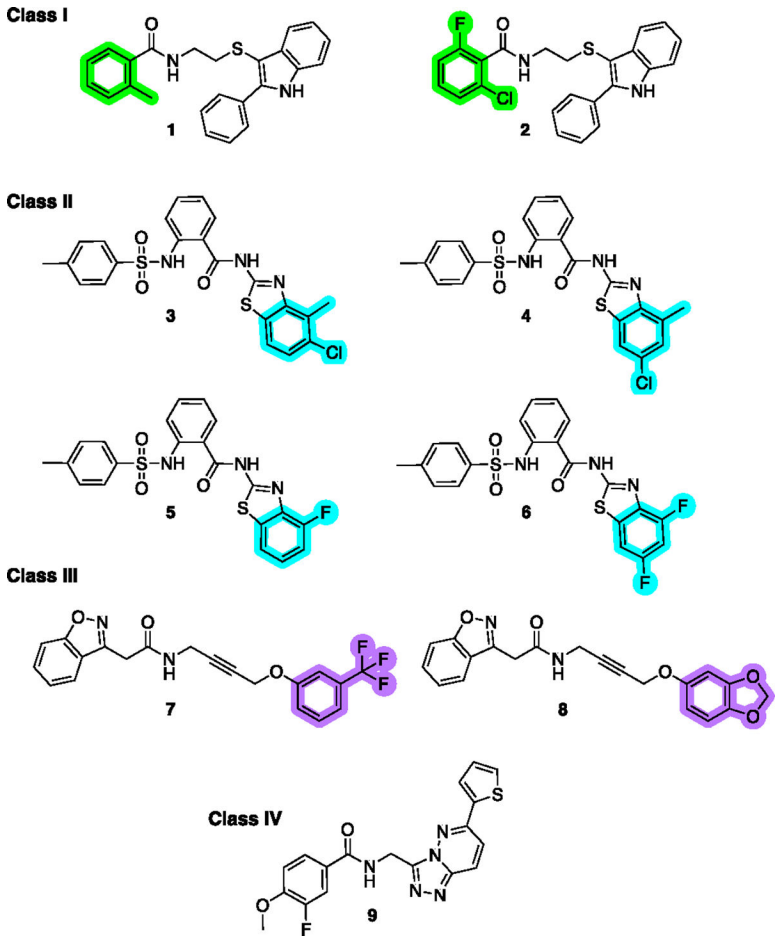


Figure 3.

Compounds identified via HTS that modulate LasR activity. These compounds can be divided into four structural classes: classes I–III (compounds **1**–**8**) antagonize LasR, while class IV (**9**) agonizes LasR. Colors highlight differences in structure in each class.

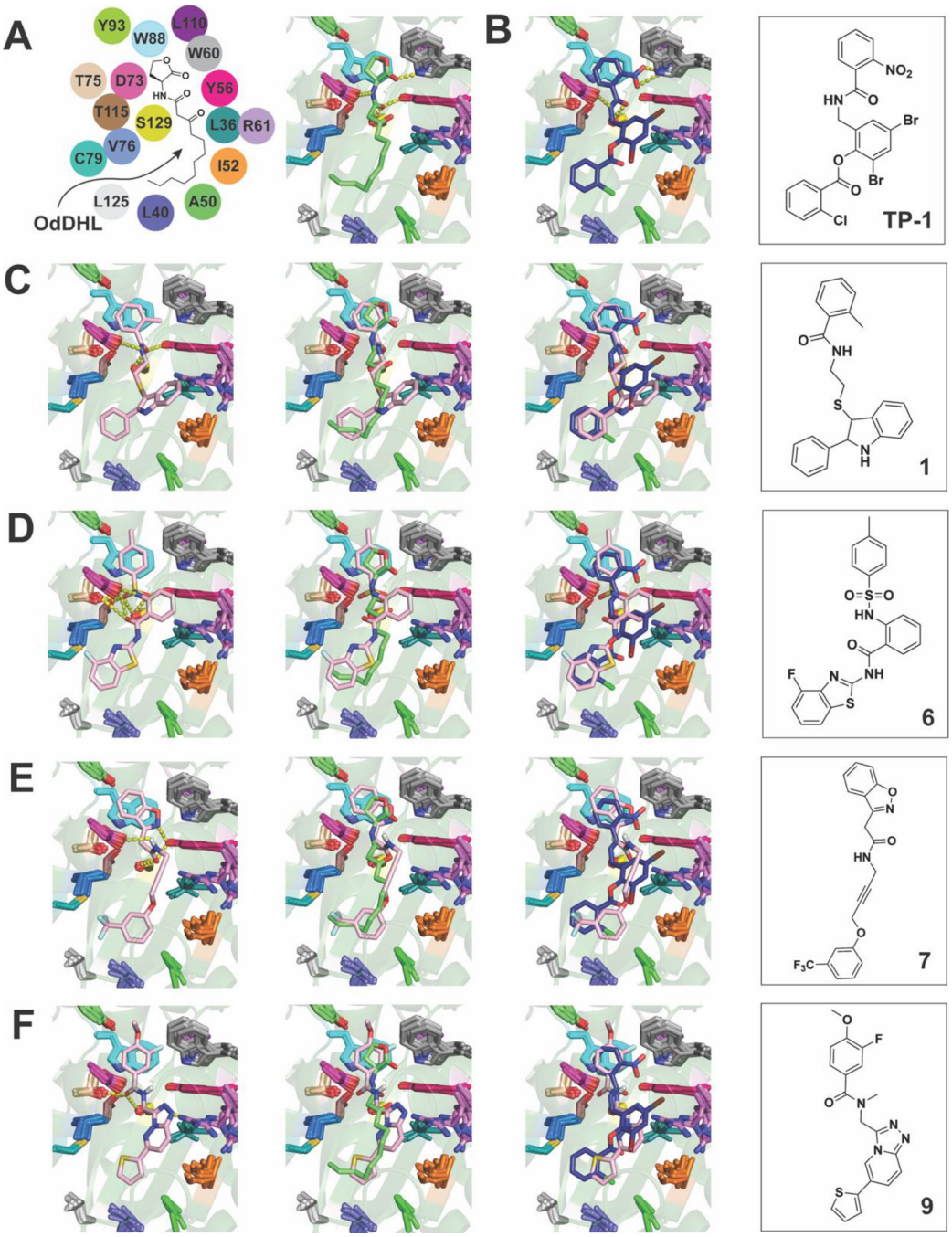


Figure 4.

Views of representative compounds from each structure class (I–IV) computationally docked into an ensemble of structurally aligned LasR LBD structures (PDB IDs: 2UV0, 3IX3, 3IX4, 3IX8, 3JPU, 6D6A, 6D6B, 6D6C, 6D6D, 6D6L, 6D6M, 6D6N, 6D6O, 6D6P, 6MWH, 6MWL, and 6MWW). (A) Two-dimensional (left) and three-dimensional (right) views of OdDHL from the [LasR LBD:OdDHL]$_2$ crystal structure.[51] (B) A view of TP-1 from the [LasR LBD:TP-1]$_2$ crystal structure[53] (left) and two-dimensional view (right). (C–F, left → right for each panel) Three-dimensional views of docked structure for compounds **1**, **6**, **7**,

and **9** with hydrogen bonding interactions → structure overlay with OdDHL → structure overlay with TP-1 → two-dimensional compound views; residues that flank the compound match those labeled in panel (A). Putative hydrogen-bonding interactions are indicated by yellow dashes. These poses represent the medoid poses for each ligand. See Methods for details of docking experiments.

Table 1.

Potency and maximum LasR antagonism and agonism activity (efficacy) for HTS compounds and selected control compounds in the *P. aeruginosa* reporter strain.[a]

Compound	IC/EC$_{50}$ (μM)[b]	95% CI (μM)[c]	Maximum Inhibition / Activation (%)[d]	Structural Class
Antagonism				
1	3.5	2.5–4.9	51	I
2	2.7	1.6–4.6	55	
3	0.8	0.3–2.1	38	II
4	2.8	1.2–4.7	38	
5	1.0	0.6–1.6	54	
6	1.8	1.4–2.4	72	
7	0.4	0.3–0.6	62	III
8	≥17[e]	--	84	
9	--[f]	--	--	IV
CL [g]	21	11–39	55	n/a
V-40 [h]	0.2	0.2–0.3	85	
TP-5 [g]	69	61–78	100	
Agonism				
1–8	--	--	--	I – III
9	0.7	0.6–1.0	100	IV
OdDHL [g]	0.1	0.1–0.2	100	n/a
TP-1 [g]	0.07	0.04–0.1	100	

[a] See Methods for details of the reporter assay and data work-up.

[b] Compounds **1–8** screened over a range of concentrations (46 nM – 100 μM); compound **9** screened for agonism over an expanded range (0.01 nM – 100 μM). Antagonism experiments conducted in competition against a fixed concentration of OdDHL (100 nM) and reported relative to activation by that concentration of OdDHL. Agonism data is reported relative to the activity of a saturating concentration of OdDHL (100 μM). IC$_{50}$ and EC$_{50}$ values calculated from three independent biological replicates (each a technical triplicate).

[c] CI = 95% confidence interval.

[d] Maximum agonism and antagonism values represent the top and bottom values of the fitted curves.

[e] Dose response curve did not fully level off over concentration range tested; see Figure S1.

[f] Inactive.

[g] Data from Moore *et al.* 2015 in the same strain and using analogous assay conditions.[15]

[h] Data from Manson *et al.* 2020 in the same strain and using analogous assay conditions.[41]