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A comparative study of non-native *N*-acyl L-homoserine lactone analogs in two *Pseudomonas aeruginosa* quorum sensing receptors that share a common native ligand yet inversely regulate virulence

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Abstract

Certain bacteria can coordinate group behaviors via a chemical communication system known as quorum sensing (QS). Gram-negative bacteria typically use *N*-acyl L-homoserine lactone (AHL) signals and their cognate intracellular LuxR-type receptors for QS. The opportunistic pathogen *Pseudomonas aeruginosa* has a relatively complex QS circuit in which two of its LuxR-type receptors, LasR and QscR, are activated by the same natural signal, *N*-(3-oxo)-dodecanoyl L-homoserine lactone. Intriguingly, once active, LasR activates virulence pathways in *P. aeruginosa*, while activated QscR can inactivate LasR and thus repress virulence. We have a limited understanding of the structural features of AHLs that engender either agonistic activity in both receptors or receptor-selective activity. Compounds with the latter active profile could prove especially useful tools to tease out the roles of these two receptors in virulence regulation. A small collection of AHL analogues was assembled and screened in cell-based reporter assays for activity in both LasR and QscR. We identified several structural motifs that bias ligand activation towards each of the two receptors. These findings will inform the development of new synthetic ligands for LasR and QscR with improved potencies and selectivities.

Graphical Abstract

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Supplementary Information

Characterization data and NMR spectra for all new or under-characterized compounds, full dose response activity curves, and mathematical description of relative selectivity analysis are available online in supplemental information.

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Keywords

Quorum sensing; *Pseudomonas aeruginosa*; *N*-(3-oxo)-dodecanoyl L-homoserine lactone; LasR; QscR

1. Introduction

Bacteria can communicate using chemical signals in a process called quorum sensing (QS) [1, 2]. In the canonical LuxI/LuxR systems found in Gram-negative bacteria, *N*-acyl L-homoserine lactones (AHLs) signals (or autoinducers) are produced by LuxI-type synthases at a basal level [3]. At a sufficiently high cell and signal density, the AHL signal will productively bind its cognate intracellular LuxR-type receptor. Thereafter, the activated complex will typically dimerize, bind to various promoters in the bacterial genome, and alter the expression of group beneficial genes. The LuxI/LuxR system is also upregulated, resulting in a positive feedback loop that is a hallmark of QS systems.

1.1. QS in *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative opportunistic pathogen that is notorious for infecting cystic fibrosis patients and other immunocompromised individuals [4]. This bacterium regulates almost 10% of its genome via QS [5], including an arsenal of virulence factors, making inhibition of its QS circuit an attractive target for both anti-virulence and fundamental chemical biology research [6–10]. However, the development of ligands that modulate QS in *P. aeruginosa* is challenging due to its relatively complex QS system (Figure 1) [11]. *P. aeruginosa* has two distinct LuxI/LuxR systems, LasR and RhlR, in addition to the unrelated LysR-type *Pseudomonas* Quinolone System (PQS) [12]. LasR, considered to lie at the top of the QS hierarchy, is activated by *N*-3-(oxo)-dodecanoyl L-homoserine lactone (OdDHL), which is produced by the LasI synthase. This receptor activates the rhl system, composed of the RhlR receptor and the RhlI synthase, the latter of which produces RhlR's cognate signal, *N*-butyryl L-homoserine lactone (BHL). Both the las and rhl systems are negatively regulated by an orphan LuxR-type receptor or receptor “solo”, QscR, which lacks its own ligand, yet binds and is maximally activated by OdDHL, the native ligand for LasR [12]. Interestingly, QscR serves as a repressor of both LasR and RhlR activity, and can suppress

virulence. The mechanism of this repression by QscR is not fully understood, but could include the formation of inactive heterodimers [13].

The three *P. aeruginosa* LuxR-type receptors have overlapping regulons (Figure 1), suggesting some redundancies in their modulation of group-related genes [14–16]. That said, LasR is the primary inducer of many major virulence factors, including elastase, endotoxin A, and alkaline protease [17], whereas RhlR primarily regulates the biosurfactant rhamnolipid [18]. QscR has been shown to directly regulate a number of genes, but their functions are currently unknown [14]. Our laboratory [19–22] and others [23–28] have devoted significant effort to developing non-native small molecules capable of targeting LasR, RhlR, and/or QscR to better delineate their individual roles in virulence progression. Several of these compounds have been shown to reduce virulence factor production in wild-type *P. aeruginosa* [29], and represent useful research tools to study *P. aeruginosa* QS pathways that can be challenging to interrogate using genetic knockouts [30]. To date, ligands relatively selective for LasR and RhlR have been identified [22]; however, the molecular features that drive the selectivity of non-native ligands for LasR over QscR, and *vice versa*, remain largely unknown [21]. Notably, compounds that display selective QscR agonism could be utilized to antagonize both the las and rhl circuits, and could be useful tools to modulate virulence; indeed, initial studies have shown that QscR activators can reduce virulence factor production in *P. aeruginosa* [31] and that a *P. aeruginosa* QscR-null mutant is hypervirulent in an insect model [32]. Delineating the structural features of AHLs that engender either agonistic activity in both QscR and LasR or receptor selective activity was one of the broad motivations for this study.

1.2. Structural differences between LasR and QscR and prior studies of AHL activity profiles in these receptors

Beyond the development of novel chemical tools for QscR and LasR, understanding the features of AHL-type compounds that selectively modulate either of these receptors is of fundamental interest, as these two receptors are activated maximally by the same natural ligand, OdDHL. This activity trend suggests that their ligand binding sites and/or modes of ligand recognition could be similar. However, structural studies of QscR and LasR have revealed that these two proteins differ in a number of ways [33, 34]. While both receptors possess the nine highly-conserved amino acids found in the ligand-binding sites of most LuxR-type receptors [35], their overall sequence similarity is only 16% [36]. X-ray crystallography of full length QscR and the ligand-binding domain (LBD) of LasR revealed that several of the hydrogen bonding contacts with OdDHL in the two receptors also involve different amino acids (Figure 2); overlays of the QscR and LasR LBDs reveal RMSD differences of 1.97 Å [33]. Additionally, QscR has been shown to be more stable and more amenable to ligand exchange *in vitro* than LasR, allowing for its biochemical manipulation with a variety of AHLs [37]. Indeed, cell-based reporter assays demonstrated that QscR is able to be activated by a wider assortment of native AHLs than LasR, as well as non-native AHLs with sterically bulkier acyl chains [21]. This more relaxed ligand binding capability may be due to QscR's larger ligand binding site and/or the different hydrogen-bonding networks available in QscR relative to LasR (Figure 2) [33, 38].

Most non-native AHLs developed to study LuxR-type receptor activity in *P. aeruginosa* have had varying acyl chains, yet maintain the native L-homoserine lactone “headgroup” [8]. Accordingly, relatively little is known about the effects of AHL analog activity in LasR and QscR with structural variations of the lactone headgroup (*vide infra*). Of the analogs with non-native headgroups that have been examined [27, 28, 39], most are weaker modulators of LasR (and other related receptors) than analogs that retain the lactone headgroup [40]. This trend has been attributed to hydrogen bonding contacts between the lactone and the receptor that are presumably essential for strong binding [41]. For example, structural data for both LasR and QscR highlight a key hydrogen bond between the OdDHL lactone carbonyl and a conserved tryptophan side chain (Figure 2) [33, 34]. The OdDHL amide proton also forms a hydrogen bonding contact with a conserved aspartate residue. Replacement of the homoserine lactone with another chemical moiety, however, would be desirable for new probe design, as the lactone is hydrolytically unstable (half-life of ~4–24 hours; depending on appended acyl chain structure [42]), which reduces their utility for deployment as tools in biologically relevant environments. Further analysis of subtle structural changes to the AHL head group, some of which retain the possibility to engage in hydrogen bonds, could result in compounds with improved stability, activity, and selectivity profiles. We sought to test this hypothesis in the current study in the context of OdDHL analogs and their comparative activity profiles in, and selectivity for, LasR and QscR.

Herein, we report the examination of a set of closely related OdDHL analogs selected to test the effects of systematic structural changes to the lactone headgroup on compound activity and selectivity for LasR and QscR. These compounds were evaluated in cell-based reporter assays that allowed for quantitative study of (i) agonism of LasR and QscR and (ii) competitive antagonism of LasR and QscR in the presence of OdDHL, providing for comparative analysis of ligand potency and selectivity in each receptor. Overall, we found that a subset of these OdDHL analogs with non-native head groups were selective for either receptor. In addition, we also identified analogs with high potencies that should have significantly improved hydrolytic stability in aqueous media relative to lactone derivatives. These compounds begin to teach us the features of AHL analogs that drive selectivity for LasR or QscR, and will guide the future development of chemical probes to study the QS circuit in *P. aeruginosa*, and likely other related bacteria.

2. Results and Discussion

2.1. Compound selection, historical background for certain molecules, and synthesis

We selected compounds for study based on prior reports of OdDHL (**1**) analogs and our own design criteria (Figure 3). All of these compounds retain the 3-oxo-dodecanoyl “tail” group (except for sulfonamide **10**, which still preserves a 12-atom side chain). Of the small set of reported OdDHL analogs with alterations to the lactone headgroup, these derivatives have each been examined in different biological assays and largely only in LasR, thus comparative activity data in LasR or relative to QscR are not available. Of this group, we selected eight compounds that contained largely systematic structural changes to the lactone headgroup (**2–6**, **8**, and **10**) for comparative analysis in the current study. Pertinent background information on these compounds is provided here. Iglewski and coworkers first

reported the homocysteine thiolactone (**3**) and γ -lactam (**4**) analogs in 1996 [43]. The thiolactone (**3**) was found to agonize LasR comparably to OdDHL (**1**), whereas the lactam was about 100-fold less potent based on EC₅₀. The Suga lab later examined the cyclopentanone (**5**) and cyclopentanol (**8**) derivatives [24]. With the carbonyl group maintained, cyclopentanone **5** retained agonistic activity in LasR but was not nearly as potent as OdDHL (**1**). The reduced analog, cyclopentanol **8**, could also agonize LasR, albeit only at very high concentrations (400 μ M). Our lab reexamined thiolactone **3** and performed initial studies on cyclopentyl derivative **6** in LasR in 2011, again confirming the high potency of the thiolactone **3** and demonstrating some agonistic activity for **6** in LasR (but no EC₅₀ was calculated) [44, 45]. Sulfonamide variant **10** was the only compounds to be tested in both LasR and QscR [20, 46, 47]. This compound was a mild agonist of QscR, yet was inactive in LasR. Ester **11** and the D-enantiomer of OdDHL, **2**, were reported in 2004 and 2006 respectively, but no biological assay data was reported in LasR [48, 49]. We expanded this set of close OdDHL analogs with tetrahydrofuran (THF) derivatives **7** and **9** (Figure 3), which are new to this study. These compounds were included to maintain an oxygen in the heterocycle yet remove the carbonyl (**7**) and lengthen the head group (**9**). The set of 10 compounds were synthesized in moderate to good yields using standard amide bond coupling procedures or previously described methods (see Experimental Section) [50–52].

The 10 OdDHL analogs selected for further analysis were roughly organized into three groups (1–3). Group 1 retained a carbonyl on the head group (Figure 3A). These compounds probed the importance of ring stereochemistry with compound **2**, and the identity of the ring heteroatom with thiolactone **3**, lactam **4**, and ketone **5**. These different atoms modify the size and shape of the ring, as well as the hydrogen bonding ability of the carbonyl and the ring atom itself. Group 2 compounds lacked a carbonyl (Figure 3B). Cyclopentane analog **6** is devoid of endocyclic heteroatoms and has no hydrogen bond acceptors, whereas THF derivative **7** retains an oxygen. Alcohol **8**, the reduced form of ketone **5**, allowed for testing the effects of a hydrogen-bond donor and acceptor versus only an acceptor (as in **5**) on the ring. Compound **9** (made as the racemate) adds a methylene to the THF derivative **7** and extends the head group. Lastly, the Group 3 compounds probed the amide linker between the head group and alkyl chain, by converting it to either a sulfonamide (**10**) or an ester (**11**) (Figure 3C). The latter compound removes a hydrogen bond donor, while the former also lacks the 3-oxo group.

2.2 Biological evaluation in LasR and QscR

2.1.1. Assay methods—Cell-based reporter gene assays are routinely used to measure LuxR-type protein activity in the presence of exogenous compound. As *P. aeruginosa* has three LuxR-type receptors that are closely interregulated (Figure 1), measuring the activity of individual receptors in the native background can be challenging. To address this issue, our lab has developed heterologous reporter systems in *E. coli* strain JLD271 (*sdhA*) for all three of the *P. aeruginosa* LuxR-type receptors [22, 30, 32, 38, 53]. Aside from standardizing the receptor expression levels and reporter plasmids, these strains also lack *E. coli*'s native LuxR-type receptor, SdhA, removing a possible ligand “sink” that could alter activity profiles. Using these LasR and QscR reporters and our previously described protocols [22, 39], we examined the agonistic activities of compounds **2–11** over a range of concentrations

(1 pM–100 μ M; see Experimental Section). Compounds that showed weak agonism (i.e., potency too low to calculate an EC₅₀ value) were also screened for their ability to antagonize LasR and QscR in competition with OdDHL (**1**) over a range of concentrations (3.2 nM–250 μ M). Screening data were analyzed by examining maximum agonistic and antagonistic activities and calculating EC₅₀ and IC₅₀ values, and are listed in Table 1.

2.1.2. Agonism assay results for Group 1 compounds (2–5)—All of the compounds in Group 1, which retained a carbonyl in the headgroup, activated LasR to nearly 100% with EC₅₀ values in the low to mid nanomolar range (Table 1). D-OdDHL **2** was the least potent activator in this group (EC₅₀ 100-fold higher than L-OdDHL (**1**)), which was unsurprising based on previous reports of the importance of lactone stereochemistry for LuxR-type receptor activation [51]. Lactam and cyclopentanone variants (**4** and **5**, respectively) were ~10–20-fold less potent than OdDHL (**1**), suggesting that these changes to the ring that either introduce an H-bond donor (lactam **5**) or remove H-bonding capability (ketone **5**) are moderately well tolerated in LasR. Thiolactone **3** was found to be the most potent non-native agonist of LasR in Group 1 (and in this study overall), with a comparable EC₅₀ to OdDHL (1.5 nM), corroborating previous reports [43, 44].

The agonism activity trends for Group 1 in QscR were both similar to and different than those in LasR. For instance, D-OdDHL **2** also showed a nearly 100-fold reduction in activity relative to OdDHL (**1**) whilst maintaining full efficacy, suggesting that the two receptors have similar intolerances for the inverted stereochemistry. Larger differences were observed upon varying the carbonyl group to thiolactone, lactam, and ketone. Thiolactone **3** was capable of full QscR activation, while the lactam and lactone showed reduced efficacy. Unlike in LasR, thiolactone **3** was five-fold less potent than OdDHL in QscR. Previous studies have suggested thiolactones to have a stabilizing effect on LasR due to their larger size and capability for hydrogen-bonding [54], so it is possible that the QscR ligand-binding site does not accommodate larger ring sizes well (assuming these closely related analogs also target the same site). This activity trend is supported by the QscR reporter assay data for lactam **4**, which displayed >200-fold reduced potency relative to OdDHL. The lactam nitrogen is closer in size to a sulfur atom than oxygen based on covalent radii [55], and will also interact with the ligand binding pocket differently because of the added hydrogen bond donor. The reduced flexibility of the amide C-N bond (relative to the C-O bond of a lactone) may also play a role in receptor binding. These differences may contribute to lactam **4**'s reduced potency in QscR. This trend may also be observed for cyclopentanone **5**; the subtly larger steric size of the cyclopentanone versus the homoserine lactone (i.e., increased covalent radius of a methylene group vs. an oxygen atom), in addition to the lack of an endocyclic H-bonding acceptor, results in a ~55-fold difference overall. Overall, LasR appeared more tolerant of these head group changes relative to QscR.

2.1.3. Agonism assay results for Group 2 compounds (6–9)—Group 2 compounds, lacking a head group carbonyl (**6–9**), exhibited slightly lower efficacies and generally lower potencies in LasR relative to Group 1 (Table 1). Cyclopentane **6**, with a 100-fold loss in potency relative to OdDHL (**1**), was the most potent compound. THF derivative **7**, whilst 600-fold less potent than OdDHL, maintains an oxygen in the head group at a

position comparable to the intact homoserine lactone, unlike compound **6**. The presence of this oxygen could possibly result in a disfavored hydrogen bonding interaction. Interestingly, a similar loss in potency is not observed in extended THF-derivative **9**. Presumably, the added methylene linker places the head group in a more favorable position for binding LasR. Alcohol **8**, with both a hydrogen bond donor and acceptor at the carbonyl position, was the weakest LasR activator in Group 2, with a ~1200-fold higher EC₅₀ relative to OdDHL. We hypothesize that this molecule makes drastically changed and/or unfavorable hydrogen binding contacts in the pocket that alter the LasR protein configuration for optimal activity.

Turning to QscR, the Group 2 compounds **6–8** had largely similar potencies in QscR and LasR. However, because OdDHL is ~10 times more potent in LasR than QscR using these reporter assays, the similar potencies of **6–8** in both receptors indicate that QscR was better able to accommodate these ligands than LasR; we return to this issue when making comparisons between the two receptors below (see also Figure 4). Cyclopentane **6** was still the most potent of the group in QscR, exhibiting only a 24-fold reduction in potency relative to OdDHL. THF variant **7** was slightly less potent than cyclopentane **6**, possibly indicating this ligand is making an undesirable contact in QscR as well as in LasR. Also similar to LasR, alcohol **8** was a very weak QscR agonist (EC₅₀ value in the micromolar range), suggesting its altered H-bonding properties were disfavored in both QscR and LasR. In contrast to LasR, however, the other THF derivative (**9**) showed negligible activity in QscR. This extended head group was apparently not tolerated for QscR agonism.

2.1.4. Agonism assay results for Group 3 compounds (10 and 11)—The two compounds in Group 3 (**10** and **11**) contained alterations to the amide linker and showed limited activity in LasR and QscR. Sulfonamide **10** was inactive in LasR and showed only weak agonistic activity in QscR, corroborating previous reported trends [47]. As some of the most potent previously reported agonists and antagonists of QscR contain steric bulk alpha to the amide linker [21], it is perhaps not surprising that the sulfonamide can be tolerated in QscR, albeit engendering very modest ligand activity. In turn, ester **11**, which lacks the ability to donate a hydrogen bond, drastically loses efficacy in LasR and QscR. This loss of activity is supported by prior mutational studies highlighting the importance of Asp73 for LasR activation [54]. The chemical experiment performed here (removing the hydrogen bond donor from the ligand rather than the protein) supports hydrogen bonding between Asp73 and Thr75 (in LasR) and Asp75 (in QscR) with a ligand as being vital to receptor activation (Figure 2) [33, 34].

2.1.5. Ant agonism assay results—We reasoned that compounds with limited to no activity in the agonism assays (**9–11**) could operate as receptor antagonists instead [56] (we note that compounds **2–8** showed no antagonism in single-point concentration antagonism assays in LasR and QscR; see Supp. Info.). Therefore, we measured the antagonism profiles of compounds **10** and **11** in LasR and **9** and **11** in QscR, each in competition against OdDHL (**1**). The data resulting from these assays are listed in Table 1. Sulfonamide **10** failed to antagonize LasR, suggesting that, in concert with its lack of agonistic activity, this linkage alternation simply destroys receptor interactions. Ester **11** was capable of weak LasR

antagonism, achieving a maximum inhibition of 53% at the highest concentration tested, but was not sufficiently potent to calculate an IC_{50} .

In QscR, extended THF analogue **9** was twice as active as ester **11**, with maximum inhibitory activities of 59% versus 23%, respectively. Neither compound was potent enough to calculate an IC_{50} , however. These data reinforce the importance of the amide N-H hydrogen bond for LasR or QscR binding and the intolerance of LasR for added steric bulk near the amide bond. Additionally, our results suggest that “elongated” head groups, such as in **9**, may be a useful motif for developing QscR-selective antagonists.

2.1.6. Relative selectivity profiles in LasR and QscR—We next scrutinized the assay data further to determine if any of the structural motifs present in our library engendered molecules with selectivity for LasR over QscR, and *vice versa*. As noted above, OddHL (**1**) is approximately 10-fold more potent in LasR than QscR. Therefore, non-native compounds with similar potencies that are above the EC_{50} value of OddHL in both receptors (i.e., **6–8**) have lost 10-fold *less* potency against QscR than LasR (relative to OddHL). To quantify this phenomenon and determine whether our molecules were selective for LasR or QscR relative to OddHL, we developed a “relative selectivity” metric (see Supp. Info. for mathematical derivation). We applied this metric to all molecules for which we could calculate an EC_{50} in both receptors (**2–8**) and excluded compounds with no activity in one receptor (**9–11**). These metric data are plotted in Figure 4 and reveal that the presence or absence of a carbonyl in a compound was the main driver of relative selectivity. Carbonyl containing compounds **3–5** showed relative selectivity for LasR, whilst compounds without carbonyls (**6–8**) showed relative selectivity for QscR. This selectivity trend suggests that receptor contacts with the carbonyl (steric and/or hydrogen-bonding) are favored in LasR over QscR for this set of close OddHL analogs.

3. Conclusions

The goal of this study was to measure the agonism and antagonism profiles of a set of head group-modified OddHL (**1**) analogues to identify molecular features important for the differential activation of LasR and QscR. These overall SARs are shown schematically in Figure 5. In general, LasR activity is more greatly affected by the removal of the homoserine lactone carbonyl than QscR. LasR also cannot tolerate steric bulk near the amide carbonyl. In terms of agonism, QscR is less amenable to elongation of the head group (i.e., as in **9**) and changes in identity of the carbonyl-bearing ring (i.e., lactone vs. thiolactone, lactam, or ketone) relative to LasR. Both receptors are equally affected by changes in ring stereochemistry and require a hydrogen bond donor on the ligand linker (i.e., an amide) for appreciable receptor activation. In addition, our study of the antagonism profiles of analogues with limited to no agonistic activity revealed that ester **11**, and THF-derivative **9** and **11**, are mild antagonists of LasR and QscR, respectively. These antagonism data suggest that the incorporation of “elongated” head groups (as in **9**) can generate QscR-selective antagonists.

Looking to the future, these investigations have revealed several head groups as potential leads for generating new probe compounds for LasR and QscR with improved hydrolytic

stability. Thiolactone **3**, lactam **4**, and cyclopentanone **5** all are relatively more stable than homoserine lactone and maintain potencies in LasR in the mid-nanomolar range. These compounds have increased selectivity for LasR over QscR, making them excellent leads for future compounds targeting this receptor. While changes in the homoserine lactone heteroatom generally result in reduced activity in both LuxR-type receptors, these activity differences may not be as critical if the compounds have longer half-lives in aqueous media, allowing them to remain active over prolonged periods in biologically relevant environments. Homocysteine thiolactone derivatives, like **3**, are particularly interesting in this regard, as we have shown them to remain intact significantly longer than the native lactone head group [44]. Alternatively, QscR selective compounds may benefit from the incorporation of a sulfonamide linker or from the removal of the homoserine lactone carbonyl. Both changes resulted in weak to modest QscR agonists, but with limited to no activity in LasR. Further development of the lead compounds and the SARs for OdDHL (**1**) reported here is ongoing.

4. Experimental

4.1. General

All chemical reagents and solvents were purchased from commercial sources and used without further purification, except for dichloromethane (DCM), which was distilled and dried over activated molecular sieves. Water (18 MΩ) was purified using a Thermo Scientific Barnstead Nanopure system. OdDHL (**1**) was purchased from Sigma Aldrich. Chlorophenol red-β-D-galactopyranoside (CPRG) was purchased from Roche. *Ortho*-nitrophenyl-β-galactoside (ONPG) was purchased from Sigma Aldrich. All media and reagents for bacterial culture were purchased from commercial sources and used according to manufacturer's instructions.

4.2. Chemistry

For compounds **2–9** and **11**, the acyl “tail group” was introduced via 2-(2-nonyl-1,3-dioxolan-2-yl) acetic acid, which was synthesized according to the method of Spring and coworkers [52]. This acid was coupled to each analog head group using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)–mediated coupling chemistry as reported previously by our laboratory [45]. The resulting product of each coupling reaction was deprotected with trifluoroacetic acid (TFA) to produce the analogs **2–4**, **6**, **7**, **9**, and **11** [52]. The head group for compound **4** ((*S*)-3-amino-2-pyrrolidinone) was prepared as previously described [57]. Enantiomerically pure (*S*, *S*)-2-aminocyclopentanol was used in the preparation of compound **8**. Formation of the amide bond between head groups and tail groups in analogues **5** and **8** required a different reaction solvent than that for **2–4**, **6**, **7**, **9**, and **11** (DMF rather than DCM), but otherwise all reaction conditions were identical. Cyclopentanone **5** was prepared via Dess-Martin oxidation of its hydroxyl precursor (**8**) as previously reported [51]. Sulfonamide **10** was synthesized as described [50]. All products were purified to homogeneity via silica gel chromatography as needed after standard aqueous work-up.

4.3. Bacteriology methods

Bacteria were cultured in Luria-Bertani medium (LB) at 37 °C. Absorbance measurements were performed in 96-well microtiter plates and path length-corrected using a Biotek Synergy 2 plate reader running Gen 5 software (version 1.05). Bacterial growth was assessed by measuring absorbance at 600 nm (OD₆₀₀).

4.4. Bacterial strains and assay protocols

The bacterial reporter strains used for this study were the (i) *E. coli* strain JLD271 (*sdhA*) harboring the LasR expression plasmid pJN105L and the *lasI-lacZ* transcriptional fusion reporter pSC11-L, and (ii) *E. coli* strain JLD271 (*sdhA*) harboring the QscR expression plasmid pJN105Q and the *pAI897-lacZ* transcriptional fusion reporter pSC11-Q. Miller-type β -galactosidase assays were performed in these two *E. coli* reporters using either CPRG or ONPG substrates as previously described [22, 39]. For agonism experiments, LasR or QscR activity was measured relative to that of 100 μ M OdDHL (**1**). Antagonism experiments were performed by competing the various compounds against OdDHL (**1**) at its EC₅₀ in LasR (1.5 nM) or QscR (15 nM), and inhibitory activity was measured relative to receptor activation at this EC₅₀. EC₅₀ and IC₅₀ values were determined by testing compounds over a range of concentrations (–250 μ M).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Simplified schematic of the three LuxR-type receptors in *P. aeruginosa* and their interregulation. LasR, QscR, and RhlR have overlapping regulons that control group beneficial genes, including many involved in virulence.

Figure 2.

Views of OdDHL (in yellow) bound to LasR (A) and OdDHL (in cyan) bound QscR (B) from their respective crystal structures [33, 34]. Key residues involved in hydrogen bonds to the homoserine lactone or amide hydrogen of OdDHL are labeled. PDB IDs: 3IX3 (LasR) and 3SZT.5 (QscR).

Figure 3.

Structures of the compounds evaluated in this study. Compounds **2–9** retained the 3-oxododecanoyl chain of OdDHL (**1**). Structures are loosely grouped based on variation to (A) the lactone stereochemistry or ring oxygen, (B) carbonyl replacement, and (C) amide linker modifications. Certain compounds were reported previously by other laboratories: **2**, Ishiguro and coworkers [49]; **3** and **4**, Iglewski and coworkers; **5**, **8**, and **11** [43]; Suga and coworkers; **6** and **10** [24, 48]; **10**, our laboratory [45].

Figure 4.

Relative selectivity profiles for compounds 2–8 in LasR and QscR. Error bars were generated from the 95% confidence intervals of the EC_{50} values in each receptor (Table 1). Bar shading included for viewing clarity only. See Supp. Info. for a mathematical definition of relative selectivity.

Figure 5.

Structural features important for the activation of LasR and QscR receptors. Features more important for LasR activation are shown in yellow. Features more important for QscR activation are shown in cyan. Changes equally detrimental for activation of both receptors are shown in red.

Table 1
Compound activity data in *E. coli* LasR and QscR reporter strains. CI = 95% confidence interval.^a

Compound	LasR			QscR		
	Agonism			Antagonism		
Compound	EC ₅₀ (nM) ^b	95% CI (nM)	Activation (%) ^c	EC ₅₀ (nM) ^b	95% CI (nM)	Activation (%) ^c
1 (OdDHL)	1.5	(0.91 – 2.5)	100	15	(6.8 – 32)	100
2	110	(81 – 150)	99	1380	(440 – 4300)	110
3	1.5	(0.71 – 3.3)	100	80	(42 – 150)	110
4	30	(11 – 79)	110	3300	(1600 – 7000)	56
5	15	(7.7 – 29)	110	830	(450 – 1500)	77
6	160	(73 – 360)	88	360	(230 – 550)	100
7	910	(760 – 1100)	81	820	(530 – 1300)	74
8	1900	(1400 – 2500)	96	3500	(1700 – 6900)	110
9	260	(130 – 500)	96	^d	–	3.2
10	–	–	6.4	1600	(1000 – 2500)	72
11	–	–	27	–	–	37

Antagonism						
Compound	IC ₅₀ (μM) ^e	95% CI (μM)	Inhibition (%) ^f	IC ₅₀ (μM) ^e	95% CI (μM)	Inhibition (%) ^f
9	–	–	–	> 250	–	59
10	–	–	–	–	–	–
11	> 250	–	53	> 250	–	23

^aFor details of reporter strains, see Experimental Section. All assays performed in triplicate; 95% CIs calculated from the SEM of n = 3 trials. Shading in table provided for clarity to highlight Groups 1–3 in the agonism data.

^bFor agonism experiments, LasR or QscR activity was measured relative to that of 100 μM OdDHL (1). EC₅₀ values determined by testing compounds over a range of concentrations (1 pM–100 μM).

^cDenotes the highest value of LasR or QscR activation observed for each compound at any concentration on the dose–response curve. Error = ±10%.

^dNot calculated.

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Antagonism experiments performed by competing the various compounds against OtdHL (1) at its EC₅₀ in LasR (1.5 nM) or QscR (15 nM), and inhibitory activity was measured relative to receptor activation at this EC₅₀. IC₅₀ values determined by testing compounds over a range of concentrations (3.2 nM–250 μM).

_{*f*} Denotes the highest value of LasR or QscR inhibition observed for each compound at any concentration on the dose–response curve. Error = ±10%. Full agonism and antagonism dose response curves are shown in the Supp. Info.