N-Phenylacetanoyl-L-Homoserine Lactones Can Strongly Antagonize or Superagonize Quorum Sensing in Vibrio fischeri

Grant D. Geske, Jennifer C. O'Neill, and Helen E. Blackwell*

Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, Wisconsin 53706-1322

B acteria can assess their local population densities using low-molecular-weight molecules (auto-inducers) and alter gene expression at high cell number to behave as a group. This process, termed quorum sensing, is widely used by bacteria to initiate group behaviors that have direct and often devastating impacts on human health and the environment (1, 2). For example, numerous bacterial pathogens use quorum sensing to initiate infection (3, 4). In contrast, symbiotic bacteria use these pathways to commence mutually beneficial relationships with their hosts (2, 5, 6). Because these important processes are controlled by chemical signals, there is intense and growing interest in the development of non-native ligands that can intercept these signals and attenuate or mimic quorum-sensing outcomes (7).

Quorum sensing is best characterized in Gram-negative proteobacteria, which use diffusible N-acylated-L-homoserine lactones (AHLs) and their cognate receptors (R proteins) for intercellular communication (Figure 1, panel a) (2, 8). Considerable research efforts have focused on the synthesis of ligands that can disrupt AHL-R protein binding and inhibit quorum sensing (9, 10), yet potent and general R protein antagonists remain scarce. Likewise, compounds exhibiting heightened activities relative to native AHLs (i.e., superagonists of quorum sensing) are also of significant interest, because they could potentially ini-

tiate bacterial group behaviors at lower cell numbers than those required in natural environments. For example, superagonists could be used to determine whether beneficial symbioses could be initiated earlier during colonization by a symbiont or whether a pathogen could be forced to initiate infection too early and be cleared by a host's immune response. Such experiments, if possible, would help illuminate the relationships between bacterial group behavior and host responses. However, to our knowledge, only two superagonists of R protein activity have been reported to date, and these compounds have yet to be tested in vivo (11, 12).

New synthesis and design strategies are needed to expand the current set of quorum-sensing modulators active in Gram-negative bacteria. Unfortunately, the structures of known antagonists and agonists vary widely, and their mechanisms of action are unclear; thus, no obvious rationales have emerged for new ligand design. To address this problem, we have been engaged in the design of focused combinatorial libraries of ligands for the modulation of quorum sensing (13–15). Here, we report the discovery of a family of non-native AHLs capable of either inhibiting or, in some cases, strongly inducing quorum sensing in the marine symbiont Vibrio fischeri. In addition, we report the first superagonist of quorum sensing in V. fischeri. These ligands provide a new blueprint for the design of both

ABSTRACT Bacteria monitor their population densities using low-molecular-weight ligands in a process known as quorum sensing. At sufficient cell densities, bacteria can change their mode of growth and behave as multicellular communities that play critical roles in both beneficial symbioses and the pathogenesis of infectious disease. The development of non-native ligands that can block quorum-sensing signals has emerged as a promising new strategy to attenuate these divergent outcomes. Here, we report that N-phenylacetanoyl-L-homoserine lactones are capable of either inhibiting or, in some cases, strongly inducing quorum sensing in the bacterial symbiont Vibrio fischeri. Moreover, simple structural modifications to these ligands have remarkable effects on activity. These studies have revealed one of the first synthetic superagonists of quorum sensing, N-(3-nitro-phenylacetanoyl)-L-homoserine lactone. Together, these ligands represent a powerful new class of chemical probes with the potential to significantly expand the current understanding of quorum sensing and its role in host/bacteria interactions.
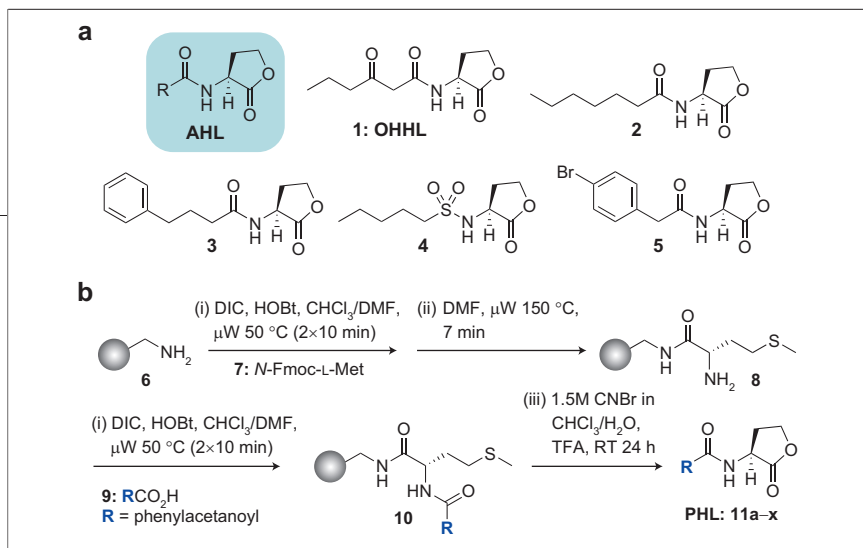
Figure 1. Ligand structures and synthesis. a) Structures of a generic AHL, OHHL (1) (the natural autoinducer ligand of *V. fischeri*), and selected known synthetic inhibitors of LuxR or other R protein function (2–5). b) Solid-phase synthetic route to PHL library 11. DIC = *N,N′*-diisopropyl-carbodiimide. HOBt = *N*-hydroxybenzotriazole. μW = temperature-controlled microwave irradiation.

quorum-sensing agonists and antagonists and represent powerful new chemical probes to investigate the mechanisms of bacterial communication.

V. fischeri colonizes the light-producing organs of certain marine fish and squids and uses quorum sensing to initiate biolu-minescence at high cell densities (6, 8). Quorum sensing is mediated in part by an AHL signal, *N*-(3-oxo-hexanoyl)-L-homoserine lactone (OHHL, 1), and its cytoplasmic recep-tor, LuxR (Figure 1, panel a) (16). OHHL is synthesized by the LuxI protein at low basal levels, and high cell densities are required to achieve a sufficient concentration of OHHL for LuxR binding (≥100 nM *in vivo* (17)); thereafter, the OHHL–LuxR complex activates the transcription of luminescence genes and other genes involved in symbio-sis and illuminates the fish or squid light organ. Quorum sensing in *V. fischeri* rep-resents the best-characterized quorum-sensing signaling pathway to date (2); how-ever, the role of quorum sensing in host–*V. fischeri* symbiosis is complex and re-mains poorly understood (6, 8, 16). As a first step toward studying the interplay be-tween quorum sensing and bacterial sym-bioses, we sought to identify non-native sig-nal molecules that could intercept quorum sensing in *V. fischeri*.

Certain AHLs with non-native acyl chains have been reported to inhibit LuxR protein function in reporter gene assays. These an-tagonists include *N*-heptanoyl-L-homoserine
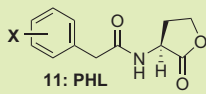
lactone (2) reported by Eberhard *et al.* (18) and Schaefer *et al.* (19) and *N*-(4-phenyl-butanoyl)-L-homoserine lactone (3) and *N*-pentylsulfonyl-L-homoserine lactone (4) re-ported by Castang *et al.* (20) (Figure 1, panel a). Recent studies in our laboratory have shown that *N*-(4-bromo-phenyl-acetanoyl)-L-homoserine lactone (4-bromo-PHL, 5) is a potent antagonist of LuxR homo-logues in several other Gram-negative bacteria (Figure 1, panel a). For example, 4-bromo-PHL, 5, inhibits R protein function in *Agrobacterium tumefaciens* at a 1:1 ratio against native AHL ligand, as determined by reporter gene assays (13). Because the pu-tative ligand binding sites of the known R proteins have considerable sequence ho-mology (70–80%) (8, 21), we hypothesized that PHLs might also modulate LuxR activity in *V. fischeri* and, if so, they represented a promising ligand class with which to initiate this study.

PHLs can be readily synthesized using a microwave-assisted, solid-phase route pre-viously reported by our laboratory (Figure 1, panel b) (13). Using this method, we synthe-sized a small focused library of 24 PHLs to systematically examine the effects of differ-ent phenylacetanoyl moieties on ligand ac-tivity. This route gave PHL products 11a–x (Table 1) in excellent purities (~95%), good isolated yields (>65%), and sufficient quan-tities (*i.e.*, 30 mg per compound) for mul-tiple biological experiments.

Non-native ligands are commonly as-sessed for R protein agonism and antago-nism using bacterial reporter strains (7, 9, 10). These strains lack AHL synthase genes, but retain their native R genes. Exogenous AHL ligand is therefore required for R protein activation, which can be measured by re-porter gene read-outs based on lumines-cence or fluorescence. The majority of syn-thetic LuxR modulators have been evaluated using the *luxR* plasmid pSB401 in various *Escherichia coli* strains (19, 20). We there-fore began our biological evaluation of PHL library 11 using the *E. coli* strain JM109 (pSB401) (22). Competitive inhibition as-says were performed in the presence of both OHHL and PHLs 11, while agonism assays were performed with PHLs alone. The known LuxR protein inhibitors 2–4 and 4-bromo-PHL 5 served as important controls for these studies (Figure 1, panel a). However, unac-ceptably large error values in the lumines-cence data (see Supplementary Figure 1) forced us to seek an alternative strain. We found that a Δ-*luxI* derivative of *V. fischeri* ES114 (16), in which the native *lux* operon behaves as the bioluminescent reporter, gave highly reproducible luminescence data in these assays. This nonstandard reporter strain was used for the subsequent primary antagonism and agonism assays in this study.

The antagonism assays in *V. fischeri* re-vealed several active PHL ligands and a number of striking structure–activity rela-tionships (SARs) (Table 1). First, the control compound 4-bromo-PHL (5) showed 79% inhibition at a 1:1 ratio with native ligand 1 (Table 1, entry 5; both ligands at 5 μM). This result supported our hypothesis that PHLs could modulate LuxR function. Indeed, 50% of the PHL library 11 exhibited ≥50% inhibition in this assay. Inhibitory activity was extremely dependent on the substitu-ents and their locations on the phenyl-acetanoyl group. For example, replacement of the 4-bromo substituent with a hydro-gen in PHL 11a abolished inhibitory ac-tivity (Table 1, entry 6). PHLs with bromo

TABLE 1. Structures and primary antagonism and agonism screening data for PHL library 11 in *V. fischeri* (Δ-*luxI*)

11: PHL

Entry	Compound	X	Inhibition (%)[a,b]	Activation (%)[a,c]
1	OHHL (**1**)	–	–	100
2	**2**	–	78	23
3	**3**	–	70	3
4	**4**	–	30	2
5	**5**	4-Br	79	3
6	**11a**	H	14	2
7	**11b**	3-Br	58	70
8	**11c**	2-Br	38	12
9	**11d**	4-F	59	2
10	**11e**	3-F	63	8
11	**11f**	2-F	49	2
12	**11g**	4-Cl	75	3
13	**11h**	3-Cl	65	61
14	**11i**	2-Cl	53	6
15	**11j**	4-I	85	4
16	**11k**	3-I	78	28
17	**11l**	2-I	63	7
18	**11m**	4-NO$_2$	47	24
19	**11n**	3-NO$_2$	−15[d]	129
20	**11o**	2-NO$_2$	23	4
21	**11p**	4-N$_3$	66	5
22	**11q**	4-Ph	79	7
23	**11r**	4-N$_2$-Ph	63	3
24	**11s**	4-CF$_3$	78	3
25	**11t**	4-CH$_3$	58	1
26	**11u**	4-NHBoc	33	2
27	**11v**	4-NH$_2$	12	3
28	**11w**	4-OH	12	2
29	**11x**	4-OMe	57	3

[a]Strain: *V. fischeri* ES114 (Δ-*luxI*). Luminescence data measured in relative light units and normalized to OHHL (**1**, red). Control compounds shown in blue. All assays performed in triplicate; error did not exceed ±10% of the mean. [b]Screen performed using 5 μM synthetic ligand against 5 μM OHHL (**1**). [c]Screen performed using 200 μM ligand. [d]PHL **11n** displayed 115% activation in this assay.

(**5**, **11a,b**), chloro (**11g–i**), and iodo substituents (**11j–l**) exhibited a ∼10% increase in antagonism as the halogen was moved from the 2- to the 3- to the 4-position on the phenyl ring. Antagonistic activity also increased slightly with increasing halogen size, with 4-iodo-PHL (**11j**) exhibiting the highest activity (Table 1, entry 15; 85%) for the halogen series.

In general, sterically large and lipophilic groups in the 4-position enhanced PHL (**11**) antagonism in *V. fischeri* (Δ-*luxI*). This is exemplified by the high activities of 4-phenyl-PHL (**11q**) and 4-trifluoromethyl-PHL (**11s**) (∼80% inhibition; Table 1). In contrast, hydrogen-bond-donating substituents in the 4-position engendered the lowest inhibitory activities (*i.e.*, 4-amino-PHL, **11v**, and

4-hydroxy-PHL, **11w**). The nitro-PHL series (**11m–o**), however, showed a more complicated activity trend, with 3-nitro-PHL (**11n**) showing no inhibitory activity relative to the 2- and 4-nitro-PHLs (entries 18–20; see below). We determined IC$_{50}$ values for the most potent PHL inhibitors identified in this assay, along with the most potent controls (**2** and **5**) for comparison. The 4-iodo-PHL (**11j**) and 4-trifluoromethyl-PHL (**11s**) exhibited the lowest IC$_{50}$ values in this study, with PHL **11s** at least two-fold more active than control compound **2** (0.6 μM *vs* 1.4 μM, respectively; Figure 2, panel a).

Similar assays were performed on PHL library **11** to screen for agonistic activity in *V. fischeri* (Δ-*luxI*). Again, we observed striking trends in the activities for PHLs with halogen and nitro groups (at 200 μM compound; Table 1). In contrast to the antagonism data, the 3-substituted compound in each of these PHL families showed the strongest activity relative to the 2- and 4-substituted derivatives, with the 3-bromo- (**11b**), 3-chloro- (**11h**), and 3-nitro-PHLs (**11n**) exhibiting at least 60% luminescence induction relative to native OHHL at the same concentration. Remarkably, simply shifting substituents on the PHL phenyl ring by a single carbon converted these ligands from LuxR antagonists to LuxR agonists. Moreover, 3-nitro-PHL (**11n**) was able to induce 29% higher luminescence than OHHL in this primary assay (Table 1, entry 19). This result was extraordinary and explained the unusual inhibition trends for the nitro-PHL series (**11m–o**; see above).

For a more quantitative comparison of ligand activity, we determined EC$_{50}$ values for our most active PHL agonists (**11b**, **11h**, and **11n**) and OHHL in *V. fischeri* (Δ-*luxI*) (Figure 2, panels b and c). These studies identified 3-nitro-PHL (**11n**) as the most active LuxR agonist, exhibiting a 10-fold lower EC$_{50}$ than OHHL (0.3 μM *vs* 3 μM, respectively). We performed analogous dose–response studies with **11n** and OHHL in wild-type *V. fischeri* ES114 (*23*) and ob-
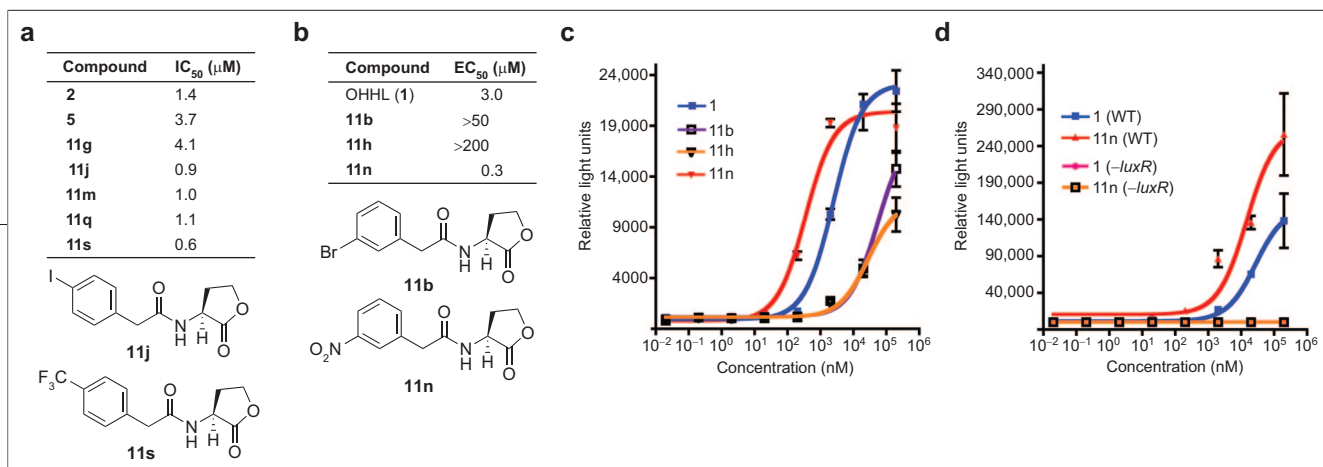
a

Compound	IC$_{50}$ (μM)
2	1.4
5	3.7
11g	4.1
11j	0.9
11m	1.0
11q	1.1
11s	0.6

11j

11s

b

Compound	EC$_{50}$ (μM)
OHHL (**1**)	3.0
11b	>50
11h	>200
11n	0.3

11b

11n

Figure 2. New synthetic modulators of LuxR. a) IC$_{50}$ table of most potent inhibitors and structures of selected compounds. b) EC$_{50}$ table of most potent activators and structures of selected compounds. See Supporting Information for dose–response curves. c) Agonism dose–response curves for OHHL (1**) and synthetic ligands 11b, 11h, and 11n in *V. fischeri* ES114 (Δ-*luxI*). d) Agonism dose–response curves for OHHL (**1**) and 11n in wild-type (WT) *V. fischeri* ES114 and *V. fischeri* ES114 (Δ-*luxR*). (Note, we were unable to achieve a maximum level of luminescence over the concentrations tested and therefore could not determine accurate EC$_{50}$ values in WT *V. fischeri*. Experiments at higher concentrations were precluded by the diminished solubilities of both OHHL (**1**) and PHL 11n at concentrations >500 μM.) Luminescence measured in relative light units. Error bars, standard deviation of the means of triplicate samples.**

served similarly heightened activity for PHL **11n** relative to OHHL (Figure 2, panel d). The superagonistic activity of PHL **11n** in *V. fischeri* relative to OHHL could be easily visualized by luminescence imaging with a CCD camera; Figure 3 shows a representative image of these experiments. We also examined the activity of **11n** and OHHL in a Δ-*luxR* derivative of *V. fischeri* ES114. Neither **11n** nor OHHL induced any detectable luminescence in this strain (Figure 2, panel d), which suggests that **11n**, like the native ligand OHHL, exerts its activity through the LuxR protein (see below). The discovery of compound **11n** is significant, because it is one of the first synthetic superagonists of quorum sensing reported (*11, 12*) and to our knowledge the first superagonist in *V. fischeri*.

In view of the structural similarity of PHLs **11** to native AHLs and the assay data and subtle SAR described above, we hypothesize that these ligands target the LuxR ligand-binding site and inhibition or activation is based on the specific binding mode and thus resulting affinity of the ligand. Further, we do not believe that these changes in activity simply reflect the different *chemical* properties of the PHLs (**11**) (*10*). This view is supported by several observations. First, the percentage of PHL **11** lactone hydrolysis (which abolishes activity for native AHLs (*24*)) is minimal and identical to that of OHHL over the time course of these luminescence assays. Second, the functionalities on PHLs **11** are unreactive under the

assay conditions tested (see Methods). Third, higher lipophilicity within the PHL series, and therefore higher potential cell permeability, does not correlate with enhanced antagonistic or agonistic activity (Table 1). This is further exemplified by the D-enantiomers of control antagonists **3** and **5**, which have identical lipophilicities as **3** and **5** yet exhibit markedly reduced activities (~20% inhibition; see Supplementary Figure 6). We have performed molecular modeling studies of several PHLs and OHHL docked into the putative ligand binding site of LuxR (built *in silico* from the one known structure of a complete R protein, TraR (*20, 21, 25, 26*); see Supplementary Figures 7–13) to further test this hypothesis. The results of these studies suggest that the LuxR binding site can readily accommodate PHLs yet is better able to accommodate 3-substituted PHLs (agonists) relative to 4-substituted PHLs (antagonists) and that activation or inhibition of LuxR may depend on the balance of favorable hydrogen bonding and unfavorable steric interactions within the binding pocket. While additional biochemical and structural studies will be required to fully elucidate PHL function in *V. fischeri*, these initial calculations provide support that PHLs **11** target LuxR.

In summary, we have discovered that PHLs **11** elicit remarkable and varied quorum-sensing responses in the bacterial symbiont *V. fischeri*. This family of ligands includes some of the most active antagonists and agonists of Gram-negative bacte-

rial quorum sensing reported to date. One significant outcome of this work is the observation that subtle alteration of substituents and their placement on the phenylacetanoyl moiety dramatically influence ligand activity. These changes do not simply abolish activity but rather convert potent antagonists into agonists. A second major outcome of this investigation is the discovery of the first synthetic superagonist of quorum sensing in *V. fischeri*, PHL **11n**. This ligand displays 10-fold higher activity relative to native autoinducer OHHL and is one of the first known superagonists of quorum sensing in Gram-negative bacteria (*11, 12*). Collectively, PHLs represent a new and valuable set of chemical tools for the study of quorum sensing in *V. fischeri* and could, with further development, provide broad insights into the roles of quorum sensing in bacterial symbioses. Preliminary experiments indicate superagonist **11n** is well tolerated by the main symbiotic partner of *V. fischeri*, the *Euprymna scolopes* squid (*6*), and is active *in vivo*; these studies will be reported in due course.

METHODS

Ligand Synthesis. PHL library **11**, OHHL (**1**), and control compounds **2**, **3**, and **5** were prepared using reported methods (*13*) (Figure 1, panel b), except the final cyclization–cleavage step was performed at RT for 24 h. Control **4** was synthesized in an analogous fashion except 4-dimethylaminopyridine and 1-pentanesulfonyl chloride replaced reagents i and **9** (Figure 1, panel b). D-AHLs were accessible using *N*-Fmoc-D-methionine in
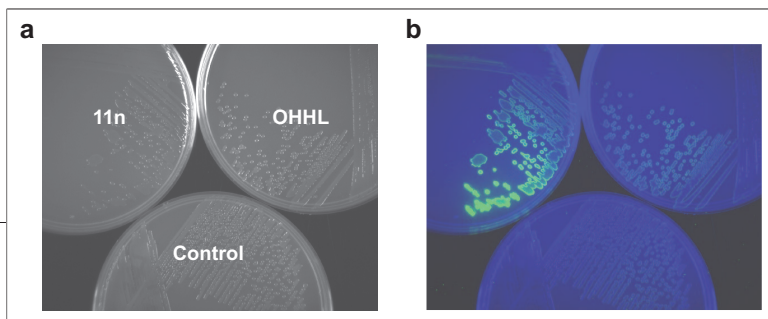
Figure 3. Bioluminescence induction by OHHL and quorum-sensing superagonist 11n **in** *V. fischeri*. **a)** *V. fischeri* ES114 (Δ-*luxI*) streaked onto agar plates containing either no compound (control), 0.5 μM OHHL (1), or 0.5 μM superagonist 11n **and grown for 8 h and imaged. b) Identical plates from panel a imaged for luminescence using a CCD imager.**

place of **7**. Compounds were submitted to biological assays following cleavage and an aqueous work-up without further purification. See Supporting Information for compound characterization data.

Compound Handling and Reagents. Stock solutions of synthetic compounds (10 mM) were prepared in DMSO. The structures of PHLs **11** and the control compounds remained unchanged after prolonged storage in DMSO (~4–6 months) and over the course of the biological assays (as determined by GC/MS, NMR, or both). Nitro-PHL derivatives (**11m**–**o**) were stored in the dark and not observed to degrade over the course of these studies. All biological reagents were purchased from Fisher Scientific. Luria–Bertani (LB) and LB salt media (LBS) were prepared as instructed with pH = 7.5 (LBS contained an additional 1.5% (w/v) NaCl, 0.3% (w/v) glycerol, and 50 nM Tris-HCl (*16*)).

Bacterial Strains. The *E. coli* strain used for these studies was JM109 (pSB401) (*22*). The *V. fischeri* strains were ES114 (*23*), ES114 (Δ-*luxI*), and ES114 (Δ-*luxR*) (*16*).

Luminescence Assays. For agonism assays, an appropriate amount of AHL stock solution was added into a 96-well plate. An overnight culture of *E. coli* or *V. fischeri* was diluted 1:10 with appropriate media (LB plus 10 μg mL^{-1} tetracycline for *E. coli*; LBS for *V. fischeri*). A 200-μL portion of the diluted culture was added to each well of the plate. Plates were grown for 4–8 h with shaking (200 rpm; 30 ºC for *E. coli* and RT for *V. fischeri*). Luminescence was measured using a multilabel plate reader and normalized to cell density. Antagonism screens were performed in an analogous manner against OHHL at its approximate EC$_{50}$ value (10 nM in *E. coli*; 5 μM in *V. fischeri*). Similar methods were used for dose–response assays, except the concentrations of PHL (**11**) or control varied between 2×10^{-2} and 2×10^5 nM. None of the PHLs (**11**) or control compounds in this study inhibited bacterial growth or displayed insolubility over the range of concentrations tested in the luminescence assays. All assays were performed in triplicate. Graphpad Prism software (version 5.0) was used to calculate IC$_{50}$ and EC$_{50}$ values. See Supporting Information for detailed assay protocols.

Supporting Information Available: This material is free of charge *via* the Internet.

Note added after print publication: Because of a production error, the DOI was incorrectly listed. This error does not affect the scientific integrity of the article. This paper was originally posted May 4, 2007, and the electronic version was corrected and reposted to the web on May 22, 2007. An Addition and Correction appears in *ACS Chem. Biol. 2* (6).

REFERENCES

1. Bassler, B. L., and Losick, R. (2006) Bacterially speaking, *Cell 125*, 237–246.

2. Waters, C. M., and Bassler, B. L. (2005) Quorum sensing: cell-to-cell communication in bacteria, *Annu. Rev. Cell Dev. Biol. 21*, 319–346.

3. de Kievit, T. R., and Iglewski, B. H. (2000) Bacterial quorum sensing in pathogenic relationships, *Infect. Immun. 68*, 4839–4849.

4. Hall-Stoodley, L., Costerton, J. W., and Stoodley, P. (2004) Bacterial biofilms: from the natural environment to infectious diseases, *Nat. Rev. Microbiol. 2*, 95–108.

5. Greenberg, E. P. (1999) Quorum sensing in Gram-negative bacteria: an important signaling mechanism in symbiosis and disease, in *Microbial Ecology and Infectious Disease* (Rosenberg, E., Ed.) pp 112–122, American Society for Microbiology, Washington, DC.

6. Ruby, E. G. (1996) Lessons from a cooperative, bacterial-animal association: the *Vibrio fischeri-Euprymna scolopes* light organ symbiosis, *Annu. Rev. Microbiol. 50*, 591–624.

7. Lyon, G. J., and Muir, T. W. (2003) Chemical signaling among bacteria and its inhibition, *Chem. Biol. 10*, 1007–1021.

8. Fuqua, C., and Greenberg, E. P. (2002) Listening in on bacteria: acyl-homoserine lactone signalling, *Nat. Rev. Mol. Cell Biol. 3*, 685–695.

9. Rasmussen, T. B., and Givskov, M. (2006) Quorum sensing inhibitors: a bargain of effects, *Microbiology 152*, 895–904.

10. Welch, M., Mikkelsen, H., Swatton, J. E., Smith, D., Thomas, G. L., Glansdorp, F. G., and Spring, D. R. (2005) Cell-cell communication in Gram-negative bacteria, *Mol. Biosyst. 1*, 196–202.

11. Muh, U., Hare, B. J., Duerkop, B. A., Schuster, M., Hanzelka, B. L., Heim, R., Olson, E. R., and Greenberg, E. P. (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.

12. Janssens, J. C., Metzger, K., Daniels, R., Ptacek, D., Verhoeven, T., Habel, L. W., Vanderleyden, J., De Vos, D. E., and De Keersmaecker, S. C. (2007) Synthesis of *N*-acyl homoserine lactone analogues reveals strong activators of SdiA, the *Salmonella typhimurium* LuxR homologue, *Appl. Environ. Microbiol. 73*, 535–544.

13. Geske, G. D., Wezeman, R. J., Siegel, A. P., and Blackwell, H. E. (2005) Small molecule inhibitors of bacterial quorum sensing and biofilm formation, *J. Am. Chem. Soc. 127*, 12762–12763.

14. Lin, Q., and Blackwell, H. E. (2006) Rapid synthesis of diketopiperazine macroarrays *via* Ugi four-component reactions on planar solid supports, *Chem. Commun.* 2884–2886.

15. Gorske, B. C., and Blackwell, H. E. (2006) Interception of quorum sensing in *Staphylococcus aureus*: a new niche for peptidomimetics, *Org. Biomol. Chem. 4*, 1441–1445.

16. Lupp, C., Urbanowski, M., Greenberg, E. P., and Ruby, E. G. (2003) The *Vibrio fischeri* quorum-sensing systems *ain* and *lux* sequentially induce luminescence gene expression and are important for persistence in the squid host, *Mol. Microbiol. 50*, 319–331.

17. Boettcher, K. J., and Ruby, E. G. (1995) Detection and quantification of *Vibrio fischeri* autoinducer from symbiotic squid light organs, *J. Bacteriol. 177*, 1053–1058.

18. Eberhard, A., Widrig, C. A., McBath, P., and Schineller, J. B. (1986) Analogs of the autoinducer of bioluminescence in *Vibrio fischeri*, *Arch. Microbiol. 146*, 35–40.

19. Schaefer, A. L., Hanzelka, B. L., Eberhard, A., and Greenberg, E. P. (1996) Quorum sensing in *Vibrio fischeri*: probing autoinducer-LuxR interactions with autoinducer analogs, *J. Bacteriol. 178*, 2897–2901.

20. Castang, S., Chantegrel, B., Deshayes, C., Dolmazon, R., Gouet, P., Haser, R., Reverchon, S., Nasser, W., Hugouvieux-Cotte-Pattat, N., and Doutheau, A. (2004) *N*-Sulfonyl homoserine lactones as antagonists of bacterial quorum sensing, *Bioorg. Med. Chem. Lett. 14*, 5145–5149.

21. Zhang, R. G., Pappas, T., Brace, J. L., Miller, P. C., Oulmassov, T., Molyneaux, J. M., Anderson, J. C., Bashkin, J. K., Winans, S. C., and Joachimiak, A. (2002) Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA, *Nature 417*, 971–974.

22. Winson, M. K., Swift, S., Fish, L., Throup, J. P., Jorgensen, F., Chhabra, S. R., Bycroft, B. W., Williams, P., and Stewart, G. S. (1998) Construction and analysis of *luxCDABE*-based plasmid sensors for investigating *N*-acyl homoserine lactone-mediated quorum sensing, *FEMS Microbiol. Lett. 163*, 185–192.

23. Boettcher, K. J., and Ruby, E. G. (1990) Depressed light emission by symbiotic *Vibrio fischeri* of the sepiolid squid *Euprymna scolopes*, *J. Bacteriol. 172*, 3701–3706.

24. Glansdorp, F. G., Thomas, G. L., Lee, J. J. K., Dutton, J. M., Salmond, G. P. C., Welch, M., and Spring, D. R. (2004) Synthesis and stability of small molecule probes for *Pseudomonas aeruginosa* quorum sensing modulation, *Org. Biomol. Chem. 2*, 3329–3336.

LETTER

25. Persson, T., Hansen, T. H., Rasmussen, T. B., Skinderso, M. E., Givskov, M., and Nielsen, J. (2005) Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic, *Org. Biomol. Chem. 3,* 253–262.

26. Frezza, M., Castang, S., Estephane, J., Soulere, L., Deshayes, C., Chantegrel, B., Nasser, W., Queneau, Y., Reverchon, S., and Doutheau, A. (2006) Synthesis and biological evaluation of homoserine lactone derived ureas as antagonists of bacterial quorum sensing, *Bioorg. Med. Chem. 14,* 4781–4791.