Published in final edited form as:

Bioorg Med Chem Lett. 2008 November 15; 18(22): 5978–5981. doi:10.1016/j.bmcl.2008.07.089.

Evaluation of a focused library of *N*-aryl L-homoserine lactones reveals a new set of potent quorum sensing modulators

Grant D. Geske[†], Margrith E. Mattmann[†], and Helen E. Blackwell^{*}

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

Abstract

A focused library of *N*-aryl L-homoserine lactones was designed around known lactone leads and evaluated for antagonistic and agonistic activity against quorum-sensing receptors in *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa*, and *Vibrio fischeri*. Several compounds were identified with significantly heightened activities relative to the lead compounds, and new structure-activity relationships (SARs) were delineated. Notably, 4-substituted *N*-phenoxyacetyl and 3-substituted *N*-phenylpropionyl L-homoserine lactones were identified as potent antagonists of TraR and LuxR, respectively.

Keywords

AHL; *N*-acylated homoserine lactone; Quorum sensing

Bacteria do not always act alone. Rather, many bacteria can assemble into multicellular communities and initiate processes as a group that they are incapable of as individual cells.¹ These group behaviors are largely under the control of a cell-cell signaling pathway called quorum sensing (QS), and can play a significant role in the establishment of both symbiotic and pathogenic relationships with eukaryotic hosts.² For example, virulence factor production and biofilm formation is under the control of QS in many clinically relevant pathogens.³ QS is mediated by a chemical language of low molecular weight signals, or autoinducers, and their cognate protein receptors. Autoinducer–receptor binding occurs once the bacteria reach a threshold cell density, and this binding event controls the transcription of genes necessary for bacterial group functions. Interception of this binding event represents a strategy to attenuate bacterial group behaviors, and has attracted considerable interest in the drug development and chemical biology fields.⁴ Our laboratory recently developed several synthetic autoinducer mimics that are capable of strongly antagonizing and agonizing autoinducer receptors in a range Gram-negative bacteria.⁵ Here, we report a third-generation set of autoinducer mimics that are derived from these initial lead compounds and that display significantly improved activities, most notably in the symbiont *Vibrio fischeri* and pathogen *Agrobacterium tumefaciens*.

^{*}Corresponding author. e-mail: blackwell@chem.wisc.edu.

[†]These authors contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Proteobacteria use *N*-acylated L-homoserine lactone signals (AHLs, Fig. 1) and cytoplasmic LuxR-type signal receptors as their primary QS circuit.⁶ One of the first approaches to modulate QS in these bacteria was the development of non-native AHLs capable of blocking or intercepting native AHL–LuxR-type receptor binding.⁷ These autoinducer mimics retained the native L-homoserine lactone, yet contained non-native acyl tails. The majority of our synthetic QS modulators are of this structural class, and selected AHLs (**1–8**) are shown in Fig. 1. Compounds **1–6** and **8** represent some of the most potent agonists and antagonists of LuxR-type receptors that we have reported.⁵ These compounds are active in *V. fischeri*, *A. tumefaciens*, and/or the opportunistic pathogen *Pseudomonas aeruginosa*, and can be classified as phenylacetyl HL (PHL, **1–5**), phenoxyacetyl HL (POHL, **6**), or phenylpropionyl HL (PPHL, **7–8**) derivatives. Our initial studies revealed that subtle structural changes to the PHL phenyl ring, some as simple as moving a substituent by one carbon, had marked effects on ligand activity;^{5,8} our examination of the POHL and PPHL structure classes was far more limited, however. In the present study, we sought to further explore the PHL, POHL, and PPHL structure classes as non-native QS modulators, and delineate additional SARs for antagonists and agonists of LuxR-type receptors in *A. tumefaciens*, *P. aeruginosa*, and *V. fischeri* (TraR, LasR, and LuxR, respectively). In addition, we sought to determine structural features that rendered ligands selective for one receptor, or more.

We designed a 39-member library (Library E) that incorporated acyl group functionalities that broadly fit into the PHL, POHL, and PPHL structure classes (shown in Fig. 2). A brief discussion of our choice of functional groups is valuable at the outset.

In examining the PHLs, we sought to study the effects of the incorporation of an expanded set of 3- and 4- substituents on agonistic and antagonistic activity against LuxR-type receptors. We focused primarily on 3-substituted PHLs, as these ligands are capable of inhibiting LasR in *P. aeruginosa*, and in turn, strongly activating LuxR in *V. fischeri*.^{5,8} In both organisms, substituents with some steric bulk and electron-withdrawing capability yielded the most active PHLs (e.g., 3-NO₂ PHL **4**; Fig. 1). We also examined a small set of PHLs with electron-withdrawing substituents in the 4-position, as these compounds are strong inhibitors of TraR in *A. tumefaciens* and LuxR in *V. fischeri* (e.g., PHLs **1**, **3**, and **5**). In this initial study of the POHL and PPHL structure classes, we also selected 3- and 4- substituted derivatives for further examination. The 4-OCF₃ POHL **6** was shown previously to be a moderate to strong inhibitor of LasR and TraR, respectively.⁵ In addition, 4-bromo PPHL **8** is a potent inhibitor of TraR, LasR, and LuxR, while the non-substituted PPHL **7** displays minimal activity against all three receptors, suggesting that phenyl ring substitution patterns are also important for activity in this structure class.

We synthesized Library E according to our previously reported microwave-assisted synthetic route to AHLs.^{5a} The 39 library members were isolated in moderate to good yields (55–80%) and high purities (91–99%) on a 10–20 mg/compound scale (see Supp. Info.)

Library E was evaluated for antagonistic and agonistic activity in TraR, LasR, and LuxR using cell-based reporter gene assays according to reported procedures.^{5,8} We utilized the following three reporter strains that lack native AHL synthases: *A. tumefaciens* WCF47 (pCF372),⁹ *E. coli* DH5 α (pJN105L pSC11),¹⁰ and *V. fischeri* ES114 (Δ -luxI).¹¹ The *A. tumefaciens* and *E. coli* strains produce the enzyme β -galactosidase upon TraR or LasR activation, respectively, and ligand activity is measured using Miller absorbance assays. LuxR activation or inhibition in the *V. fischeri* strain is reported by luciferase production. Antagonism assays were performed in the presence of library compound and native AHL ligand (at its approximate EC₅₀ value), while agonism assays were performed with library compound alone (see Supp. Info.) The native AHL ligands for *A. tumefaciens* (OOHL), *P. aeruginosa* (OdDHL), and *V. fischeri* (OHHL) and compounds **1–8** (Fig. 1) were used as controls for these assays.

The reporter gene assays of Library E revealed that 36 of the 39 library members exhibited strong antagonistic or agonistic activity against one of more LuxR-type receptor. This high percentage of “hits” (92%) serves to validate our third-generation library design focused on PHLs, POHLs, and PPHLs. Thirty-four compounds were identified that displayed inhibitory activities of >70% against TraR, LasR, and/or LuxR. In turn, six compounds were identified as either LasR or LuxR agonists, with activities of >50% in LuxR and/or >20% in LasR (see Supp. Info. for primary assay data.) To obtain more quantitative data about the activity of these hits, we performed dose response assays in the three reporter strains and determined either IC₅₀ or EC₅₀ values for the 36 compounds. These values are listed in Table 1 and Table 2, respectively. Here, we focus on the most active LuxR-type receptor antagonists and agonists identified in Library E for brevity.

The most active TraR antagonists in Library E were POHLs and PPHLs containing electron-withdrawing groups in the 4-position (Table 1), with POHLs representing the largest subset of actives.¹³ 4-CF₃ PPHL **E35** and 4-NO₂ POHL **E24** were the most potent TraR antagonists overall, with IC₅₀ values 2- to 4-fold lower than control PPHL **8** and control POHL **6** (~0.25 μM).¹² Notably, **E35** and **E24** were capable of inhibiting TraR by 50% at a ~1:1 ratio with the native ligand OOHL, and represent the most active TraR inhibitors that we have identified to date. The next three most active TraR antagonists in Library E were all 4-halo POHLs (**E20–E22**), and displayed increasing antagonistic activity as halogen size increased (Cl→Br→I). We previously found that 4-halo PHLs exhibit the same pattern for TraR antagonistic activity,⁵ and interestingly this trend is continued in these one-atom longer homologs. This activity trend was less apparent in the 4-halo PPHL series, however. In terms of TraR agonists, no compounds with appreciable agonistic activity were found in Library E (Table 2), again suggesting that this receptor has stringent ligand-binding requirements (*i.e.*, specific to OOHL) to adopt an active conformation.⁵

Similar to TraR, the most active LasR antagonists in Library E were POHLs and PPHLs (Table 1). However, PPHLs were the largest set of actives overall, with 3-I PPHL **E33** being the most active LasR antagonist in the library (IC₅₀ = 1.8 μM). The four next most active compounds were, in contrast to **E33**, all 4-substituted derivatives: 4-Br and 4-I POHLs (**E21** and **E22**), and 4-Cl and 4-NO₂ PPHLs (**E30** and **E37**). The 4-halo POHLs exhibited the same activity trend in LasR as they did in TraR, with 4-I POHL **E22** being the most active antagonist in the series (IC₅₀ = 2.0 μM) and 2-fold more active than control POHL **6**. Notably, POHL **E22** was also identified as a strong TraR antagonist (see above). The 4-halo PPHL series, however, did not exhibit a consistent antagonistic activity trend relative to the POHLs, similar to the data for TraR. Surprisingly, the PHLs in Library E failed to exhibit appreciable antagonistic activity against LasR. Indeed, the overall activities of the LasR antagonists identified in Library E were low relative to the controls, most notably PPHL **8** and PHL **4**. These data, along with those from an earlier study,^{5a} suggest that the PHL, POHL, and PPHL structure classes contain more individual, potent LasR antagonists as opposed to families of highly activity ligands (as seen for TraR above and LuxR below).

Turning next to LasR agonists, we identified four compounds in Library E that showed weak LasR agonistic activity, one PHL and 3 POHLs (Table 2). The 4-SCF₃ PHL **E8** was the most active agonist overall, capable of activating LasR to 65% the level of native ligand (OdDHL) at 300-fold higher concentration. Interestingly, PHL **E8** is also a weak *antagonist* of both TraR and LuxR (Table 1). To our knowledge, no PHL activator of LasR has been reported to date, so the identification of **E8**, despite its low activity, is noteworthy.

In contrast to the TraR and LasR screening data, the most active LuxR inhibitors identified in Library E were all PPHLs (Table 1). Further, the most active subset of these compounds had electron-withdrawing substituents in the 3-*position*, as opposed to the 4-*position* (*e.g.*, **E31**,

E33, **E36**, and **E38**). The three most potent PPHLs (3-I **E33**, 3-CF₃ **E36**, and 3-Br **E31**) all had lower IC₅₀ values than that of control PHL **5** and control PPHL **8** (~0.47 vs. 0.61 and 1.35 μM, respectively), and were capable of inhibiting LuxR by 50% at a ~6-fold lower concentration than native ligand (OHHL). These ligands are among the most potent LuxR antagonists that we have discovered to date. Of note, 3-I PPHL **E33** was also identified as the strongest LasR inhibitor in this study (see above), further highlighting the utility of PPHLs as a general class of LuxR-type receptor modulators. This activity trend for PPHL antagonists is intriguing, as we had previously observed that the one-carbon shorter homologs, *i.e.*, PHLs, exhibit higher LuxR inhibitory activity with substituents in the 4-*position* as opposed to the 3-position (see above).⁷ Indeed, the PHLs in Library E, the majority of which were 3-substituted, were all weaker LuxR antagonists relative to the 3-substituted PPHLs (see Table 1).

The 3-substituted PHLs did provide some exciting results in the LuxR *agonism* assays, however (Table 2). Here, two 3-substituted PHLs were uncovered as highly potent LuxR agonists (3-CN PHL **E5** and 3,5-CF₃ PHL **E9**), with EC₅₀ values ~10 fold lower than that of the native ligand (OHHL). Notably, PHL **E9** has an EC₅₀ value comparable to our previously reported LuxR activator, 3-NO₂ PHL **4**, yet does not activate to the same threshold level (70% vs. ~100%).⁸ (Intriguingly, the mono-substituted 3-CF₃ PHL **E3** failed to activate LuxR, and was a moderate antagonist instead; Table 1). Few synthetic LuxR-type receptor activators have been reported; thus, the discovery of LuxR agonists **E5** and **E9** in Library E is significant. In addition, this result serves to further refine our previous SAR data for LuxR activators to include PHLs with specific electron-withdrawing groups in the 3- or the 3- and 5-positions.⁸

In summary, analysis of Library E has yielded a new set of synthetic LuxR-type receptor antagonists and agonists. The most active antagonists were largely POHLs or PPHLs with electron-withdrawing groups in the 4- or 3-positions, respectively. Several antagonists had markedly improved activity relative to our initial lead compounds, most notably **E24**, **E33** and **E35** in TraR or LuxR. In addition, PHLs **E5** and **E9** were identified as strong activators of LuxR. These results serve to further underscore the utility of screening focused AHL libraries for the optimization of existing and the identification of new LuxR-type receptor modulators. Ongoing work in our laboratory is focused on evaluating the Library E hits in phenotypic QS assays and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Financial support for this work was provided by the NIH (AI063326-01), Greater Milwaukee Foundation, Shaw Scientist Program, Burroughs Wellcome Fund, Camille & Henry Dreyfus Foundation, Research Corporation, Johnson & Johnson, and 3M. H.E.B. is an Alfred P. Sloan Foundation Fellow. We gratefully acknowledge Professors Stephen Winans (Cornell University), Peter Greenberg (U Washington), and Edward Ruby (UW–Madison) for donations of bacterial strains, and Patrick Shipway for synthetic assistance.

References and Notes

1. Camilli A, Bassler BL. Science 2006;311:1113. [PubMed: 16497924]
2. Greenberg EP. Nature 2003;424:134. [PubMed: 12853935]
3. Smith RS, Iglewski BH. Curr. Opin. Microbiol 2003;6:56. [PubMed: 12615220]
4. For a recent commentary, see: Bjarnsholt T, Givskov M. Phil. Trans. R. Soc. B 2007;362:1213. [PubMed: 17360273]

5. (a) Geske GD, O'Neill JC, Miller DM, Mattmann ME, Blackwell HE. *J. Am. Chem. Soc* 2007;129:13613. [PubMed: 17927181] (b) Geske GD, O'Neill JC, Miller DM, Wezeman RJ, Mattmann ME, Lin Q, Blackwell HE. *ChemBioChem* 2008;9:389. [PubMed: 18224645]
6. Lazdunski AM, Ventre I, Sturgis JN. *Nat. Rev. Microbiol* 2004;2:581. [PubMed: 15197393]
7. For a review, see: Geske GD, O'Neill JC, Blackwell HE. *Chem. Soc. Rev* 2008;37:1432. [PubMed: 18568169]
8. Geske GD, O'Neill JC, Blackwell HE. *ACS Chem. Biol* 2007;2:315. [PubMed: 17480049]
9. Zhu J, Beaber JW, Moré MI, Fuqua C, Eberhard A, Winans SC. *J. Bacteriol* 1998;180:5398. [PubMed: 9765571]
10. Lee JH, Lequette Y, Greenberg EP. *Mol. Microbiol* 2006;59:602. [PubMed: 16390453]
11. Lupp C, Urbanowski M, Greenberg EP, Ruby EG. *Mol. Microbiol* 2003;50:319. [PubMed: 14507383]
12. Note, IC₅₀ data for controls **1–8** were obtained at ~2-fold lower concentration of native ligand relative to Library E. This does not prevent the comparison of relative IC₅₀ values in Library E, and indeed provides a more conservative interpretation of the activities of the hits relative to controls **1–8**.
13. We note that the dose response curves for some of the antagonists in this study start to upturn at higher concentrations (~100 μ M). Ongoing studies are focused on fully characterizing this phenomenon. However, we believe that these compounds can still be termed antagonists over a specific concentration range, and that this upturn pattern does not preclude our comparison of ligand activity within each receptor data set.

Figure 1. Generic structure for AHLs (top left), structures of selected naturally occurring AHLs (top right), and structures of our previously reported non-native AHLs (PHLs **1–5**, POHL **6**, and PPHL **7–8**; bottom). Acyl chain carbon numbers are shown for clarity for native AHLs.

Figure 2. Structures of the acyl groups of the AHLs in Library E (**E1–E39**). All 39 AHLs have 1- stereochemistry.

Table 1IC₅₀ values for most active antagonists across the three strains.^a

Entry	Compd	<i>A. tumefaciens</i> TraR (μM) ^b	<i>E. coli</i> LasR (μM) ^c	<i>V. fischeri</i> LuxR (μM) ^d
1	1	1.25 ^e	1.72 ^{ef}	0.86 ^e
2	2	-- ^g	4.63	--
3	3	2.25 ^e	--	0.96 ^e
4	4	--	0.61 ^{ef}	--
5	5	0.81 ^e	--	0.61
6	6	0.46 ^{ef}	4.67 ^{ef}	--
7	8	0.92 ^e	0.34 ^{ef}	1.35
8	E1	--	--	2.0 (91%) ^h
9	E2	--	--	2.5 (85%) ^h
10	E3	--	--	1.4
11	E6	8.4	--	2.1
12	E7	--	--	1.5
13	E8	4.7	--	1.3
14	E10	--	--	1.0 ^{ef}
15	E11	2.4 ^e	--	--
16	E12	4.2	--	0.94
17	E13	--	--	1.3
18	E15	1.8 (84%) ^h	--	--
19	E16	1.1 ^e	7.8 ^e	--
20	E17	4.3	--	--
21	E19	2.7 ^{ef}	--	--
22	E20	0.62^e	4.7 ^e	--
23	E21	0.51^{ef}	2.1 (67%)^h	3.0
24	E22	0.44^{ef}	2.0 (63%)^h	1.9
25	E23	1.6 ^{ef}	--	--
26	E24	0.29^{ef}	--	--
27	E25	--	--	2.4
28	E26	--	4.3 ^{ef}	2.2
29	E27	--	8.9 ^{ef}	2.1
30	E28	--	6.8 ^{ef}	--
31	E29	--	12 ^{ef}	2.7
32	E30	3.3	2.2^{ef}	0.97
33	E31	--	3.3 ^{ef}	0.53
34	E32	2.1	--	0.63
35	E33	--	1.8^{ef}	0.39
36	E34	1.1 ^e	--	1.7
37	E35	0.21^e	--	2.2
38	E36	--	--	0.50
39	E37	1.6 ^e	3.0 (67%)^h	0.99
40	E38	--	3.4 ^{ef}	0.88
41	E39	--	3.4	--

^aSee text for details of reporter strains. Values determined by testing compounds over a range of concentrations (0.01–1×10⁶ nM) against native ligand. All assays performed in triplicate. See Supp. Info. for plots of dose response curves. Shaded compounds are controls; key data for Library E is bolded. Control IC₅₀ data from ref. 5a; see also ref. 12.

^bDetermined against 200 nM OOH_L.

^cDetermined against 20 nM OdDHL.

^dDetermined against 3 μM OHHL.

^eDose response curve upturned at higher concentrations; IC₅₀ value calculated from partial antagonism dose response curve. See ref. 13.

^fDose response curve did not fully level off over the concentrations tested due to upturn.

^gNot determined.

^hDose response curve levels off, yet not at 100% inhibition. This could indicate eventual upturn. Maximal inhibition level is indicated.

Table 2EC₅₀ values for most active agonists across the three strains.^a

Entry	Compd	<i>A. tumefaciens</i> TraR (μM)	<i>E. coli</i> LasR (μM)	<i>V. fischeri</i> LuxR (μM)
1	OOHL	0.20	-- ^b	--
2	OdDHL	--	0.01	--
3	OHHL	--	--	3.0
4	4	--	--	0.35
5	6	--	6.3 (30%) ^c	--
6	E5	--	--	0.30 (75%) ^c
7	E8	--	3.4 (65%) ^c	--
8	E9	--	--	0.37 (70%) ^c
9	E32	--	>10 ^d	--
10	E35	--	>10 ^d	--
11	E39	--	>10 ^d	--

^a See footnote a for Table 1. Control EC₅₀ data from ref. 5a.^b Not determined.^c Dose response curve reached a plateau over the concentrations tested, yet the level of maximal induction was lower than that for the native ligand; EC₅₀ value calculated from this dose response curve. Value in parentheses equals the maximum induction value achievable (at 100 μM ligand) relative to native ligand.^d Dose response curve did not fully plateau over the concentrations tested.