JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

C4-Alkoxy-HPD: A Potent Class of Synthetic Modulators Surpassing Nature in AI-2 Quorum Sensing

Kyoji Tsuchikama, Jie Zhu, Colin A. Lowery, Gunnar F. Kaufmann, and Kim D. Janda*

The Skaggs Institute for Chemical Biology and Departments of Chemistry, Immunology & Microbial Science, and Worm Institute for Research and Medicine (WIRM), The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

Supporting Information

ABSTRACT: Bacteria have developed cell-to-cell communication mechanisms, termed quorum sensing (QS), that regulate bacterial gene expression in a cell population-dependent manner. Autoinducer-2 (AI-2), a class of QS signaling molecules derived from (4*S*)-4,5-dihydroxy-2,3-pentanedione (DPD), has been identified in both Gram-negative and Gram-positive bacteria. Despite considerable interest in the AI-2 QS system, the biomolecular communication used by distinct bacterial species still remains shrouded. Herein, we report the synthesis and evaluation of a new class of DPD analogues, C4-alkoxy-5-hydroxy-2,3-pentanediones, termed C4-alkoxy-HPDs. Remarkably, two of the analogues were more potent QS agonists than the natural ligand, DPD, in *Vibrio harveyi*. The findings presented extend insights into ligand–receptor recognition/signaling in the AI-2 mediated QS system.

Bacteria have developed unique systems to coordinate their behavior in a cell density-dependent manner, a process that has been termed quorum sensing (QS).[1,2] QS is mediated by a series of signal production/secretion/response events utilizing diffusible signaling molecules called autoinducers. When the bacterial population, and thus concentration of autoinducer, reaches a threshold level, gene expression is synchronized in a concerted manner. In many species, this process often results in pathogenic events such as biofilm formation and virulence factor production. Therefore, the modulation of QS has emerged as a potential therapeutic approach for combating microbial infection.[3−6]

Autoinducer-2 (AI-2), one of the classes of autoinducers, is produced by both Gram-negative and Gram-positive bacterial species.[7−10] AI-2 signaling molecules share a single common precursor, (4*S*)-4,5-dihydroxy-2,3-pentanedione (DPD), that exists in a multiplexed equilibrium between its linear and cyclic forms (Figure 1).[11] Collectively, these findings have led to the hypothesis that bacteria use AI-2 as a "universal language" for interspecies monitoring as well as intraspecies communication.[10]

Our group and others have reported upon the synthesis and potency of a series of DPD analogues including C1-alkyl-DPDs,[12−16] 2,5-dihydroxy-2-methylcyclopentanones (DHMPs),[17] and C5-methyl-DPDs[18] as QS modulators (Figure 2). In sum, these reports have provided evidence that

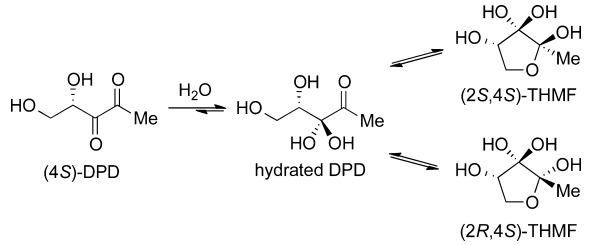


Figure 1. A plausible equilibrium of DPD in aqueous solution.

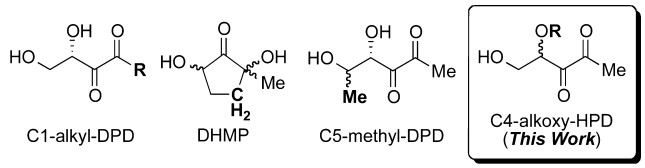


Figure 2. Structures of DPD analogues for the modulation of AI-2 based QS.

minor structural alterations of DPD can have a significant impact on the function of AI-2 in QS mediated processes. However, even with these studies, a clear gap still exists between our understanding of DPD's complex chemical equilibrium and leveraging structural information for agonist/antagonist design between bacterial species.[11]

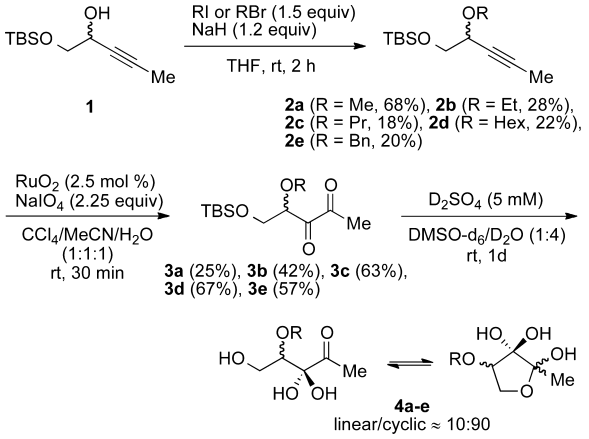
To help better define the chemical determinants critical within the sphere of the AI-2 QS system, we were drawn to the QS activity seen with the C1-alkyl derivatives, particularly their oscillatory agonist/antagonist activity based upon the addition of single methylene units. Thus, we designed and synthesized a new group of synthetic DPD analogues, C4-alkoxy-5-hydroxy-2,3-pentanedione (C4-alkoxy-HPD, Figure 2). In this communication, we present data demonstrating that these C4-alkoxy-HPDs are capable of more potent QS agonism than the natural signal used in *Vibrio harveyi*.

The synthesis of the C4-alkoxy-HPDs is shown in Scheme 1, which began from readily available racemic 1-((*tert*-butyldimethylsilyl)oxy)pent-3-yn-2-ol (**1**).[19] Alkyl groups (methyl, ethyl, propyl, hexyl, and benzyl) were installed on the C4-hydroxy group under standard conditions of ether synthesis; however, incomplete consumption of the starting material and significant degradation of substrate and products

Received: June 7, 2012

   dx.doi.org/10.1021/ja305532y | *J. Am. Chem. Soc.* XXXX, XXX, XXX–XXX

Scheme 1. Synthesis of C4-Alkoxy-HPDs 4a−e



were observed and propargyl ethers **2a−e** were obtained in low to moderate yields. Subsequently, ruthenium-catalyzed oxidation of ethers **2a−e** was accomplished to give diketones **3a−e**. Finally, precursors **3a−e** were solubilized in dilute sulfuric acid to afford C4-alkoxy-HPDs **4a−e** in quantitative yield. NMR analysis of compounds **4a−e** showed that they exist as a mixture of linear/cyclic forms in a ratio of approximately 10:90. Given the reported linear/cyclic ratio of DPD (approximately 20:80),[11,20,21] these results illustrate that installation of "capping" substituents on the C4-hydroxy group within DPD does not significantly impact cyclization.

The obtained C4-alkoxy-HPDs **4a−e** were evaluated for the modulation of QS signaling using two established reporter assays: bioluminescence emission in *V. harveyi*[22] and induction of *β*-galactosidase expression in *Salmonella enterica* serovar Typhimurium.[23] First, we examined the effects of the synthetic analogues on bioluminescence of the *V. harveyi* strain MM32 (ATCC BAA-1117, Δ*luxS*, Δ*luxN*), a strain unable to produce its own DPD due to the lack of the LuxS synthase and also lacking the acylhomoserine lactone receptor LuxN. The use of this strain allows for the measurement of QS responses only via the AI-2 pathway in the presence of exogenous autoinducers. Cultures were incubated in the presence of DPD or each synthetic analogue at varying concentrations (20−0.02 μM).

Analogues **4a−c** induced bioluminescence emission, whereas C4-HexO-HPD **4d** and C4-BnO-HPD **4e** exhibited weak or no induction at 20 μM (Figure 3). The induction was strongly dependent on the length of the C4-hydroxy alkylation. Thus, while C4-MeO-HPD **4a** presented modest QS activity, remarkably, C4-EtO-HPD **4b** and C4-PrO-HPD **4c** appeared to be better ligands than DPD. Notably, analogue **4c**, a racemate, was almost 10-fold more potent than the natural ligand DPD with a submicromolar EC_{50} value of 0.15 ± 0.03 μM, whereas the EC_{50} value of DPD was 1.07 ± 0.06 μM under our assay conditions (Table 1).[24] It is reasonable to speculate that a single enantiomer of analogue **4c** may provide even greater enhanced activity.

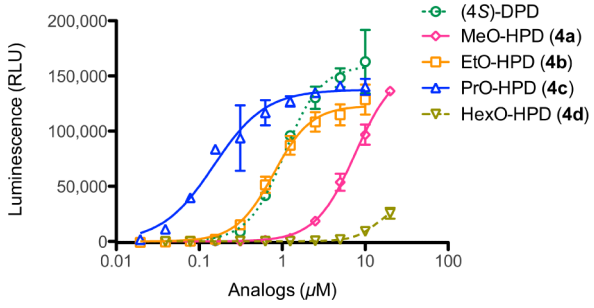


Figure 3. Bioluminescence of *V. harveyi* strain MM32 (Δ*luxN*, Δ*luxS*) in the presence of the analogues. Bioluminescence was normalized to cell density. All assays were performed in triplicate, and error bars represent standard deviation. All curves were fit to variable-slope sigmoidal curves using GraphPad Prism software. BnO-HPD **4e** showed no agonistic effect at 20 μM.

Table 1. Summary of QS Modulation by C4-Alkoxy-HPDs[a]

compound (C4-RO-HPD)	EC_{50} in *V. harveyi* assay (mean ± SD, μM)[b]	*β*-galactosidase activity in *S.* Typhimurium (mean ± SD, %)
(4*S*)-DPD	1.07 ± 0.06	100.0 ± 2.9
R = Me (**4a**)	7.60 ± 0.45	<5
R = Et (**4b**)	0.79 ± 0.05	<5
R = Pr (**4c**)	**0.15 ± 0.03**	<5
R = Hex (**4d**)	N.D.[c]	<5
R = Bn (**4e**)	N.D.[c]	<5

[a]All assays were performed in triplicate. [b]All EC_{50} values were calculated from the sigmoidal curves obtained in Figure 3. [c]Assays were performed in the presence of 50 μM compound. SD, standard deviation; N.D., not determined.

To our knowledge, these compounds are the first examples of synthetic DPD analogues that are better at initiating an AI-2 mediated process than the natural ligand.[25] In the AI-2 based QS of *V. harveyi*, it is a borate diester derived from the cyclic isomer (2*S*,4*S*)-THMF that the periplasmic AI-2 receptor protein LuxP recognizes (Figure 1), which triggers a protein phosphorylation cascade that ultimately results in altered gene expression.[26,27] With this mechanism in mind, the slightly higher ratio of cyclic forms in the equilibrium of C4-alkoxy-HPDs (Scheme 1) may help drive their enhanced activity; however, this is unlikely to be the sole contributing factor given the varying agonist activity of the panel of C4-alkoxy-HPDs examined. We surmise that LuxP has an additional structural hydrophobic modulatory site, which analogue **4c** has advantageously accessed to produce superior QS activity. Indeed, a docking model of LuxP and analogue **4c** appears to support our speculation (Figure S5).[28] On the other hand, congeners with large hydrophobic groups such as the hexyl (**4d**) and benzyl (**4e**) derivatives may be too bulky for a productive interaction.

To continue our studies, analogues **4a−e** were screened for QS modulation in *S.* Typhimurium strain Met844 (Δ*luxS*, *lsr-lacZ* fusion), a strain incapable of producing its own DPD.[29] The *lsr-lacZ* fusion encodes *β*-galactosidase under the AI-2-regulated *lsr* promoter, and enables the monitoring of AI-2-dependent *lsr* expression based on the residual *β*-galactosidase activity. Assays were performed using the analogues at 50 μM in the absence of DPD (agonist assay) or in the presence of 50 μM DPD (antagonist assay). These experiments revealed no significant agonist or antagonist activity (Table 1 and Figure S3). This is in contrast to previous reports on a panel of C1-alkyl-DPD analogues, in which several compounds exhibited antagonist effects in AI-2 based QS of *S.* Typhimurium.[12,15,16]

In the AI-2 QS circuit of *S.* Typhimurium, DPD is transported into the cytoplasm and the C5-hydroxy group within the linear form is phosphorylated by the kinase LsrK to

dx.doi.org/10.1021/ja305532y | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

afford the chemical species thought to be responsible for regulating gene expression.[30,31] A recent report highlights how LsrK also phosphorylates a series of unnatural C1-alkyl-DPDs to provide active modulators of QS acting on the *lsr* operon.[15] For the purpose of further dissecting the lack of analogue activity, LsrK-mediated phosphorylation assays of C4-alkoxy-HPDs were performed (Figures 4 and S4). As anticipated based

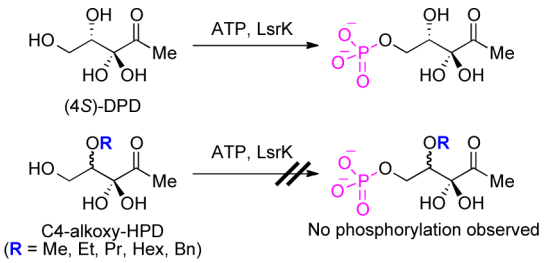


Figure 4. LsrK-mediated phosphorylation of DPD and C4-alkoxy-HPDs.

on their QS inactivity, none of the analogues were phosphorylated by LsrK, suggesting that the C4-hydroxy group in DPD is a critical factor in the mechanism of LsrK-mediated phosphorylation.

In conclusion, we have detailed the synthesis of a panel of DPD derivatives, C4-alkoxy-HPDs. These DPD analogues were evaluated for the modulation of bacterial QS in established assays, and unanticipated, yet remarkable, effects on AI-2 QS circuits were observed in a species-dependent manner. Indeed, the present work describes the discovery of the most potent agonist known for *V. harveyi* QS signaling. While potent antagonistic activity was not uncovered with this series of structures, the nuance between agonist and antagonist is a fine line and thus our findings should enable future chemical structure-based approaches for accessing such alternative activity. In a broader sense, the present work, coupled with previous findings with C1-alkyl-DPD analogues, highlights how manipulation of the DPD scaffold can provide valuable tools for in-depth studies of the ligand–receptor interactions making up AI-2-mediated QS.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic protocols, assay methods, additional biological assays, and characterization of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

kdjanda@scripps.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge Prof. Bonnie Bassler (Princeton University) for providing us with *S.* Typhimurium strain Met844, Dr. Daniel Globisch for the LuxP-analogue docking model, and Dr. Karen C. McCague for insightful discussions. We also acknowledge financial support from the NIH (Grant AI077644 to K.D.J.), the Skaggs Institute for Chemical Biology, and the Japan Society for the Promotion of Science (fellowship to K.T.).

■ REFERENCES

(1) Engebrecht, J.; Nealson, K.; Silverman, M. *Cell* **1983**, *32*, 773–81.
(2) Fuqua, W. C.; Winans, S. C.; Greenberg, E. P. *J. Bacteriol.* **1994**, *176*, 269–75.
(3) Suga, H.; Smith, K. M. *Curr. Opin. Chem. Biol.* **2003**, *7*, 586–91.
(4) Bjarnsholt, T.; Jensen, P. O.; Rasmussen, T. B.; Christophersen, L.; Calum, H.; Hentzer, M.; Hougen, H. P.; Rygaard, J.; Moser, C.; Eberl, L.; Hoiby, N.; Givskov, M. *Microbiology* **2005**, *151*, 3873–80.
(5) Lowery, C. A.; Salzameda, N. T.; Sawada, D.; Kaufmann, G. F.; Janda, K. D. *J. Med. Chem.* **2010**, *53*, 7467–89.
(6) Zhu, P.; Li, M. *Curr. Med. Chem.* **2012**, *19*, 174–86.
(7) Bassler, B. L.; Wright, M.; Silverman, M. R. *Mol. Microbiol.* **1994**, *13*, 273–86.
(8) Bassler, B. L.; Greenberg, E. P.; Stevens, A. M. *J. Bacteriol.* **1997**, *179*, 4043–5.
(9) Vendeville, A.; Winzer, K.; Heurlier, K.; Tang, C. M.; Hardie, K. R. *Nat. Rev. Microbiol.* **2005**, *3*, 383–96.
(10) Waters, C. M.; Bassler, B. L. *Annu. Rev. Cell. Dev. Biol.* **2005**, *21*, 319–46.
(11) Globisch, D.; Lowery, C. A.; McCague, K. C.; Janda, K. D. *Angew. Chem., Int. Ed.* **2012**, *51*, 4204–8.
(12) Lowery, C. A.; Park, J.; Kaufmann, G. F.; Janda, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 9200–1.
(13) Lowery, C. A.; Abe, T.; Park, J.; Eubanks, L. M.; Sawada, D.; Kaufmann, G. F.; Janda, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 15584–5.
(14) Ganin, H.; Tang, X.; Meijler, M. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3941–4.
(15) Roy, V.; Smith, J. A.; Wang, J.; Stewart, J. E.; Bentley, W. E.; Sintim, H. O. *J. Am. Chem. Soc.* **2010**, *132*, 11141–50.
(16) Gamby, S.; Roy, V.; Guo, M.; Smith, J. A.; Wang, J.; Stewart, J. E.; Wang, X.; Bentley, W. E.; Sintim, H. O. *ACS Chem. Biol.* **2012**, *7*, 1023–30.
(17) Tsuchikama, K.; Lowery, C. A.; Janda, K. D. *J. Org. Chem.* **2011**, *76*, 6981–9.
(18) Rui, F.; Marques, J. C.; Miller, S. T.; Maycock, C. D.; Xavier, K. B.; Ventura, M. R. *Bioorg. Med. Chem.* **2012**, *20*, 249–56.
(19) Mohamed, Y. A. M.; Inagaki, F.; Takahashi, R.; Mukai, C. *Tetrahedron* **2011**, *67*, 5133–41.
(20) Meijler, M. M.; Hom, L. G.; Kaufmann, G. F.; McKenzie, K. M.; Sun, C.; Moss, J. A.; Matsushita, M.; Janda, K. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2106–8.
(21) Semmelhack, M. F.; Campagna, S. R.; Federle, M. J.; Bassler, B. L. *Org. Lett.* **2005**, *7*, 569–72.
(22) Taga, M. E.; Xavier, K. B. *Curr. Protoc. Microbiol.* **2011**, *Chapter 1*, No. Unit1C.1.
(23) Griffith, K. L.; Wolf, R. E., Jr. *Biochem. Biophys. Res* **2002**, *290*, 397–402.
(24) A similar EC_{50} value of DPD was reported: Winzer, K.; Hardie, K. R.; Burgess, N.; Doherty, N.; Kirke, D.; Holden, M. T.; Linforth, R.; Cornell, K. A.; Taylor, A. J.; Hill, P. J.; Williams, P. *Microbiology* **2002**, *148*, 909–22.
(25) (4*S*,5*R*)-4,5-Dihydroxy-2,3-hexanedione (ref 18) was the most potent agonist of the synthetic analogues reported to date in the AI-2 based QS of *V. harveyi*, although it showed approximately 10-fold higher EC_{50} value than that of DPD.
(26) Neiditch, M. B.; Federle, M. J.; Pompeani, A. J.; Kelly, R. C.; Swem, D. L.; Jeffrey, P. D.; Bassler, B. L.; Hughson, F. M. *Cell* **2006**, *126*, 1095–108.
(27) Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B. L.; Hughson, F. M. *Nature* **2002**, *415*, 545–9.
(28) The existence of an additional cavity proximal to the DPD binding site within LuxP has also been discussed in ref 18.
(29) Taga, M. E.; Miller, S. T.; Bassler, B. L. *Mol. Microbiol.* **2003**, *50*, 1411–27.
(30) Xavier, K. B.; Miller, S. T.; Lu, W.; Kim, J. H.; Rabinowitz, J.; Pelczer, I.; Semmelhack, M. F.; Bassler, B. L. *ACS Chem. Biol.* **2007**, *2*, 128–36.
(31) Xue, T.; Zhao, L.; Sun, H.; Zhou, X.; Sun, B. *Cell Res.* **2009**, *19*, 1258–68.