OL | Organic Letters

Letter

Cite This: Org. Lett. 2019, 21, 7274–7278

pubs.acs.org/OrgLett

Discovery and Total Synthesis of Doscadenamide A: A Quorum Sensing Signaling Molecule from a Marine Cyanobacterium

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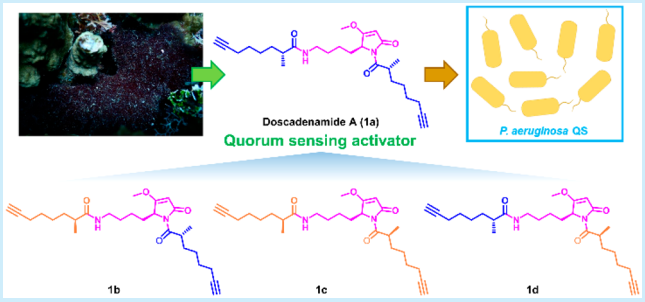
Supporting Information

ABSTRACT: Quorum sensing (QS) plays a critical role in the regulation of bacterial pathogenesis. Doscadenamide A (**1a**) was isolated from a marine cyanobacterium, its structure elucidated by NMR, and its activity linked to QS induction. The total synthesis of **1a** was developed, and the absolute configuration confirmed through comparison of the isolated natural product with synthetic diastereomers. Our preliminary investigation indicated that **1a** could activate QS signaling in a LasR-dependent manner.



Quorum sensing (QS) is an intercellular communication process adopted by a number of bacteria to regulate diverse physiological activities. This process involves the production and release of diffusible extracellular signaling molecules named autoinducers (AIs), which would accumulate with an increase in bacterial population density.[1−3] QS plays a pivotal role in regulating bacterial pathogenesis. For example, QS modulates the production of virulence factors such as pyocyanin and elastase in *Pseudomonas aeruginosa* during bacterial growth and infection.[4] Thus, QS signaling pathways are an attractive target for the development of antimicrobial therapeutic agents. *P. aeruginosa* is a Gram-negative opportunistic pathogen that can cause serious lung infections in cystic fibrosis patients[5] and microbial keratitis during contact lens wear.[6] The AIs that control QS signaling in *P. aeruginosa* include two acylhomoserine lactones with varying alkyl chain lengths and oxidation states at C-3 [C4-HSL and 3-oxo-C12-HSL (Figure 1)] and a group of quinolone compounds [*Pseudomonas* quinolone signal (PQS)]. They can diffuse freely across cell membranes and bind intracellularly with corresponding receptor proteins (R proteins).[7] These signaling systems form a complex hierarchical quorum sensing network, where the Las system is considered to be the apex of the hierarchy.[8] Therefore, the LasR receptor is usually considered to be the target for antagonist and agonist development in *P. aeruginosa*.[8,9] For example, a synthetic non-native AHL, QSI-1, was demonstrated to be a potent LasR antagonist.[10] The structurally unrelated mimic of AHL, TP-1, is a highly selective superagonist of the LasR quorum sensing system, while its derivative TP-5 turned into a moderate QS antagonist.[11,12] Moreover, there is evidence showing that *N*-octanoyl homoserine lactone [C8-HSL (Figure 1)] can be produced in the cyanobacterium culture of *Gloeothece* PCC6909, and its accumulation corresponds to a characteristic pattern of autoinduction.[13]

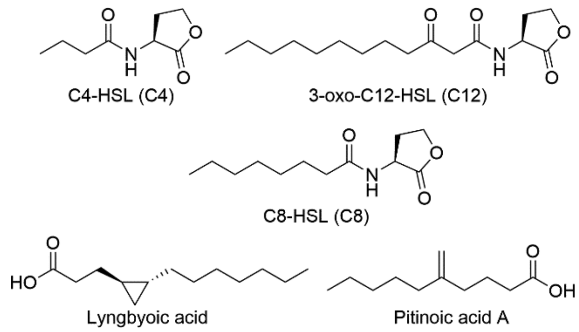


Figure 1. Structures of the endogenous quorum sensing signaling molecules in *P. aeruginosa* (C4-HSL and 3-oxo-C12-HSL), C8-AHL produced in a cyanobacterium culture, and natural quorum sensing inhibitors from marine cyanobacteria, lyngbyoic acid and pitinoic acid A.

Marine cyanobacteria have been a valuable source for the discovery of biologically active and structurally unique natural products, including peptides, polyketides, and peptide−polyketide hybrids.[14] It is noteworthy that marine cyanobacteria also produce various AHL-dependent QS inhibitors.[15] For instance, lyngbyoic acid (Figure 1), a small cyclopropane-

Received: July 19, 2019
Published: August 15, 2019

DOI: 10.1021/acs.orglett.9b02525
Org. Lett. 2019, 21, 7274–7278

containing fatty acid, was isolated from *Lyngbya* cf. *majuscula* and proven to strongly inhibit the activity of LasR.[7] Pitinoic acid A (Figure 1) was also reported to be a *P. aeruginosa* quorum sensing inhibitor.[16] In addition to these QS inhibitors, here we report the isolation, total synthesis, and preliminary biological investigation of a structurally unprecedented QS modulator, doscadenamide A [**1a** (Figure 2)], as a non-HSL QS agonist in a LasR-dependent manner.

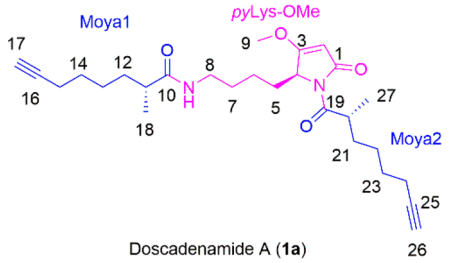


Figure 2. Structure of doscadenamide A (**1a**).

The cyanobacterium *Moorea bouillonii* was collected at Fingers Reef, Guam, and previous investigation of this cyanobacterium led to the isolation of apratoxin A,[17] lyngbyaloside, 2-*epi*-lyngbyaloside, 18*E*-lyngbyaloside C, 18*Z*-lyngbyaloside C,[18] and apratyramide.[19] The cyanobacterial sample was fractionated as described previously,[18] and the isolation was achieved by silica gel column chromatography and several rounds of reversed-phase HPLC to yield doscadenamide A (**1a**) {white solid, $[\alpha]^{20}_{D}$ + 40 (*c* 0.07, MeOH)}. The HRESIMS spectrum of **1a** in the positive mode exhibited a $[M + H]^+$ peak at *m*/*z* 457.3066, which suggested a molecular formula of $C_{27}H_{40}N_2O_4$ with nine degrees of unsaturation. The structure of **1a** was elucidated using a combination of one-dimensional (1D) and two-dimensional (2D) NMR techniques. The ^{1}H and ^{13}C NMR spectra of **1a** in $CDCl_3$ (Figures S1 and S2) indicated the presence of several characteristic signals corresponding to one *O*-methyl group (δ_H 3.85 ppm, δ_C 58.9 ppm), two alkyne groups (δ_H 1.92−1.94 ppm, δ_C 68.5, 68.5, 84.6, 84.7 ppm), one α-proton (δ_H 4.64−4.66 ppm, δ_C 59.2 ppm), two α-methyl groups (δ_H 1.12−1.14 ppm, δ_C 16.3−18.1 ppm), and several methylene groups (δ_H 1.30−1.90, 2.06−2.18 ppm, δ_C 18.4−39.4 ppm). Examination of the 2D NMR spectra [COSY, TOCSY, HSQC, HMBC, and NOESY (Figures S3−S7 and Table S1)] in $CDCl_3$ revealed the structural skeleton of **1a**, which features two linear alkyne amide side chains [Moya1 and Moya2 (Figure 2)] and one pyrrolinone core [*py*Lys-OMe (Figure 2)]. To establish the absolute configuration, a portion of **1a** (2 mg) was treated with ozone at 25 °C for 30 min, followed by oxidative workup and acid hydrolysis (Scheme S1). The hydrolysate was concentrated and partitioned between water and EtOAc. The resulting aqueous phase was analyzed by chiral HPLC-MS revealing the presence of L-Lys, establishing the *S* configuration of C4 at the *py*Lys-OMe moiety. The organic residue was coupled with (*S*)- and (*R*)-phenylglycine methyl ester (PGME)[20] (Scheme S2) to analyze the configuration of the α-methine in the side chain of **1a**. The $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$) for the PGME derivatives (Figure 3) indicated that the configurations of the α-methine in both side chains of **1a** are highly likely *R* and that the overall configuration is 4*S*,11*R*,20*R*. However, during the investigation, we found a minor diastereomer signal in addition to the major NMR signal

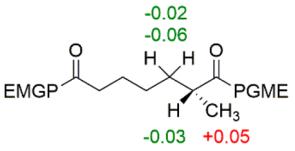
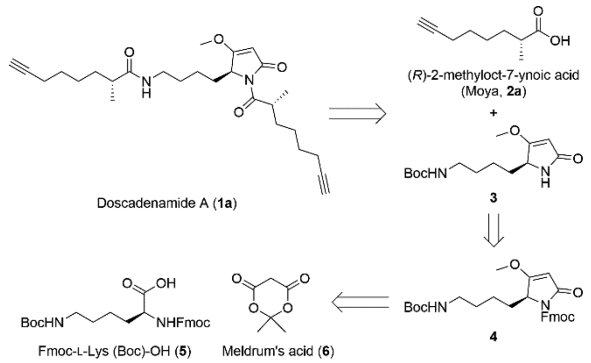


Figure 3. PGME analysis. $\Delta\delta$ ($\delta_S - \delta_R$) values for PGME derivatives of **1a**.

corresponding to the α-methyl group, which could be observed in the COSY spectra of the two PGME derivatives (Figures S8 and S9). To further validate the configuration of **1a** and provide sufficient material for thorough biological investigation, the total synthesis of **1a** was performed.

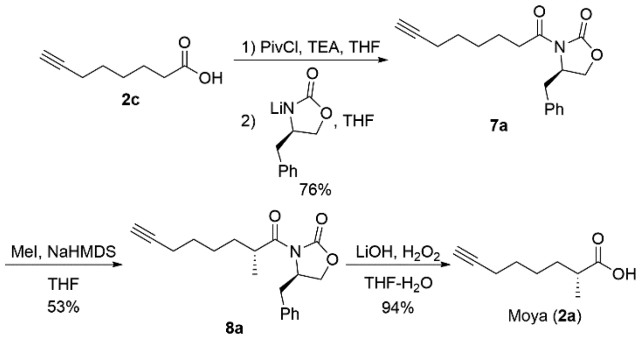
As depicted in Scheme 1, the retrosynthetic analysis of **1a** relied on the disconnection at the two amide linkages between

Scheme 1. Retrosynthetic Analysis of Doscadenamide A (1a)



the pyrrolinone ring and two side chain carboxylic acids, which in the case of **1a** are the same as (*R*)-2-methyloct-7-ynoic acid (Moya, **2a**). The pyrrolinone ring can be obtained via the reaction between the doubly protected amino acid Fmoc-L-Lys (Boc)-OH (**5**) and Meldrum's acid (**6**). As for the synthesis of **2a**, the target compound can be achieved in 14 steps using a method reported in 2005.[21] To improve the efficiency and introduce more flexibility into the production of diverse carboxylic acids with α-substituted alkyl groups, we developed an optimized synthetic method (Scheme 2), in which **2a** can be obtained in three steps in 38% overall yield. The commercially available oct-7-ynoic acid (**2c**) was activated using pivaloyl chloride followed by addition of the lithium salt of the oxazolidinone chiral auxiliary at −78 °C. The resulting **7a** was methylated under conventional conditions to yield **8a** as a single diastereomer.[22,23] Target compound **2a** was

Scheme 2. Optimized Synthesis of Moya (2a)

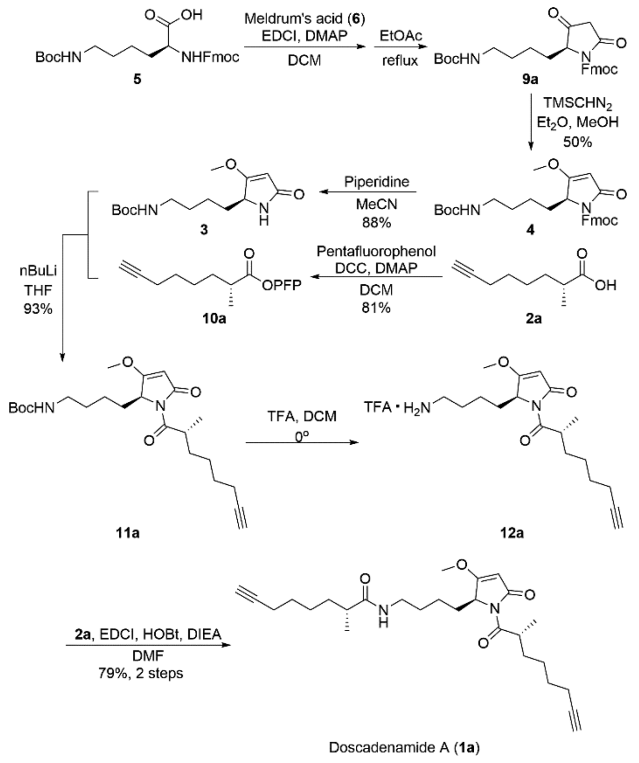


DOI: 10.1021/acs.orglett.9b02525
Org. Lett. 2019, 21, 7274−7278

obtained following alkaline hydrolysis by lithium hydroperoxide.[24]

The total synthesis of **1a** was accomplished using synthetic carboxylic acid **2a** (Scheme 3), including generation of the

Scheme 3. Total Synthesis of Doscadenamide A (1a)



pyrrolinone core and two coupling processes to assemble the structure. Pyrrolidine-2,4-dione **9a** was prepared through condensation of **5** with **6** in the presence of EDCI and DMAP, followed by thermolysis.[25] This intermediate was used in the next step without purification. Conversion of **9a** into its O-methylated derivative **4** was achieved by treatment with trimethylsilyldiazomethane. Subsequently, the *N*-Fmoc protecting group in **4** was removed using piperidine to yield the secondary amide of tetramic acid **3**. The first coupling was accomplished by condensation of the anion derived from deprotonation of **3** by nBuLi and active ester **10a** derived from activation of **2a** by pentafluorophenol to yield **11a**.[26] On the basis of the ^{1}H NMR spectrum of **11a**, there was <5% impurity (Figure S12). In addition, from comparison of the ^{1}H NMR spectra of **11a** and its epimer at C20, **11b**, we found that this impurity may be introduced due to minor epimerization at C20 (Figure S13). After removal of the Boc protecting group with TFA, intermediate **12a** was condensed with **2a** using typical coupling conditions to afford target compound **1a** in six steps in 30% overall yield from **3**. The ^{1}H NMR spectrum of **1a** indicated <5% impurity and that synthetic **1a** had a diastereomeric purity that was higher than that of the natural product doscadenamide A (see the methyl region in Figure 4).

To further validate the configuration of the stereocenters in the side chain, the three other diastereomers [**1b**−**1d** (Figure 4)] of **1a** were also synthesized using the described synthetic method. Similarly, there is <5% impurity shown in the ^{1}H NMR spectra of **1b**−**1d**. Specifically, the absolute configurations of the three stereocenters are 4*S*,11*R*,20*R* for **1a**, 4*S*,11*S*,20*R* for **1b**, 4*S*,11*S*,20*S* for **1c**, and 4*S*,11*R*,20*S* for **1d**. From a comparison of the NMR spectra of all four diastereomers with those of the isolated natural product doscadenamide A (Figure 4 and Figures S10 and S11), the four diastereomers displayed different ^{1}H NMR signals at δ 5.42−5.55 ppm, δ 3.11−3.28 ppm, δ 2.04−2.21 ppm, δ 1.90−1.95 ppm, δ 1.32−1.56 ppm, and δ 1.09−1.22 ppm. Only the ^{1}H and ^{13}C NMR spectra of synthetic **1a** matched those of the natural product. Meanwhile, **1a** and **1b** exhibited virtually the same ^{13}C NMR spectrum as well as the natural product, while **1c** and **1d** displayed ^{13}C NMR spectra different from those of the isolated doscadenamide A (Figure S11). In addition, the optical rotation value of **1a** {$[\alpha]^{20}_{D}$ +54.3 (*c* 0.07, MeOH)} further confirmed that the absolute configuration of synthetic diastereomer **1a** is consistent with the isolated doscadenamide A, verifying the proposed configuration of doscadenamide A.

Doscadenamide A (**1a**) and QS signaling molecule 3-oxo-C12-HSL [C12 (Figure 1)] share structural similarities because they both feature a five-membered ring core and long alkyl side chains. In addition, **1a** superficially resembles tetramic acid derivative compounds, and C12 has been previously shown to undergo rearrangement to form a tetramic acid derivative.[27] Thus, we proposed **1a** may exhibit QS modulatory activities. Our preliminary screening results indicated that **1a** can activate the 3-oxo-C12-HSL-responsive reporter plasmid pSB1075,[28] a plasmid encoding LasR and containing a light-producing *luxCDABE* cassette expressed in *E. coli* (Figure 5A). However, **1a** was not able to activate the related reporter pTIM5319,[7] which is identical to pSB1075 but lacks the AHL-binding site LasR (Figure 5B), which suggests that **1a** activates QS via the AHL-binding site. To validate the activity of **1a**, we tested its effect on wild-type *P. aeruginosa* using activating doses (10, 100, and 1000 μM) based on reporter assay results. Because 10 μM already caused almost maximal induction of the QS pigment pyocyanin production (Figure S15), we selected 10 μM for subsequent comparative biological investigations. After further optimization of the assay condition using a more concentrated cell density, we included the other three diastereomers. As shown in Figure 5C, after treatment with **1a**−**1d**, pyocyanin levels were elevated in response to all diastereomers after 6 h. Our preliminary results indicated that the C11 and C20 configurations are not crucial for QS activation by the doscadenamide scaffold but may play a slight modulatory role. However, the assay is sensitive to cell density, timing, and other factors. In-depth SAR studies are ongoing to understand the molecular basis. The observed agonistic activities may provide a starting point for the development of potential superagonists with new structural skeletons. Those superagonists are expected to artificially regulate virulence factor production such as pyocyanin and activate QS at lower bacterial cell populations and, thus, to stimulate the host immune system to clear the infection when fewer bacterial cells are present.[3]

In summary, we have isolated a new quorum sensing modulating agent, doscadenamide A (**1a**), from a marine cyanobacterium. Its total synthesis has been accomplished through development of an efficient and flexible scheme. Specifically, we developed an efficient method for synthesizing α-alkylated carboxylic acids. The quorum sensing activating activity of **1a** was verified in the Gram-negative bacterium *P. aeruginosa*. Doscadenamide A (**1a**) could serve as a new template for the development of QS superagonists with new

DOI: 10.1021/acs.orglett.9b02525
Org. Lett. 2019, 21, 7274−7278

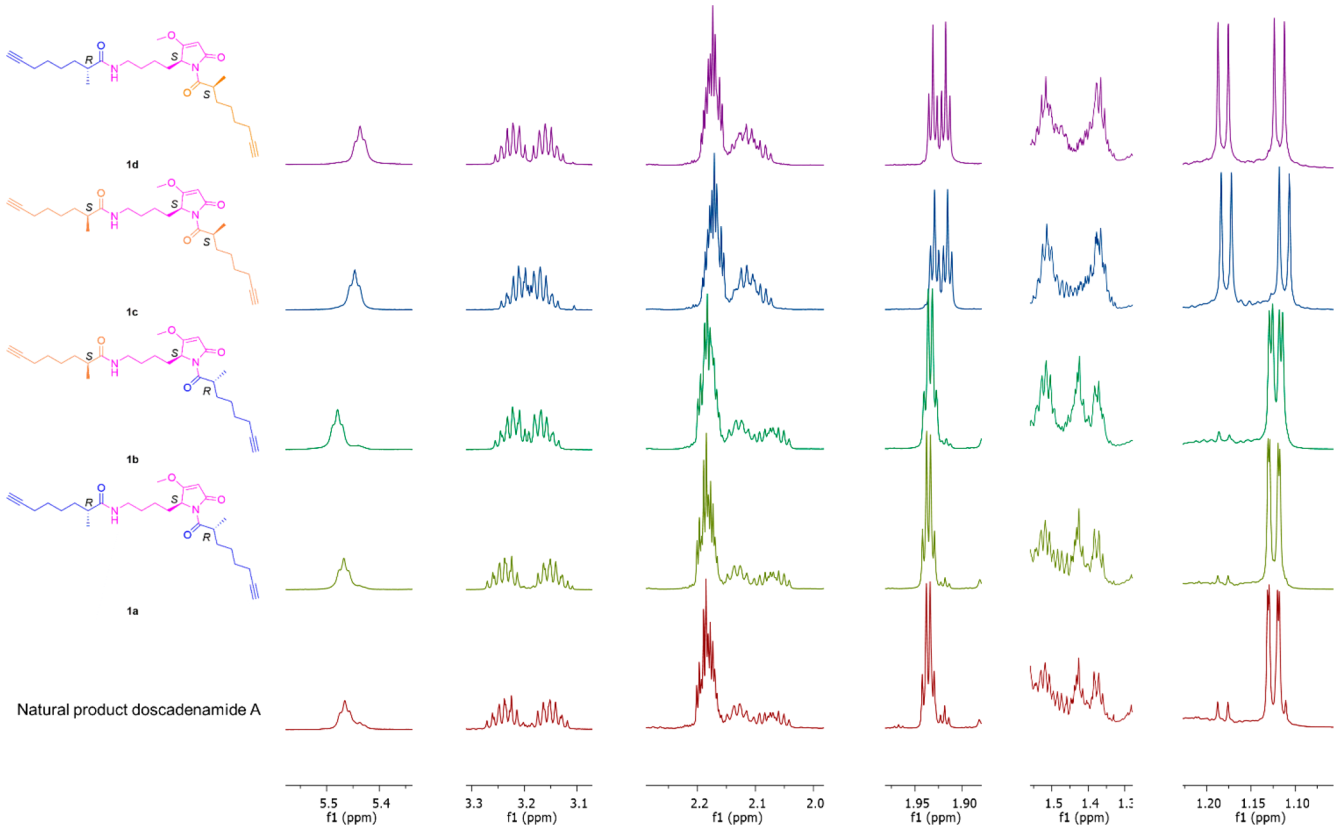


Figure 4. Comparison of selected regional ^{1}H NMR spectra of the isolated natural product doscadenamide A and synthetic diastereomers **1a**−**1d** (maroon, olive, green, navy, and purple bottom to top, respectively) in $CDCl_3$ (600 MHz) at 27 °C, with structures of synthetic diastereomers **1a**−**1d** shown on the left.

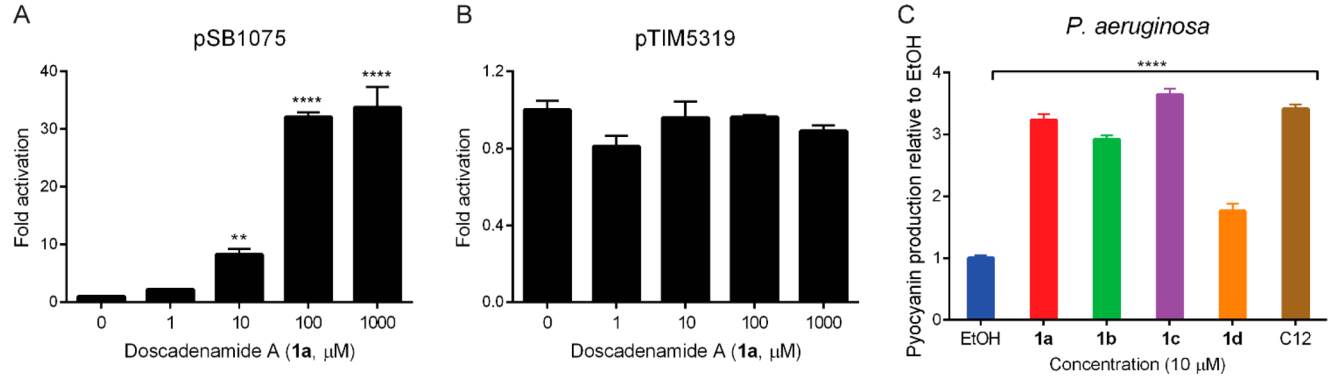


Figure 5. Preliminary determination of the activating activity of doscadenamide A (**1a**) in (A) pSB1075, the *lasR-luxCDABE* reporter constructs expressed in *E. coli*, and (B) related reporter pTIM5319, which lacks a functional AHL-binding domain. The bacterial cultures were treated with **1a** in a dose−response manner and solvent control at 37 °C for 6 h before luminescence was measured. Results are expressed as fold activation compared to the solvent control. Effect of doscadenamide A (**1a**) and its diastereomers (**1b**−**1d**) as well as positive control 3-oxo-C12-HSL (C12) at 10 μM on the (C) production of pyocyanin in wild-type *P. aeruginosa* after being shaken for 6 h at 37 °C. Data are presented as means ± the standard deviation (**P < 0.01; ****P < 0.0001) compared to the solvent control using one-way ANOVA (n = 3).

skeletons to explore the potential of these activators as therapeutics or chemical tools.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b02525.

Experimental Procedures, Table S1, Figures S1−47, including NMR spectra and assay data, and Supplementary References (PDF)

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DOI: 10.1021/acs.orglett.9b02525
Org. Lett. 2019, 21, 7274−7278

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Author Contributions

X.L.: total synthesis, biological experiments with *P. aeruginosa*, experimental design, and manuscript writing. S.M.: isolation and structure elucidation. Q.-Y.C.: supervision of the total synthesis experimental design. J.C.K.: biological evaluation using reporter assays. V.J.P.: sample collection and identification and manuscript editing. H.L.: project supervision, experimental design, and manuscript editing. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): The University of Florida has filed a patent application related to the subject of the manuscript.

■ ACKNOWLEDGMENTS

The authors acknowledge financial support from the National Institutes of Health, National Cancer Institute Grant R01CA172310 and National Institute of General Medical Sciences Grant P41GM086210, and the Debbie and Sylvia DeSantis Chair professorship (H.L.). The authors thank Dr. Max Teplitski (formerly Soil and Water Sciences Department, University of Florida Institute of Food and Agricultural Sciences, Gainesville, FL) for providing reporter strains and support with the reporter assays.

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