Chemistry & Biology
Article

Honaucins A–C, Potent Inhibitors of Inflammation and Bacterial Quorum Sensing: Synthetic Derivatives and Structure-Activity Relationships

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DOI 10.1016/j.chembiol.2012.03.014

SUMMARY

Honaucins A–C were isolated from the cyanobacterium *Leptolyngbya crossbyana* which was found overgrowing corals on the Hawaiian coast. Honaucin A consists of (*S*)-3-hydroxy-γ-butyrolactone and 4-chlorocrotonic acid, which are connected via an ester linkage. Honaucin A and its two natural analogs exhibit potent inhibition of both bioluminescence, a quorum-sensing-dependent phenotype, in *Vibrio harveyi* BB120 and lipopolysaccharide-stimulated nitric oxide production in the murine macrophage cell line RAW264.7. The decrease in nitric oxide production was accompanied by a decrease in the transcripts of several proinflammatory cytokines, most dramatically interleukin-1β. Synthesis of honaucin A, as well as a number of analogs, and subsequent evaluation in anti-inflammation and quorum-sensing inhibition bioassays revealed the essential structural features for activity in this chemical class and provided analogs with greater potency in both assays.

INTRODUCTION

Marine cyanobacteria have emerged as one of the richest sources of biologically active and structurally unique natural products (Tidgewell et al., 2010). To date, there has been a strong focus on the anticancer properties of cyanobacterial natural products, perhaps due in part to funding priorities in biomedical research and in part to a competitive advantage that these cytotoxic metabolites may confer on their producers by deterring grazing (Nagle and Paul, 1999). Regardless, it is likely that continued interrogation of this natural-product-rich group will result in the discovery of compounds with applications in other therapeutic areas, such as management of inflammation, infection, and neurological diseases.

Inflammation, in particular, is an attractive therapeutic target due to its pervasive impacts on human health. In addition to well-known chronic inflammatory disorders such as rheumatoid arthritis and asthma, it is now recognized that many diseases not previously thought to have an autoimmune basis do involve inflammation, including cancer, heart diseases, skin diseases, and disorders of the bowel (Grivennikov et al., 2010; Tousoulis et al., 2011; Cheung et al., 2011). The ability to effectively treat chronic inflammation is thus of great importance from the perspective of both disease prevention and management as well as reduction of health care costs. However, both of the most commonly prescribed anti-inflammatory drug classes, corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs), have undesirable side effects, including hypertension and osteoporosis in the former case and gastrointestinal irritation and renal damage in the latter (Moghadam-Kia and Werth, 2010; Conaghan, 2011). Thus, an unmet medical demand exists for novel anti-inflammatory agents that exert their effects through different modes of action, and the natural products of marine cyanobacteria may offer one possible source of such compounds. Indeed, diverse marine organisms including marine cyanobacteria have already been found to be sources of anti-inflammatory metabolites that operate by novel mechanisms (Terracciano et al., 2006; Gautam and Jachak, 2009; Villa and Gerwick, 2010; Flachsmann et al., 2010).

The ability of small molecules to regulate quorum sensing (QS) among pathogenic microorganisms represents a second relatively unexplored area of drug discovery from marine cyanobacteria. QS is a population-density-based signaling process through which prokaryotes coordinate diverse cellular responses, including initiation of sporulation, swarming, horizontal gene transfer, production or repression of virulence factors and other secondary metabolites (to initiate pathogenic or encourage mutualistic interactions), bioluminescence, and biofilm formation (Pappas and Winans, 2003; Zhang et al., 2002; Ni et al., 2009). QS is regulated by the production and chemoreception of signaling molecules known as "autoinducers." When the density of an organism is sufficient for the concentration of the autoinducer to surpass a threshold, gene expression related to the above physiological responses is triggered (Teng et al., 2011). Because QS can be a factor in the pathogenicity of infectious microorganisms, inhibitors of this process have garnered interest for their potential therapeutic

applications. QS inhibition of pathogenic microbes is especially appealing, because it has the potential to impair the ability of the pathogen to cause disease yet is not overtly lethal and thus is unlikely to lead to the development of resistant phenotypes (Galloway et al., 2011).

Several marine natural products that possess anti-inflammatory properties have been found in marine invertebrates such as corals and sponges, as well as in marine microorganisms (Terracciano et al., 2006; Gautam and Jachak, 2009; Villa and Gerwick, 2010; Flachsmann et al., 2010). With respect to marine microorganisms, which in many cases are likely the actual producers of the anti-inflammatory compounds isolated from invertebrates, it is interesting to speculate that they may have acquired the ability to produce anti-inflammatory compounds in response to evolutionary pressures to overcome the innate immune response mounted by their hosts (Villa and Gerwick, 2010; Ogier et al., 2010; McFall-Ngai et al., 2010).

Recent studies of bacterial communication and host-bacteria interaction reveal that bacterial QS modulators have diverse and sometimes contradictory effects on host cell physiology. Under different circumstances, these can include either inhibition or stimulation of the immune response, suggesting that microbial QS signaling molecules are key modulators of intra- and interkingdom interactions (Rumbaugh and Kaufmann, 2012). However, the exact relationship between bacterial QS and host immunity is still unclear. Therefore, identification of natural products with the capacity to modulate both bacterial QS and host immune responses will provide useful chemical tools for studying the relationships between these two ecological phenomena and new lead molecules for drug discovery.

In these regards, we have evaluated the extracts and metabolites of marine cyanobacteria as potential inhibitors of both the mammalian innate immune system and bacterial QS signaling. As a result, we describe here a group of newly discovered natural products with both of these biological properties, honaucins A–C (1–3). These were isolated from the Hawaiian cyanobacterium *Leptolyngbya crossbyana*, which was found overgrowing coral reefs in the Hōnaunau Bay of Kona (Smith et al., 2008). The structures of honaucins A–C were elucidated by standard spectroscopic techniques and confirmed by total organic synthesis. Each of these new metabolites inhibited nitric oxide (NO) production as well as the expression of selected proinflammatory cytokines in RAW264.7 murine macrophage cells stimulated with lipopolysaccharide (LPS). In addition, these new natural products were found to inhibit QS-signaling-dependent phenotypes in *Vibrio harveyi* BB120 and *Escherichia coli* JB525. Synthesis of a minipanel of honaucin analogs was followed by evaluation in the appropriate bioassays and revealed several critical structural features for biological activity in this structure class. Moreover, this effort resulted in the discovery of honaucin derivatives with improved anti-inflammatory and QS inhibitory properties.

RESULTS

Isolation and Structure Elucidation of Honaucins A–C

The colonial cyanobacterium *Leptolyngbya crossbyana* (HI09-1) was collected in January 2009 by SCUBA from Hōnaunau reef off the island of Hawaii and extracted repetitively with 2:1 CH_2Cl_2-CH_3OH to yield 1.7 g of crude extract. A portion of the organic extract (1.4 g) was subjected to silica gel vacuum-liquid chromatography (VLC) using a stepwise gradient of hexanes/EtOAc/MeOH to produce nine fractions (A–I). Fraction E, eluting with 40% hexanes in EtOAc, was found to potently inhibit production of NO in LPS-stimulated macrophages. This fraction was subjected to bioactivity-guided fractionation using silica Sep-Pak chromatography and reverse-phase high-performance liquid chromatography (RP HPLC) to afford three pure anti-inflammatory compounds, honaucins A (**1**, 5.4 mg, 0.39%), B (**2**, 1.4 mg, 0.10%), and C (**3**, 1.1 mg, 0.08%).

Honaucin A (**1**) was obtained as a colorless oil and high-resolution electrospray ionization mass spectroscopy (HR ESIMS) showed a molecular ion cluster at *m/z* 205.0262/207.0240 at a ratio of 3:1, indicating the presence of one chlorine atom (Figure S1 available online). The molecular formula of **1** was determined as $C_8H_9ClO_4$ by HR ESI time-of-flight (TOF) MS (HR ESITOFMS) ($[M + H]^+$ *m/z* 205.0262), and the infrared (IR) spectrum displayed absorption bands at 1784 and 1724 cm^{-1} for the presence of γ-lactone and α,β-unsaturated ester functionalities. The ^1H and ^{13}C NMR spectra of **1** displayed two downfield quaternary carbons (δ_C 174.5 and 165.0), two olefinic protons (δ_H 7.06 and 6.13), an *O*-substituted methine (δ_H 5.53), and an *O*-substituted methylene (δ_H 4.55 and 4.42) (Table 1; Figure S1). NMR data from gradient correlation spectroscopy (gCOSY), heteronuclear single-quantum coherence, and heteronuclear multiple-bond coherence (gHMBC) experiments allowed construction of β-*O*-substituted γ-butyrolactone and 4-chlorocrotonic acid moieties. Finally, a gHMBC correlation from H-3 to C-1′ revealed a connection between these partial structures, thus providing the planar structure of honaucin A (**1**) (Figure 1).

Honaucin B (**2**) showed an isotope pattern similar to that of **1** by positive-ion HR ESITOFMS ($[M + Na]^+$ *m/z* 273.0499), indicating a molecular formula of $C_{10}H_{15}ClO_5$ (Figure S2). The IR spectrum of **2** displayed absorption bands at 3454, 1724, and 1659 cm^{-1}, indicating the presence of alcohol, α,β-unsaturated ester, and isolated ester functionalities, respectively. The ^1H and ^{13}C NMR spectra of **2** showed several resonances similar to those in **1** (Table 1; Figure S2), thus confirming the presence of 4-chlorocrotonic acid and a 3,4-di-*O*-substituted butanoic acid moiety (Figure 1). A gHMBC correlation from H-4 to C-1′ indicated an ester linkage between these positions, whereas a correlation from H-5 to C-1 indicated that an ethyl group was linked to C-1 via an ester bond.

Honaucin C (**3**, *m/z* 259.0346 for $C_9H_{13}ClO_5$) also displayed an isotope pattern consistent with one chlorine atom by positive-ion HR ESITOFMS (Figure S3). The one- and two-dimensional (2D) NMR spectra of **3** were almost identical to those of **2**, although the methyl triplet (H_3-6, δ_H 1.28) and the methylene (H_2-5, δ_H 4.18) in the ethyl group in **2** were replaced by a methyl singlet (H_3-5, δ_H 3.74) in **3** (Table 1; Figure S3). Finally, gHMBC correlations from H-5 to C-1 and from H-4 to C-1′ led to the planar structure of honaucin C (**3**).

The absolute configuration at C-3 in **1** was determined as *S* by comparing the optical rotation of **1** ($[\alpha]_D^{22}$ −80.5) with literature values of (*R*/*S*)-3-hydroxy-γ-butyrolactone

Table 1. NMR Spectroscopic Data for Honaucin A–C in $CDCl_3$

	Honaucin A (**1**)				Honaucin B (**2**)				Honaucin C (**3**)	
Position	δ_C, Multi	δ_H, Multi (*J* in Hz)	COSY	HMBC[a]	δ_C, Multi	δ_H, Multi (*J* in Hz)	COSY	HMBC[a]	δ_C, Multi	δ_H, Multi (*J* in Hz)
1	174.5, qC				172.2, qC				172.2, qC	
2a	34.8, CH_2	2.91, dd (18.5, 6.7)	2b, 3	1, 4	38.0, CH_2	2.57, s	3	1, 3	38.0, CH_2	2.59, s
2b		2.67, d (18.5)	2a	1, 3, 4		2.56, d (3.46)	3			2.57, d (2.82)
3	70.3, CH	5.53, t (5.7)	2a, 4a	1, 4, 1′	66.5, CH	4.32, m	2, 4	2, 4	66.5, CH	4.31, m
3-OH						3.14, brs		4		3.08, brs
4a	73.1, CH_2	4.55, dd (11.1, 4.7)	3, 4b		67.4, CH_2	4.22, m	3	2, 3	67.4, CH_2	4.21, m
4b		4.42, d (11.1)	4a	1, 2, 3						
5					61.3, CH_2	4.18, m	6	1	61.3, CH_3	3.74, s
6					14.4, CH_3	1.28, t (6.70)	5	5		
1′	165.0, qC				165.7, qC				165.7qC	
2′	122.9, CH	6.13, d (15.4)	3′, 4′	1′, 3′, 4′	123.6, CH	6.16, d (15.4)	3′, 4′	1′, 3′, 4′	123.6, CH	6.16, d (15.2)
3′	144.1, CH	7.06, dt (15.4, 5.9)	2′, 4′	1′, 2′, 4′	142.9, CH	7.04, dt (15.3, 5.9)	2′, 4′	1′, 2′, 4′	142.9, CH	7.03, dt (15.3, 5.8)
4′	42.5, CH_2	4.20, d (5.9)	3′	1′, 2′, 3′	42.6, CH_2	4.19, m	3′	2′, 3′	42.6, CH_2	4.16, m

Measured at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR.
[a]From ^1H to the indicated ^{13}C.

(Uchikawa et al., 1988). This assignment was confirmed by total synthesis of honaucin A, as well as its C-3 epimer (**17**). Synthetic (−)-honaucin A was prepared by Steglich esterification of 4-chlorocrotonic acid (**7**) and (*S*)-3-hydroxy-γ-butyrolactone (**4**) (see Supplemental Experimental Procedures). Similarly, *epi*-honaucin A (**17**) was synthesized from 4-chlorocrotonic acid (**7**) and (*R*)-3-hydroxy-γ-butyrolactone (**5**). Synthetic honaucin A was found to have a specific rotation of −77.5, similar to that of the natural compound, whereas *epi*-honaucin A (**17**) showed a specific rotation of +66.0. The absolute configuration at C-3 in metabolites **2** and **3** was also determined as *S* by comparing the specific rotations of **2** and **3** with the literature value for the 4-*O*-tritylated derivative of ethyl (*S*)-3,4-dihydroxybutanoate (Prasad et al., 1990).

Anti-Inflammatory Activity of Honaucins A–C

The degree of NO production in LPS-stimulated macrophages provides a measurement of induced inflammation via the activation of TLR4, followed by intracellular signaling via the MyD88-dependent and MyD88-independent pathways and the subsequent activation of the inducible nitric oxide synthase gene (iNOS). We exploited these pathways in an initial phenotypic screen to assess the anti-inflammatory potential of honaucins A–C (**1**–**3**). NO production by LPS-stimulated RAW264.7 murine macrophages was inhibited by compounds **1**–**3** with half-maximal inhibitory concentration (IC_{50}) values of 4.0, 4.5, and 7.8 μM, respectively. By contrast, the IC_{50} value for the cytotoxicity of honaucin A in the cells was greater than 49 μM, giving a greater than 10-fold therapeutic window between its NO inhibitory effect and cytotoxicity (Figure 2A; Table 2).

To determine whether decreased NO production in the presence of honaucin A (**1**) was due to changes at the mRNA level or through radical oxygen scavenging, the antioxidant activity of honaucin A was evaluated. However, no antioxidant activity was observed up to 146 μM (Figure S4). Quantitation of the transcript levels of key proinflammatory cytokines TNF-α, IL-1β, IL-6, and iNOS in honaucin-A-treated versus untreated LPS-stimulated RAW 264.7 cells was accomplished by quantitative real-time polymerase chain reaction. Cells treated with compound **1** had reduced transcript levels of all four cytokines after 6 hr of treatment (reduction in transcripts of TNF-α, IL-1β, IL-6 and iNOS were −9.3, −154.6, −19.0, and −11.8 fold, respectively; Figure 2B).

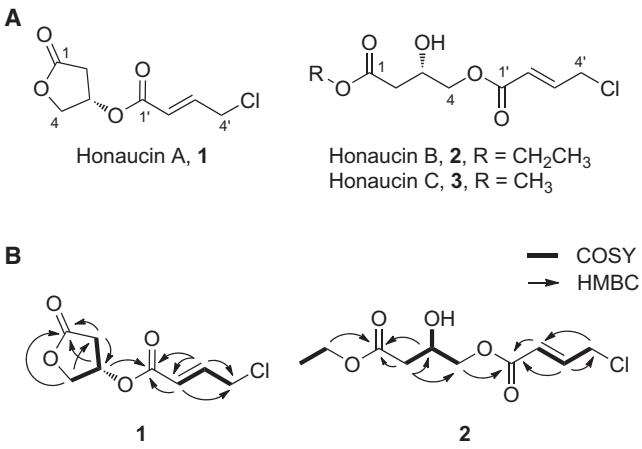

Figure 1. Structures of Honaucins A–C
(A) Structures of honaucins A–C (**1**–**3**).
(B) Structure elucidation of **1** and **2** based on NMR data.
See also Figures S1, S2, and S3.

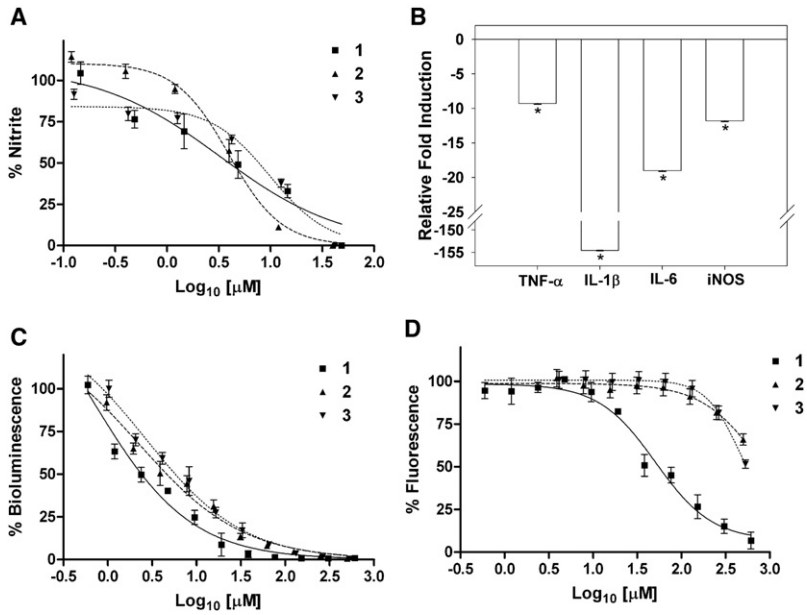

Figure 2. Biological Properties of Honaucins A–C

Error bars indicate the mean ± SD for n = 3.

(A) Inhibition of LPS-stimulated NO production on murine macrophages.

(B) Downregulation of proinflammatory cytokines in LPS-stimulated murine macrophages by honaucin A (*$p < 0.002$ compared to LPS treatment alone).

(C) Inhibition of QS signaling in *V. harveyi* BB120.

(D) Inhibition of QS signaling in *E. coli* JB525.

See also Figure S4.

Inhibition of QS Phenotypes by Honaucins A–C

Honaucins A–C (**1**–**3**) were also screened for inhibition of QS-regulated phenotypes in two different bacterial species, in part due to the structural resemblance of honaucins A–C to other small lactone microbial signaling molecules. The wild-type strain *V. harveyi* BB120 uses QS to regulate various phenotypes, including bioluminescence (Henke and Bassler, 2004). Chemical communication by *V. harveyi* is accomplished via three parallel signaling pathways that utilize the autoinducers HA1-1, CAI-1, and AI-2, respectively (Henke and Bassler, 2004). In the HAI-1 system, membrane-bound histidine kinase (LuxN-type) proteins detect the native autoinducer 3-hydroxybutanoyl-L-homoserine lactone. These are detected on the cell surface and information is relayed into the cell via an intracellular signaling cascade (Freeman et al., 2000; Swem et al., 2008, 2009). Genetic knockouts of the HAI-1 system display a 99.9% reduction in bioluminescence (Henke and Bassler, 2004). All three natural honaucins (**1**–**3**) demonstrated a dose-dependent inhibition of bioluminescence with IC_{50} values of 5.6, 17.6, and 14.6 μM, respectively (Figure 2C), thus indicating the capacity of the honaucins to antagonize this QS system. The γ-butyrolactone functionality is not an essential feature for inhibition of QS-mediated bioluminescence in *V. harveyi* BB120, although it does have an impact on potency.

A second reporter strain, *E. coli* strain JB525, was used to further test for anti-QS activity of the honaucins (Andersen et al., 2001). This strain has been engineered to produce the *V. fischeri* LuxR receptor for acyl homoserine lactones (AHLs). In contrast to the HAI-1 system of *V. harveyi* where the AHL binds to a membrane bound receptor, the AHL in *V. fisheri* diffuses into the cell to bind its cytoplasmic receptor for QS initiation (Andersen et al., 2001; Swem et al., 2009). Once activated by exogenously supplied autoinducer (3-oxo-hexanoyl homoserine lactone, OHHL), LuxR positively affects expression of a *luxI* promoter that initiates production of an unstable green fluorescent protein (GFP). Thus, measurement of GFP fluorescence reflects the quantity of LuxR with bound AHL (Andersen et al., 2001). Honaucin A (**1**) inhibited production of GFP in *E. coli* JB525 in a dose-dependent manner with an IC_{50} value of 38.5 μM (Figure 2D). Honaucins B (**2**) and C (**3**) were essentially inactive in this assay (IC_{50} values are greater than 500 μM), indicating the importance of the γ-butyrolactone pharmacophore in antagonism of QS-regulated GFP production in *E. coli* JB525. No growth inhibitory effects were observed for metabolites **1–3** in either assay at the highest concentrations tested over 500 μM.

Structurally Modified Honaucin A Derivatives

Within its relatively small structure, honaucin A (**1**) possesses three distinct functional groups; an (*S*)-3-hydroxy-γ-butyrolactone, an esterified α,β-unsaturated carboxylate, and a primary halogen atom (Figure 3A). The γ-butyrolactone is a conserved functional group in AHLs, the primary class of QS molecule in Gram negative bacterial signaling. Previous efforts to develop synthetic inhibitors of AHL-mediated QS signaling have mostly retained the homoserine lactone feature (Lowery et al., 2010). However, to our knowledge β-hydroxy-γ-butyrolactones have not previously been evaluated for QS inhibition or anti-inflammatory activity. Similarly, although Gershon et al. (1976) assayed a series of 4-substituted crotonic acid esters for antifungal activity, this structural moiety and related derivatives have not been examined for QS-inhibitory or anti-inflammatory activities.

Thus, with the goal of identifying structural features responsible for the anti-inflammatory and QS-inhibitory activities of the honaucins, we prepared analogs of the subunits forming the honaucins and then combined these in a systematic fashion. Modifications included alterations to the configuration and position of the hydroxy substituent on the γ-butyrolactone ring (**4**–**6, 16**–**17**), presence and position of the double bond, carbon chain length and nature of the halogen atom in the crotonic acid portion (**7**–**15, 18**–**26**) (Figure 3C). The resulting analogs were then assayed in the QS inhibitory and anti-inflammatory assays (Table 2).

Anti-Inflammatory Activities of Synthetic Honaucin A Derivatives

Of the three structural subunits representing the lactone portion, (*S*)-3-hydroxy-γ-butyrolactone (**4**), (*R*)-3-hydroxy-γ-butyrolactone (**5**), and (*R*/*S*)-2-hydroxy-γ-butyrolactone (**6**), the fragment representing natural honaucin A (**1**) was the most

Table 2. Biological Activities of Natural and Synthetic Honaucin A Derivatives

Compounds	NO Inhibition (IC_{50} ± SD[a], μM)	p Values for IC_{50} Values from NO Inhibition Compared to Compound **1**	Cell Survival at IC_{50} of NO Inhibition (%)	Cytotoxicity in Raw264.7 Cells (IC_{50}, μM)	QS Inhibition (IC_{50} μM)	
					V. harveyi	*E. coli*
1	4.0 ± 2.3		100	>49	5.6	38.5
2	4.5 ± 1.0	0.7473	96.8	16	17.6	908
3	7.8 ± 1.4	0.0709	85.2	30	14.6	576
4	21.0 ± 10.8	0.0560	100	>98	–[d]	–[d]
5	70.7 ± 15.7	0.0019	100	>98	–[d]	–[d]
6	–[b]	–[c]	–[b]	>98	–[d]	–[d]
7	28.0 ± 4.3	0.0010	95.5	80	72.5	152
8	–[b]	–[c]	–[b]	>81	–[d]	–[d]
9	–[b]	–[c]	–[b]	>116	–[d]	–[d]
10	–[b]	–[c]	–[b]	>116	–[d]	–[d]
11	–[b]	–[c]	–[b]	>100	–[d]	–[d]
12	55.8 ± 5.9	0.0001	95.0	>67	–[d]	–[d]
13	11.6 ± 4.3	0.0540	100	>288	167	>304
14	3.5 ± 0.5	0.7316	94.7	35	10.5	–[e]
15	1.2 ± 0.2	0.1036	96.4	10	1.2	–[e]
16	9.7 ± 3.6	0.0819	80.0	56.5	–[d]	–[d]
17	39.3 ± 3.7	0.0001	94.0	>49	22.1	260
18	19.6 ± 5.3	0.0015	100	>49	15.9	221
19	–[b]	–[c]	–[b]	>49	–[d]	–[d]
20	20.8 ± 3.3	0.0019	100	>54	–[d]	–[d]
21	–[b]	–[c]	–[b]	>59	–[d]	–[d]
22	–[b]	–[c]	–[b]	>59	–[d]	–[d]
23	6.2 ± 1.5	0.2375	100	>43	–[d]	–[d]
24	4.3 ± 0.6	0.8377	85.0	128	168	342
25	1.5 ± 0.0	0.0001	91.7	22	0.24	0.51
26	0.9 ± 0.1	0.0801	100	23	0.13	0.70

[a]Standard deviation was calculated from three technical replicates.
[b]No activity was observed with the compound up to the highest concentration tested (30 μg/ml).
[c]Inhibition of NO production in LPS-stimulated Raw 264.7 cells did not reach to 50% at the highest test concentration (30 μg/ml), and it was not possible to calculate the p value.
[d]No activity was observed with the compound up to the concentration of 125 μg/ml.
[e]Due to the antibiotic effects of these compounds, their IC_{50} values could not be reliably measured.

potent (IC_{50} of **4** = 21.0 μM, Table 2; Figure 4A). Both the configuration and position of the hydroxy group in the lactone moiety are important to the anti-inflammatory potency of these γ-butyrolactones. The second subunit, 4-chlorocrotonic acid (**7**), showed modest anti-inflammatory activity by itself (IC_{50} = 28.0 μM, Table 2; Figure 4B). The chain extended derivative (**12**) showed similar activity to **7**, whereas modification of the conjugated double bond of 4-chlorocrotonic acid (**8**, **10**, **11**) significantly decreased anti-inflammatory activity. The inhibition of NO production by compounds **8**, **10**, and **11** in LPS-stimulated Raw 264.7 cells did not reach to 50% at the highest test concentration (30 μg/ml), so their IC_{50} values could not be determined. The presence of a halogen atom at C-4 of crotonic acid (**9**, **13**–**15**) also led to a dramatic change in anti-inflammatory activity. While crotonic acid (**9**) displayed no activity up to 116 μM, 4-fluorocrotonic acid (**13**), 4-bromocrotonic acid (**14**), and 4-iodocrotonic acid (**15**) were potent NO inhibitors with IC_{50} values of 11.6 μM, 3.5 μM, and 1.2 μM, respectively (Table 2; Figure 4C). Therefore, the conjugated double bond and halogen atom present in the carboxylic acid moiety appear to be quite important to the anti-inflammatory properties of the honaucins.

In synthetic analogs of honaucin A containing both the γ-butyrolactone and crotonic acid portions, modifications to the point of attachment between the two subunits [*epi*-honaucin A (**17**) and 2-hydroxy honaucin A (**18**)] generally led to less potent molecules. As shown for compounds **8**, **10**, and **11**, most modifications (**19, 21**–**22**) of the double-bond portion of the 4-chlorocrotonic acid moiety of honaucin A resulted in decreased anti-inflammatory activity, and they did not inhibit 50% NO production in LPS-stimulated murine macrophages at the highest concentration tested (30 μg/ml). The exception to this trend was substitution of the halogen atom; 4′-bromohonaucin A (**25**) and 4′-iodohonaucin A (**26**) exhibited improved

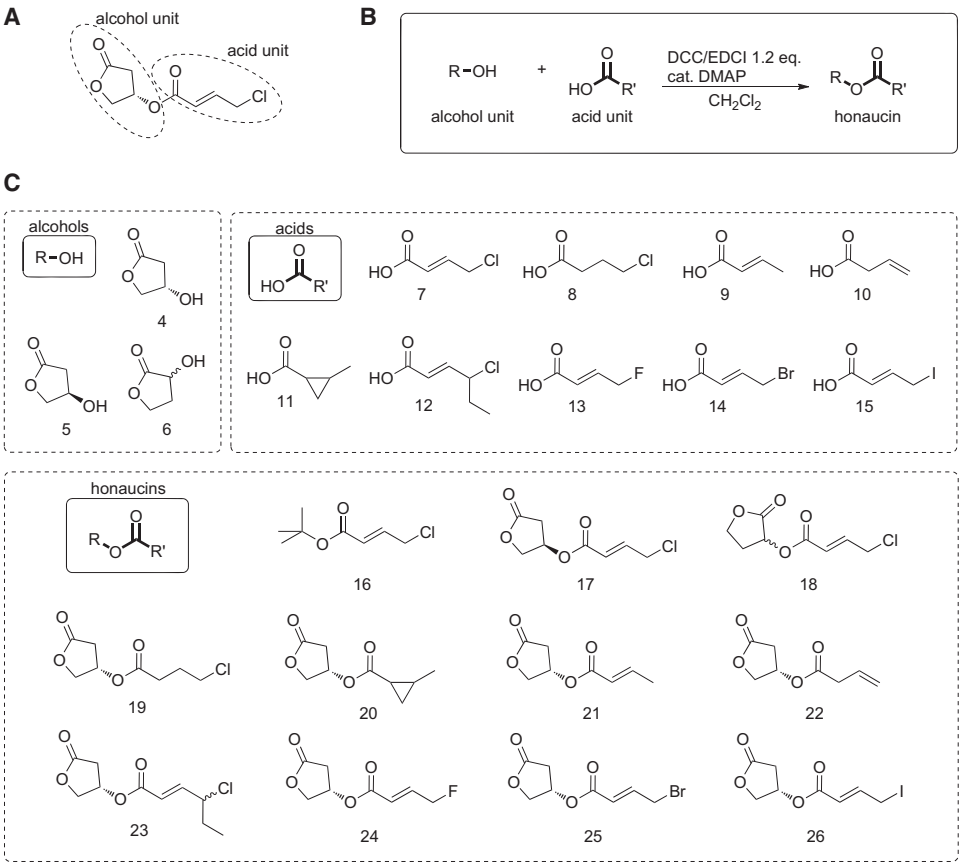

Figure 3. Synthesis of Honaucin A and Its Synthetic Analogs

(A) The two building units of honaucin A (**1**).

(B) Synthetic scheme of honaucin A and its analogs.

(C) Synthetic honaucin A analogs and their building units.

anti-inflammatory activities with IC_{50} values of 1.5 μM and 0.9 μM, respectively (Table 2; Figure 4D).

Inhibition of QS Phenotypes by Synthetic Analogs of Honaucin A

The QS-mediated antagonism of *V. harveyi* bioluminescence by the naturally occurring analogs honaucins B (**2**) and C (**3**) indicated that the butyrolactone functional group was not necessary for activity. The various γ-butyrolactones (**4**–**6**) by themselves showed no inhibitory activity in either the *V. harveyi* BB120 or *E. coli* JB525 assays, indicating that this moiety by itself was also not sufficient for QS antagonism (Table 2). By contrast, the 4-chlorocrotonic acid (**7**) subunit retained partial inhibitory activity of *V. harveyi* bioluminescence with an IC_{50} of 72.5 μM.

However, modification of the halogen atom at the 4-position of the crotonic acid fragment greatly modulated QS-inhibitory activity. While 4-fluorocrotonic acid (**13**) had decreased inhibitory activity against *V. harveyi* BB120 (IC_{50} = 167 μM), the 4-bromocrotonic acid fragment (**14**) showed a marked increase in activity (IC_{50} = 10.5 μM), and 4-iodocrotonic acid (**15**) exhibited a 60-fold increase in activity (IC_{50} = 1.2 μM) in comparison with 4-chlorocrotonic acid (**7**). However, it is noteworthy that both **7** and **14** were mildly antibiotic at high concentrations (antibiotic effects were observed at 14- and 72-fold-greater concentrations, when comparing the minimum inhibitory concentration (MIC) of growth-inhibitory effects to QS-inhibitory IC_{50} values, respectively). The most potent crotonic acid fragment, **15**, showed no growth-inhibitory effects up to 32 μM.

This same trend was observed in the inhibition of GFP production by *E. coli* JB525. 4-Chlorocrotonic acid (**7**) inhibited GFP production with an IC_{50} of 152 μM, while 4-fluorocrotonic acid (**13**) was more than 2-fold less active. The brominated and iodinated analogs (**14** and **15**) were the most potent, but antibiotic effects (MIC = 188 and 18 μM, respectively) precluded confident measurement of their IC_{50} concentrations.

Of derivatives containing both core moieties of the honaucins, 3-*epi*-honaucin A (**17**) and 2-hydroxy honaucin A (**18**) were 3- to 4-fold less active than honaucin A against both sensor strains, suggesting that the 3*S* attachment represents the optimal position and configuration. Halogen substitution had a dramatic effect on the QS inhibitory activity of the intact honaucin A analogs (Figure 5). Against *V. harveyi*, the fluorinated derivative (**24**) was 30-fold less potent, whereas the brominated and iodinated analogs (**25** and **26**) were an impressive 23- and 43-fold more active (IC_{50} = 0.24 μM and 0.13 μM), respectively. A similar trend was observed against the *E. coli* JB525 sensor

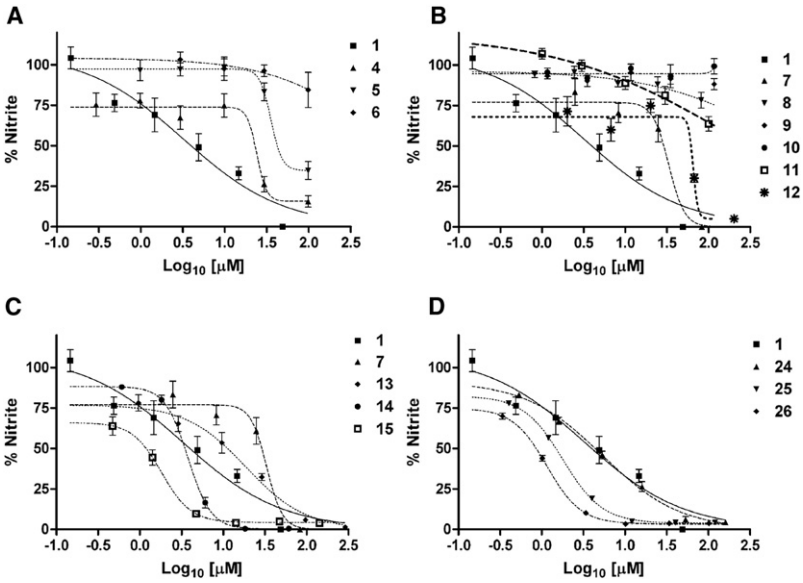

Figure 4. Inhibition of LPS-Stimulated Production of NO in Murine Macrophages by Honaucin A Analogs and Their Structural Units

Error bars indicate the mean ± SD for n = 3.

(A) Inhibition of LPS-stimulated NO production by **1** compared to hydroxy-γ-butyrolactones **4**–**6**.

(B) Inhibition of LPS-stimulated NO production by **1** compared to carboxylic acids **7**–**12**.

(C) Inhibition of LPS-stimulated NO production by **1** compared to 4-halogenated crotonic acids **7** and **13**–**15**.

(D) Inhibition of LPS-stimulated NO production by **1** compared to selected honaucin A derivatives **24**–**26**.

See also Figure S5.

strain; **24** was 9-fold less potent than **1**, while **25** and **26** possessed IC_{50} concentrations of 0.51 μM and 0.70 μM (76- and 55-fold more active), respectively. Of derivatives containing both core moieties, antibacterial effects were only observed for compounds **25** and **26**, which only modestly affected bacterial growth in both assays (MIC = 251 μM and 422 μM, respectively, against *E. coli* JB525; MIC = 125 μM and 105 μM, respectively, against *V. harveyi* BB120).

DISCUSSION

Structure-Activity Relationships of the Honaucins

Anti-inflammatory and QS-inhibition assay of the naturally occurring honaucins and their synthetic analogs indicated that each of the functional groups comprising the honaucins is critical for anti-inflammatory and QS-inhibition activity, with the exception that the chlorine atom can be substituted with either bromine or iodine, but not fluorine, and retain high potency. Most modifications to the ring structure, such as the position and configuration of the substitutions on the lactone ring, as well as the absence of a conjugated double bond or halogenation in the crotonic acid fragment, decreased activity in both assay systems (Table 2). Exceptions to these trends were 4′-bromohonaucin A (**25**) and 4′-iodohonaucin A (**26**), which both showed potent anti-inflammatory and QS-inhibitory activities without appreciable toxicity. Their potency, compared to that of honaucin A (**1**), was increased nearly 30-fold in the QS inhibition and 3- to 5-fold in the anti-inflammatory assay. The correlation of biological activity with the ease of displacement of the halogen leaving group suggests that the activity of the honaucins may involve nucleophilic reactions at C-4′. However, inconsistent with this hypothesis, both 4-fluorocrotonic acid (**13**) and 4′-fluorohonaucin A (**24**), in which the fluorine atoms are not easily displaced, showed similar or more potent anti-inflammatory activities than 4-chlorocrotonic acid (**7**) or honaucin A (**1**), respectively. This suggests that honaucin inhibition of the innate immune response occurs by mechanisms other than, or in addition to, halogen-atom nucleophilic displacement. 4′-Iodohonaucin A (**26**) was the most active compound among this series of honaucins. However, it was found to be quite labile, presumably through a facile displacement of the iodine atom, and therefore, 4′-bromohonaucin A (**25**) may be the most promising candidate for further development.

Honaucin A (**1**) displayed much more potent anti-inflammatory and QS inhibitory activities than either of its active component fragments, (*S*)-3-hydroxy γ-butyrolactone (**4**) and 4-chlorocrotonic acid (**7**). These results suggest that a synergism is achieved when these two structural units are combined within a single molecule, and it is therefore conceivable that the honaucins have resulted through an evolutionary process that is conceptually similar to fragment-based drug design.

Honaucin A and Inhibition of Quorum Sensing

Intraspecies QS in Gram negative bacteria is most commonly regulated by AHLs (Galloway et al., 2011). Honaucin A is structurally similar to the AHLs, retaining both the γ-butyrolactone and a linear acyl side chain. However, the small structural alterations present in the honaucins appear to modulate their binding characteristics in such a way that they act as antagonists, rather than inducers, of QS-regulated phenotypes.

A handful of QS antagonists have been isolated from various marine organisms (Clark et al., 2008; Dobretsov et al., 2010; Givskov et al., 1996; Kwan et al., 2010, 2011; Teasdale et al., 2009, 2011), and four such inhibitors have been previously isolated from marine cyanobacteria. However, the honaucins are chemically distinct from each of these. Lyngbyoic acid, isolated from *Lyngbya* cf. *majuscula,* modestly inhibited QS in *P. aeruginosa*, reducing elastase and pyocyanin production (Kwan et al., 2011). The tumonoic acids obtained from *Blennothrix cantharidosmum* also showed modest activity, inhibiting bioluminescence production in *V. harveyi* with an IC_{50} of 62 μM (Clark et al., 2008). Malyngamide C and 8-*epi*-malyngamide C moderately inhibited LasR-based QS in *P. aeruginosa* (Kwan et al., 2010). Finally, malyngolide which was obtained from *Lyngbya majuscula*, modestly inhibited (IC_{50} = 110 μM) the production of violacein, a QS-regulated phenotype in *Chromobacterium violaceum* (Dobretsov et al., 2010). In comparison, honaucins A–C are considerably more potent in their inhibition of *V. harveyi*-regulated bioluminescence, with IC_{50}s ranging from 5.6 to 17.6 μM. Furthermore, honaucin

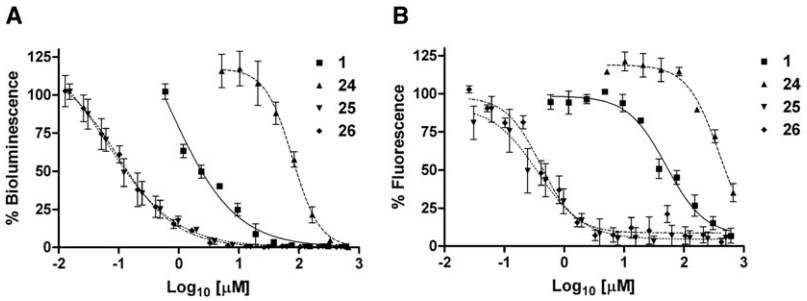

Figure 5. Inhibition of QS Signaling by Honaucin A and Three of Its Derivatives

Error bars indicate the mean ± SD for n = 3.

(A) Inhibition of QS-signaling phenotype in *Vibrio harveyi* BB120.

(B) Inhibition of QS-signaling phenotype in *Escherichia coli* JB525.

Honaucin A, **1**; 4′-Fluorohonaucin A, **24;** 4′-Bromohonaucin A, **25**; 4′-Iodohonaucin A, **26.**

See also Figure S5.

A inhibited the *V. fischeri* LuxI/R QS pathway in *E. coli* JB525 with an IC_{50} of 38.5 μM. Together, these results suggest that honaucin A inhibits AHL-mediated QS pathways, but further studies will be required to clarify the precise molecular mechanism of action of this new metabolite class.

Honaucin A and Inhibition of Eukaryotic Inflammation

Honaucin A and its brominated and iodinated analogs inhibited NO production in LPS-stimulated macrophages with IC_{50}s ranging from 0.8 to 4.0 μM. In addition to their potent biological activity and modest cytotoxicity, these molecules are small, structurally concise, and synthetically tractable. These qualities present the honaucins as attractive potential anti-inflammatory drug leads.

There is considerable need to develop new anti-inflammatory treatments that have alternative molecular targets compared to the more traditional agents, which target cyclooxygenases (COX) 1 and 2. Many patients are allergic to, or suffer adverse side effects from, COX inhibitors, limiting their treatment options (Farooque and Lee, 2009; Sánchez-Borges et al., 2004; Lanas, 2010). While the molecular target of the honaucins is yet to be identified, continuing efforts are underway to define the precise mechanism of action for these new modulators of inflammation.

Quorum-Sensing Inhibition and Anti-Inflammatory Activity

The honaucins and several of their synthetic analogs were potent inhibitors of both bacterial QS and NO production by LPS-stimulated macrophages. These two bioactivities may be mechanistically related. A scatter plot of these two activities of the honaucins displayed a positive correlation (Figure S5). Ours is not the first report of a biomolecule having dual involvement in QS and inflammation. Kravchenko et al. (2008) observed attenuated inflammation in activated bone-marrow-derived macrophages in the presence of the *P. aeruginosa* QS signaling molecule *N*-(3-Oxododecanoyl)-L-homoserine lactone. This compound was found to exert anti-inflammatory effects by impairing the activation of NF-κB-dependent gene expression. However, not all accounts describe attenuation of the immune response; there have also been reports of immune activation by inhibition of the transcriptional activity of peroxisome-proliferator-activated receptor gamma (PPARγ) (Jahoor et al., 2008; Cooley et al., 2010; Teplitski et al., 2011). This discrepancy may be explained by a biphasic role for AHLs in immunomodulation in which they exert anti-inflammatory effects at low concentrations and stimulate inflammation at higher concentrations (Hughes and Sperandio, 2008).

Manoalide is another example of a natural product that is a QS inhibitor and possesses anti-inflammatory properties. The sesterpene metabolite, originally isolated from the Indo-Pacific sponge *Luffariella variabilis* (de Silva and Scheuer, 1980), was found to exert anti-inflammatory effects through an irreversible inhibition of PLA_2, as well as antimicrobial activity through a strong inhibition of QS signaling. This latter activity was observed in both a promiscuous LuxR-regulated QS system cloned into *E. coli* and a *P. aeruginosa*-specific QS screen (Skindersoe et al., 2008). Thus, a commonality in the signaling pathways for prokaryotic QS and eukaryotic inflammation is potentially responsible for the pleiotropic effects of the honaucins and their analogs (Jahoor et al., 2008), a phenomenon described as ''evolutionary molecular modeling''(Wink, 2003).

SIGNIFICANCE

Three structurally novel metabolites, honaucins A–C (1–3), were isolated from the bloom-forming cyanobacterium *Leptolyngbya crossbyana* and show potent anti-inflammatory and quorum-sensing (QS) inhibitory properties. Honaucin A (1) consists of two distinct structural units, (*S*)-3-hydroxy-γ-butyrolactone and 4-chlorocrotonic acid. In honaucin B (2), the lactone of honaucin A is opened to form the ethyl ester and the 4-chlorocrotonic acid is esterified to the C-4 hydroxy group rather than the C-3 hydroxy group as in honaucin A. Honaucin C (3) was found to be the methyl ester equivalent of honaucin B (2). These three metabolites inhibited lipopolysaccharide (LPS)-stimulated nitric oxide production and repressed expression of proinflammatory cytokines in murine macrophages. Furthermore, all three compounds were effective at inhibiting QS signaling-dependent phenotypes in *V. harveyi* BB120 and *E. coli* JB525. Biological screening of synthetic analogs of honaucin A revealed that the halogen atom at the 4-position of the crotonic acid subunit was crucial for both anti-inflammatory and QS inhibitory properties. The analogs 4′-bromohonaucin A (25) and 4′-iodohonaucin A (26) were discovered to have more potent anti-inflammatory and QS inhibitory effects compared to the natural honaucins. Due to its stability and potent biological properties, 4′-bromohonaucin A (25) may represent a valuable lead for the development of drugs with dual anti-inflammatory and bacterial QS inhibitory properties.

EXPERIMENTAL PROCEDURES

General Experimental Procedures

See Supplemental Experimental Procedures.

Collection and Identification of Marine Cyanobacteria

The colonial cyanobacterium *L. crossbyana* (HI09-1) was collected in January 2009 by SCUBA from Hōnaunau reef, Hawai'i, at a depth of 20 m (GPS coordinates N19°42.338′, W155°91.292′). Samples were stored in 70% EtOH at −20°C prior to extraction. The collected specimen was morphologically and phylogenetically identified in a previous study (Choi et al., 2010).

Extraction and Isolation

The cyanobacterial tissue was extracted repetitively with 2:1 CH_2Cl_2-CH_3OH to yield 1.7 g of crude extract. A portion of the extract (1.4 g) was fractionated by silica gel VLC with a stepped gradient elution of hexanes, EtOAc, and MeOH. The fraction eluting with 40% hexanes in EtOAc was dried under reduced pressure, dissolved in hexanes, and extracted with MeOH. The MeOH-soluble material was fractionated using a Silica Sep-Pak column. The fraction eluting with 50% hexanes in EtOAc was subsequently subjected to RP HPLC (Phenomenex Fusion RP 4 μ C18, 250 × 10 mm, 50% CH_3CN/H_2O at 3 mL/min) to give pure honaucin A (**1**, 5.4 mg, 0.39%) and honaucin B (**2**, 1.4 mg, 0.10%). The fraction eluting with 75% hexanes in EtOAc on Silica Sep-Pak chromatography was also subjected to RP HPLC (Phenomenex Fusion RP 4 μ C18, 250 × 10 mm, 50% CH_3CN/H_2O at 3 mL/min) to give pure honaucin C (**3**, 1.1 mg, 0.08%).

Honaucin A (**1**): colorless oil; $[\alpha]_D^{22}$ −80.5 (*c* 0.2, MeOH); ultraviolet (UV) (MeCN) λ_{max} 205 nm (log ε 4.17); IR (neat) ν_{max} 3010, 2955, 1784, 1724, 1316, 1270, 1167, 991 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, see Table 1; HR ESITOFMS *m/z* $[M + H]^+$ 205.0262 (calculated for $C_8H_{10}ClO_4$ 205.0262).
Honaucin B (**2**): colorless oil; $[\alpha]_D^{22}$ −8.0 (*c* 0.2, CH_2Cl_2); UV (MeCN) λ_{max} 206 nm (log ε 4.33); IR (neat) ν_{max} 3454, 2983, 1724, 1659, 1316, 1272, 1182, 1028 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, see Table 1; HR ESITOFMS *m/z* $[M + Na]^+$ 273.0499 (calculated for $C_{10}H_{15}ClNaO_5$ 273.0500).
Honaucin C (**3**): colorless oil; $[\alpha]_D^{22}$ −8.7 (*c* 0.2, CH_2Cl_2); UV (MeCN) λ_{max} 206 nm (log ε 4.31); IR (neat) ν_{max} 3451, 2982, 1724, 1659, 1316, 1272, 1182, 1029 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR ESITOFMS *m/z* $[M + Na]^+$ 259.0346 (calculated for $C_9H_{13}Cl\ NaO_5$ 259.0344).

Preparation of Synthetic Honaucin A and Its Analogs

See Supplemental Experimental Procedures for preparation and analytical data of synthetic honaucin A and its analogs.

Assay for the Detection of NO Production by Murine Macrophages

Measurement of NO production by murine macrophages stimulated with lipopolysaccharide, in the presence and absence of natural products and synthetic analogs, followed our previously published procedures (Villa et al., 2010). This process is described in detail in the Supplemental Experimental Procedures.

Assessment of the Antioxidant Capacity of Honaucin A

See Supplemental Experimental Procedures for a detailed experimental description of assessment of the antioxidant capacity of honaucin A.

RNA Isolation and cDNA Synthesis

See Supplemental Experimental Procedures for a detailed experimental description of RNA isolation and cDNA synthesis.

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time polymerase chain reaction of cDNA isolated from cells treated with honaucin A or other experimental conditions followed established protocols that are given in detail in the Supplemental Experimental Procedures.

Quorum-Sensing Reporter Strains and Assays

Quorum-sensing assays were run in two microbial systems, *V. harveyi* BB120 (Bassler et al., 1997), a wild-type, bioluminescent strain, and *Escherichia coli* JB525 (*E. coli* MT102 harboring the *gfp* plasmid pJBA132), which produces an unstable GFP in response to C_6-C_8 AHL autoinducers (Andersen et al., 2001). Detailed descriptions of the experimental protocols are given in the Supplemental Experimental Procedures.

Statistics

All experiments included three replicates. Data are presented as the mean ± SD for the indicated number of technical replicates. Student's t test was used for the determination of statistical significance, with $p < 0.05$ being considered significant. Prism software (Graphpad Software Inc., San Diego, CA) was used to generate figure graphs displaying SD.

ACCESSION NUMBERS

The 16S rRNA gene sequence of *L. crossbyana* is available in the DDBJ/EMBL/GenBank databases under accession number GU111930.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.chembiol.2012.03.014.

ACKNOWLEDGMENTS

We gratefully acknowledge support of this research from UC San Diego and NIH NS053398. We thank the B.S. Moore laboratory (UCSD) for a sample of 4-fluorocrotonic acid.

Received: January 20, 2012
Revised: March 23, 2012
Accepted: March 27, 2012
Published: May 24, 2012

REFERENCES

Andersen, J.B., Heydorn, A., Hentzer, M., Eberl, L., Geisenberger, O., Christensen, B.B., Molin, S., and Givskov, M. (2001). gfp-based N-acyl homoserine-lactone sensor systems for detection of bacterial communication. Appl. Environ. Microbiol. *67*, 575–585.

Bassler, B.L., Greenberg, E.P., and Stevens, A.M. (1997). Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. J. Bacteriol. *179*, 4043–4045.

Cheung, K.P., Taylor, K.R., and Jameson, J.M. (2011). Immunomodulation at epithelial sites by obesity and metabolic disease. Immunol. Res., in press. Published online December 13, 2011. 10.1007/s12026-011-8261-7.

Choi, H., Engene, N., Smith, J.E., Preskitt, L.B., and Gerwick, W.H. (2010). Crossbyanols A-D, toxic brominated polyphenyl ethers from the Hawai'ian bloom-forming Cyanobacterium *Leptolyngbya crossbyana*. J. Nat. Prod. *73*, 517–522.

Clark, B.R., Engene, N., Teasdale, M.E., Rowley, D.C., Matainaho, T., Valeriote, F.A., and Gerwick, W.H. (2008). Natural products chemistry and taxonomy of the marine cyanobacterium *Blennothrix cantharidosmum*. J. Nat. Prod. *71*, 1530–1537.

Conaghan, P.G. (2011). A turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy, and toxicity. Rheumatol. Int. 10.1007/s00296-011-2263-6.

Cooley, M.A., Whittall, C., and Rolph, M.S. (2010). Pseudomonas signal molecule 3-oxo-C12-homoserine lactone interferes with binding of rosiglitazone to human PPARgamma. Microbes Infect. *12*, 231–237.

de Silva, E.D., and Scheuer, P.J. (1980). Manoalide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis*. Tetrahedron Lett. *21*, 1611–1614.

Dobretsov, S., Teplitski, M., Alagely, A., Gunasekera, S.P., and Paul, V.J. (2010). Malyngolide from the cyanobacterium *Lyngbya majuscula* interferes with quorum sensing circuitry. Environ. Microbiol. Rep. *2*, 739–744.

Farooque, S.P., and Lee, T.H. (2009). Aspirin-sensitive respiratory disease. Annu. Rev. Physiol. *71*, 465–487.

Flachsmann, F., Schellhaas, K., Moya, C.E., Jacobs, R.S., and Fenical, W. (2010). Synthetic pseudopterosin analogues: a novel class of antiinflammatory drug candidates. Bioorg. Med. Chem. *18*, 8324–8333.

Freeman, J.A., Lilley, B.N., and Bassler, B.L. (2000). A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in *Vibrio harveyi*. Mol. Microbiol. *35*, 139–149.

Galloway, W.R.D.J., Hodgkinson, J.T., Bowden, S.D., Welch, M., and Spring, D.R. (2011). Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. Chem. Rev. *111*, 28–67.

Gautam, R., and Jachak, S.M. (2009). Recent developments in anti-inflammatory natural products. Med. Res. Rev. *29*, 767–820.

Gershon, H., Shanks, L., and Gawiak, D.E. (1976). Antifungal activity of 4-substituted crotonic acid esters. J. Med. Chem. *19*, 1069–1072.

Givskov, M., de Nys, R., Manefield, M., Gram, L., Maximilien, R., Eberl, L., Molin, S., Steinberg, P.D., and Kjelleberg, S. (1996). Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. J. Bacteriol. *178*, 6618–6622.

Grivennikov, S.I., Greten, F.R., and Karin, M. (2010). Immunity, inflammation, and cancer. Cell *140*, 883–899.

Henke, J.M., and Bassler, B.L. (2004). Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. J. Bacteriol. *186*, 6902–6914.

Hughes, D.T., and Sperandio, V. (2008). Inter-kingdom signalling: communication between bacteria and their hosts. Nat. Rev. Microbiol. *6*, 111–120.

Jahoor, A., Patel, R., Bryan, A., Do, C., Krier, J., Watters, C., Wahli, W., Li, G., Williams, S.C., and Rumbaugh, K.P. (2008). Peroxisome proliferator-activated receptors mediate host cell proinflammatory responses to Pseudomonas aeruginosa autoinducer. J. Bacteriol. *190*, 4408–4415.

Kravchenko, V.V., Kaufmann, G.F., Mathison, J.C., Scott, D.A., Katz, A.Z., Grauer, D.C., Lehmann, M., Meijler, M.M., Janda, K.D., and Ulevitch, R.J. (2008). Modulation of gene expression via disruption of NF-κB signaling by a bacterial small molecule. Science *321*, 259–263.

Kwan, J.C., Teplitski, M., Gunasekera, S.P., Paul, V.J., and Luesch, H. (2010). Isolation and biological evaluation of 8-*epi*-malyngamide C from the Floridian marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. *73*, 463–466.

Kwan, J.C., Meickle, T., Ladwa, D., Teplitski, M., Paul, V., and Luesch, H. (2011). Lyngbyoic acid, a "tagged" fatty acid from a marine cyanobacterium, disrupts quorum sensing in *Pseudomonas aeruginosa*. Mol. Biosyst. *7*, 1205–1216.

Lanas, A. (2010). A review of the gastrointestinal safety data—a gastroenterologist's perspective. Rheumatology (Oxford) *49* (*Suppl 2*), ii3–ii10.

Lowery, C.A., Salzameda, N.T., Sawada, D., Kaufmann, G.F., and Janda, K.D. (2010). Medicinal chemistry as a conduit for the modulation of quorum sensing. J. Med. Chem. *53*, 7467–7489.

McFall-Ngai, M., Nyholm, S.V., and Castillo, M.G. (2010). The role of the immune system in the initiation and persistence of the Euprymna scolopes—*Vibrio fischeri* symbiosis. Semin. Immunol. *22*, 48–53.

Moghadam-Kia, S., and Werth, V.P. (2010). Prevention and treatment of systemic glucocorticoid side effects. Int. J. Dermatol. *49*, 239–248.

Nagle, D.G., and Paul, V.J. (1999). Production of secondary metabolites by filamentous tropical marine cyanobacteria: ecological functions of the compounds. J. Phycol. *35*, 1412–1421.

Ni, N., Choudhary, G., Peng, H., Li, M., Chou, H.-T., Lu, C.-D., Gilbert, E.S., and Wang, B. (2009). Inhibition of quorum sensing in *Vibrio harveyi* by boronic acids. Chem. Biol. Drug Des. *74*, 51–56.

Ogier, J.C., Calteau, A., Forst, S., Goodrich-Blair, H., Roche, D., Rouy, Z., Suen, G., Zumbihl, R., Givaudan, A., Tailliez, P., et al. (2010). Units of plasticity in bacterial genomes: new insight from the comparative genomics of two bacteria interacting with invertebrates, *Photorhabdus* and *Xenorhabdus*. BMC Genomics *11*, 568.

Pappas, K.M., and Winans, S.C. (2003). A LuxR-type regulator from *Agrobacterium tumefaciens* elevates Ti plasmid copy number by activating transcription of plasmid replication genes. Mol. Microbiol. *48*, 1059–1073.

Prasad, K., Chen, K.-M., Repic, O., and Hardtmann, G.E. (1990). A highly stereoselective route to the four stereoisomers of a six-carbon synthon. Tetrahedron Asymmetry *1*, 307–310.

Rumbaugh, K.P., and Kaufmann, G.F. (2012). Exploitation of host signaling pathways by microbial quorum sensing signals. Curr. Opin. Microbiol. *15*, 162–168. Published online December 26, 2011. 10.1016/j.mib.2011.12.003.

Sánchez-Borges, M., Capriles-Behrens, E., and Caballero-Fonseca, F. (2004). Hypersensitivity to non-steroidal anti-inflammatory drugs in childhood. Pediatr. Allergy Immunol. *15*, 376–380.

Skindersoe, M.E., Ettinger-Epstein, P., Rasmussen, T.B., Bjarnsholt, T., de Nys, R., and Givskov, M. (2008). Quorum sensing antagonism from marine organisms. Mar. Biotechnol. (NY) *10*, 56–63.

Smith, J.E., Kuwabara, J., Flanaga, K., duPlessis, S., Coney, J., Beets, J., Takabayashi, M., Barnes, S., Turner, J., Brown, D., et al. (2008). An unusual cyanobacterial bloom in Hawai'i. Coral Reefs *27*, 851.

Swem, L.R., Swem, D.L., Wingreen, N.S., and Bassler, B.L. (2008). Deducing receptor signaling parameters from in vivo analysis: LuxN/AI-1 quorum sensing in *Vibrio harveyi*. Cell *134*, 461–473.

Swem, L.R., Swem, D.L., O'Loughlin, C.T., Gatmaitan, R., Zhao, B., Ulrich, S.M., and Bassler, B.L. (2009). A quorum-sensing antagonist targets both membrane-bound and cytoplasmic receptors and controls bacterial pathogenicity. Mol. Cell *35*, 143–153.

Teasdale, M.E., Liu, J., Wallace, J., Akhlaghi, F., and Rowley, D.C. (2009). Secondary metabolites produced by the marine bacterium *Halobacillus salinus* that inhibit quorum sensing-controlled phenotypes in gram-negative bacteria. Appl. Environ. Microbiol. *75*, 567–572.

Teasdale, M.E., Donovan, K.A., Forschner-Dancause, S.R., and Rowley, D.C. (2011). Gram-positive marine bacteria as a potential resource for the discovery of quorum sensing inhibitors. Mar. Biotechnol. (NY). *13*, 722–732. Published online December 9, 2010. 10.1007/s10126-010-9334-7.

Teng, S.-W., Schaffer, J.N., Tu, K.C., Mehta, P., Lu, W., Ong, N.P., Bassler, B.L., and Wingreen, N.S. (2011). Active regulation of receptor ratios controls integration of quorum-sensing signals in *Vibrio harveyi*. Mol. Syst. Biol. *7*, 491. 10.1038/msb.2011.30.

Teplitski, M., Mathesius, U., and Rumbaugh, K.P. (2011). Perception and degradation of N-acyl homoserine lactone quorum sensing signals by mammalian and plant cells. Chem. Rev. *111*, 100–116.

Terracciano, S., Aquino, M., Rodriquez, M., Monti, M.C., Casapullo, A., Riccio, R., and Gomez-Paloma, L. (2006). Chemistry and biology of anti-inflammatory marine natural products: molecules interfering with cyclooxygenase, NF-kappaB and other unidentified targets. Curr. Med. Chem. *13*, 1947–1969.

Tousoulis, D., Kampoli, A.M., Papageorgiou, N., Androulakis, E., Antoniades, C., Toutouzas, K., and Stefanadis, C. (2011). Pathophysiology of atherosclerosis: the role of inflammation. Curr. Pharm. Des. *17*, 4089–4110.

Tidgewell, K., Clark, B.T., and Gerwick, W.H. (2010). The natural products chemistry of cyanobacteria. In Comprehensive Natural Products Chemistry, Second Edition, B. Moore and P. Crews, eds. (Oxford, UK: Elsevier), pp. 141–188.

Uchikawa, O., Okukado, N., Sakata, T., Arase, K., and Terada, K. (1988). Synthesis of (*S*)- and (*R*)-3-hydroxy-4-butanolide and (2*S*,4*S*)-, (2*R*,4*S*)-, (2*S*,4*R*)-, and (2*R*,4*R*)-2-hydroxy-4-hydroxymethyl-4-butanolide and their satiety and hunger modulating activities. Bull. Chem. Soc. Jpn. *61*, 2025–2029.

Villa, F.A., and Gerwick, L. (2010). Marine natural product drug discovery: Leads for treatment of inflammation, cancer, infections, and neurological disorders. Immunopharmacol. Immunotoxicol. *32*, 228–237.

Villa, F.A., Lieske, K., and Gerwick, L. (2010). Selective MyD88-dependent pathway inhibition by the cyanobacterial natural product malyngamide F acetate. Eur. J. Pharmacol. *629*, 140–146.

Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry *64*, 3–19.

Zhang, R.G., Pappas, K.M., 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.