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Oxazaborolidine derivatives inducing autoinducer-2 signal transduction in *Vibrio harveyi*

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Abstract—The bioluminescence of the marine bacterium *Vibrio harveyi* is controlled by quorum sensing. This effect is mediated by production, accumulation, and auto-detection of the species-specific autoinducer 1 (AI-1), autoinducer 2 (AI-2), and the *V. cholerae* autoinducer 1 (CAI-1). The *V. harveyi* AI-2 was recently identified as furanosyl borate diester. We synthesized several oxazaborolidine derivatives that chemically resemble the structure of AI-2. Five oxazaborolidine derivatives (BNO-1 to BNO-5) were tested, however only BNO-1 (3,4-dimethyl-2,5-diphenyl-1,3,2-oxazaborolidine), and BNO-5 (2-butyl-3,4-dimethyl-5-phenyl-1,3,2-oxazaborolidine) strongly induced *V. harveyi* bioluminescence in *V. harveyi* mutant (BB170) lacking sensor 1. A dose-dependent relationship between those oxazaborolidine derivatives and bioluminescence induction was observed with this *V. harveyi* strain (BB170). BNO-1 and BNO-5 did not affect *V. harveyi* BB886 lacking sensor 2. Using a mutant strain which produces neither AI-1 nor AI-2 (*V. harveyi* MM77) we showed that the presence of spent medium containing AI-2 is essential for BNO-1 and BNO-5 activity. This effect was similar when introducing the spent medium and the BNOs together or at a 3-h interval. A comparable induction of bioluminescence was observed when using synthetic DPD (pre-AI-2) in the presence of BNO-1 or BNO-5. The mode of action of BNO-1 and BNO-5 on bioluminescence of *V. harveyi* is of a co-agonist category. BNO-1 and BNO-5 enhanced AI-2 signal transduction only in the presence of AI-2 and only via sensor 2 cascade. BNO-1 and BNO-5 are the first oxazaborolidines reported to affect AI-2 activity. Those derivatives represent a new class of borates which may become prototypes of novel agonists of quorum sensing mediated by AI-2 in *V. harveyi*.

1. Introduction

Regulation of bacterial properties by population density is a manifestation of bacterial cell–cell communication (quorum sensing) and is mediated by signal molecules known as autoinducers (AIs).[6,29,36] Quorum sensing is associated with genetic competence, bacterial colonization, biofilm formation,[24,30] virulence,[5,37,40] and other bacterial properties, by regulating the expression of the corresponding genes.[29]

Typically, oligopeptides play the role of AIs in Gram-positive bacteria,[19,22] Acylated homoserine lactones (AHL) act as AIs in Gram-negative bacteria,[14] whereas AI-2s are considered signal molecules, affecting a broad spectrum of bacteria.[6,36,41] The AIs are recognized by membranal or intracellular receptors which control numerous physiological pathways of the bacteria.[36]

Although AI-2 cascade has been found in many species,[35] the free living marine bacterium *Vibrio harveyi* has been proposed as a model for investigating quorum sensing related to AI-1, AI-2[2,3] and CAI.[16] *V. harveyi* produces three types of AIs that regulate its bioluminescence: species-specific AHL designated AI-1,[3,7] AI-2,[2,3,34,39,41] and the recently described CAI-1 (*V. cholerae* AI).[16] Both AI-1 and AI-2 are perceived by two-component signal transduction systems: Sensor 1, the membrane-bound histidine kinase LuxN, binds AI-1,[3,13] while the AI-2 cognate sensor 2 includes the periplasmic binding protein LuxP and the membrane-bound histidine protein kinase LuxQ.[28] In the absence of AI-1 and AI-2, the kinase activity of the sensors results in activation (phosphorylation) of the central bioluminescence repressor LuxO. The phosphorylated active repressor, in conjugation with the transcription factor σ^{54}, activates transcription of five regulatory small RNAs which interact with a RNA chaperon, called

Keywords: Quorum sensing; Autoinducers; *Vibrio harveyi*; Luminescence; Oxazaborolidines.
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Hfq, and destabilized the mRNA encoding the transcriptional activator LuxR which is required to activate transcription of the luciferase operon *luxCDABE*. In the presence of AIs, the phosphatase activity of the sensors causes inactivation (dephosphorylation) of the central repressor LuxO, LuxR is activated, promoting transcription of the luciferase operon, and allows bioluminescence of the bacteria.[4,11,12,36,39]

AI-2 is an unstable molecule which was recently crystallized in a complex with LuxP and was identified as a furanosyl borate diester in which the boron plays a pivotal role in the structure of the AI-2 molecule of *V. harveyi*.[8,9,26] AI-2 is synthesized by *V. harveyi* in a series of enzymatic reactions.[32] In the final steps, LuxS converts the ribose moiety of *S*-ribosylhomocysteine into 4,5-dihydroxy-2,3-pentanedione (DPD).[25,31,34,38] In *V. harveyi*, DPD reacts in its cyclic form with boric acid to form AI-2.[9] However, not all AI-2 are boron containing compounds as was identified for the AI-2 of *Salmonella typhimurium*.[27]

The *luxS* gene, encoding the LuxS enzyme synthesizing DPD, plays a crucial role in quorum sensing of *V. harveyi*.[10,36,41] Clearly, DPD (pre-AI-2) and the furanosyl borate diester (AI-2) are potential prototypes for analog designs due to their pivotal role in the quorum sensing cascade.

Oxazaborolidines are five-membered heterocyclic boron compounds containing oxygen and nitrogen atoms. No information on the biological activity of oxazaborolidines was available until our recent study on the effect of several oxazaborolidines on the viability of *S. mutans*.[17,18]

A structural relationship between *V. harveyi* AI-2 and oxazaborolidines is evident (Fig. 1). Both are heterocyclic hydrated complexes, containing negatively charged tetra-coordinated boron atom that has the ability to form hydrogen bonds.

Therefore, it appeared to us that those molecules might be suitable candidates for studying quorum sensing via the AI-2 receptors of *V. harveyi*. We reasoned, based on the chemical structure of oxazaborolidines and the biological activity displayed by other boron compounds with B–N bonds, that oxazaborolidines might selectively bind to a receptor like sensor-2 (AI-2 receptor of *V. harveyi*) thereby triggering an agonistic response.

2. Materials and methods

2.1. Synthesis of oxazaborolidines

The oxazaborolidines were synthesized as described by Jabbour et al.[18] Briefly, BNO-1 to BNO-5 (Fig. 1a) were synthesized by reacting an amino alcohol with a boronic acid. The synthesis of BNO-1 and BNO-5 and their hydrated forms is illustrated in Figure 2. The purity of the oxazaborolidines was determined by ^{1}H, ^{13}C, ^{11}B NMR, microanalysis and mass-spectral analysis.[18] The maximal solubility of oxazaborolidines in double distilled water (saturated solution) at 37 °C (mM): BNO-1, 71; BNO-2, 486; BNO-3, 358; BNO-4, 121; BNO-5, 123.

2.2. Bacterial strains and growth conditions

All *V. harveyi* strains and mutants (Table 1), kindly provided by B. Bassler (Princeton University), were grown aerobically at 30 °C with constant shaking in AB medium.[15]

2.3. Determination of oxazaborolidines, MIC for *V. harveyi*

Minimal inhibitory concentration (MIC) was tested according to the CLSI (formerly NCCLS) guidelines. *V. harveyi* BB170, MM77, and the BB886 strains (adjusted to 0.5 Macfarlane standard suspension) were grown at 30 °C, in the presence of BNO-1, BNO-2, BNO-3, BNO-4, and BNO-5 at concentrations ranging

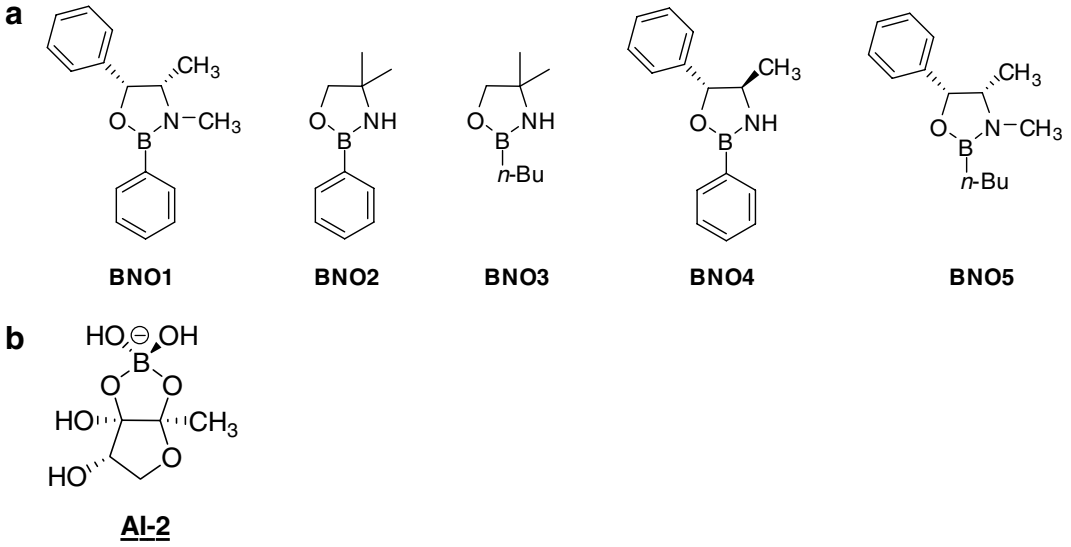

Figure 1. (a) Structure of BNO-1 to BNO-5. (b) Structure of AI-2.

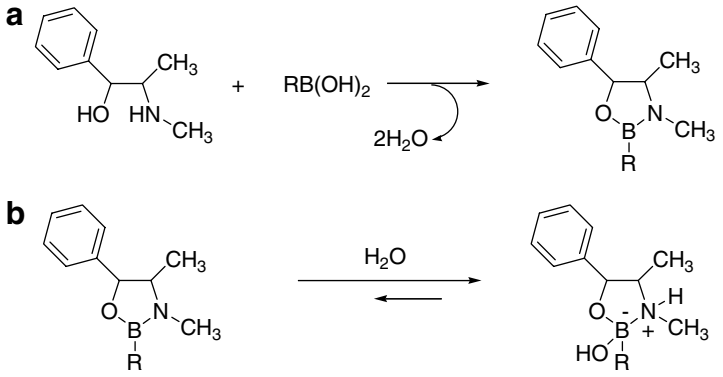

Figure 2. (a) Synthesis of BNO-1 (R = Phenyl) and BNO-5 (R = Butyl) by reacting (−)-ephedrine with either phenyl or butyl boronic acid with the azeotropic removal of water. (b) Hydrated complex formation of BNO-1 (R = Phenyl) and BNO-5 (R = Butyl).

Table 1. *V. harveyi* strains

V. harveyi strain	Relevant genotype	Relevant phenotype
BB170	(*BB7*) luxN:: Tn5	sensor -1$^-$, sensor-2$^+$; AI-1$^+$, AI-2$^+$
BB886	(*BB7*) *luxP*:: Tn5	sensor-1$^+$, sensor-2$^-$; AI-1$^+$, AI-2$^+$
MM77	(*BB7 or BB120*) *luxLM*:: Tn5, *luxS*:: Cm^r	sensor-1$^+$, sensor-2$^+$; AI-1$^-$, AI-2$^-$
BB152	(*BB7 or BB120*) *luxLM*::Tn5	sensor-1$^+$, sensor-2$^+$; AI-1$^-$, AI-2$^+$
MM30	(*BB120*) *luxS*:: Tn5	sensor-1$^+$, sensor-2$^+$; AI-1$^+$, AI-2$^-$

from 0.5 mM to 150 mM, in 96-well transparent plates (NUNC, Denmark). After 18-h of incubation, bacterial growth was determined by absorbance at OD_{595} (GENios reader TECAN, Austria). Each experiment was conducted in triplicate and repeated three times.

2.4. Rapid screening of oxazaborolidines

We screened five different oxazaborolidine derivatives (BNO-1, BNO-2, BNO-3, BNO-4, and BNO-5) for their effect on bioluminescence of *V. harveyi* strains. The mutant *V. harveyi* strains (BB170, BB886) were grown to cell density of $1.5–2.5 \times 10^9$ CFU/ml (15–16 h) in AB medium at 30 °C with constant shaking. The fresh cultures were then diluted 5000-fold in fresh AB medium containing the tested compound at a concentration of 200 μM. Each compound was exposed to spent medium of *V. harveyi* MM30 (AI-1$^+$, AI-2$^-$) containing AI-1 (in the case of strain BB886) or to spent medium of *V. harveyi* BB152 (AI-1$^-$, AI-2$^+$) containing AI-2 (in the case of strain BB170) to examine possible effect on bioluminescence of the tested compound. Each sample was tested also without the spent medium in order to examine potential self-induction. The negative control contained bacteria in fresh AB medium and the positive control contained bacteria, fresh AB medium, and 10% v/v spent medium with the appropriate autoinducer (*V. harveyi* BB152 or MM30 spent medium). The test was conducted in white 96-well plates (NUNC). Luminescence was measured automatically by a GENios reader (TECAN) every 30 min. The end point of the bacterial density was determined (OD_{595}). Each sample was tested in three separate biological experiments, each conducted in triplicate. Fold induction was determined above the endogenous level of luminescence expressed by *V. harveyi* BB170 or BB886 at minimum luminescence time point after 3–4 h of incubation.[2,30,34] For the above rapid screening, the induction was determined only according to the luminescence. The screening assays were performed at concentration of 200 μM of each tested agent. This concentration was chosen as it is about 100 times less than the MIC and no significant effect was recorded on bacterial growth at this concentration (data not shown). Based on those screening results we have further investigated agents which have demonstrated a potential effect on quorum sensing (BNO-1, BNO-5).

2.5. Induction of *V. harveyi* bioluminescence by BNO-1 and BNO-5

Due to the fact that in our screening assay, BNO-1 and also BNO-5 have been found to be the most effective in inducing bioluminescence of *V. harveyi*, their induction activity was further explored. The tested *V. harveyi* strains (BB170 and BB886) were grown to cell density of $1.5–2.5 \times 10^9$ CFU/ml (15–16 h). Next the suspension was diluted (1:5000 or 1:10,000) in fresh AB medium supplemented with BNO-1 or BNO-5 at various concentrations below the MIC. Positive and negative controls were used as described before.

Bacterial cultures were incubated at 30 °C for approximately 7 h, and the luminescence emitted during growth was measured with a Lumac/3M Biocounter M2010 (Lumac BV, Schaesberg, Netherlands) or GENios reader (TECAN). The relative intensity of light emission was calculated in proportion to cell density, represented by number of CFU or relatively to the sample OD_{595}. Fold induction at each compound's concentration was deter-

mined at minimum luminescence time point after 3–4 h, above the endogenous level of luminescence expressed by *V. harveyi* BB170 or BB886, as was conducted by Bassler et al.[2]

2.6. The combined effect of BNO-1 and BNO-5 on bioluminescence

The potential combined effect of spent medium containing AI-2, spent medium containing AI-1 or synthetic DPD (pre-AI-2)[25] (The Scripps Research Institute, USA) together with BNO-1 or with BNO-5 on luminescence induction was determined using *V. harveyi* MM77 (sensor 1^+, sensor 2^+; AI-1^-, AI-2^-). Bacteria were grown as described above. Response curves were recorded for 0.4% (v/v) spent medium of *V. harveyi* BB152 (AI-1^-, AI-2^+), 10% (v/v) spent medium of *V.harveyi* MM30 (AI-1^+, AI-2^-) or synthetic DPD at various concentrations, ranging from 0.0002 to 200 nM, with BNO-1 or with BNO-5 at constant concentration of 800 μM. The effects of those combinations on luminescence induction were examined when the bacteria were exposed to the tested compounds and spent media or DPD, immediately upon the beginning of bacterial growth period. Further examination was performed on *V. harveyi* BB152 spent medium and BNO-1 or BNO-5 which were added together 3 h after the beginning of bacterial growth or when one of the tested agents was introduced to the bacteria upon the beginning of bacterial growth and the other agent 3 h later. The readings were taken in triplicates in 96-well white plates with an optic bottom (NUNC) in a GENios reader (TECAN) at 30 °C. Luminescence measurements were recorded every 30 min parallel with absorbance measurement (OD_{595}). The value of each reading (bacteria in various conditions) was divided by the absorbance values to normalize the luminescence value of each sample to its cell density and avoid dissimilarities caused by differences in growth rates.

Fold induction, above non specific luminescence background, was determined at the end of bacterial growth after approximately 7 h of growth. We used this time period because in our experimental system the most significant inductive effect occurred then.

3. Results

3.1. Aqueous stability of oxazaborolidines

The B–N bond of the oxazaborolidines is susceptible to nucleophilic attack and upon the addition of 1 mole of H_2O immediately forms a hydrated complex with H_2O (^{11}B $\delta \sim 7$ ppm) (Fig. 2). We have investigated the hydrated complexes of tested oxazaborolidines and found them to be very stable to hydrolysis in the pH range 5–13, meaning that no free boronate (boronic acid) is released from these compounds as was determined by ^{11}B NMR.[18]

3.2. Induction of *V. harveyi* bioluminescence by BNOs

Of the five oxazaborolidine derivatives we tested, only BNO-1 and BNO-5 showed the strongest effect on *V. harveyi* bioluminescence (Fig. 3). The effective concen-

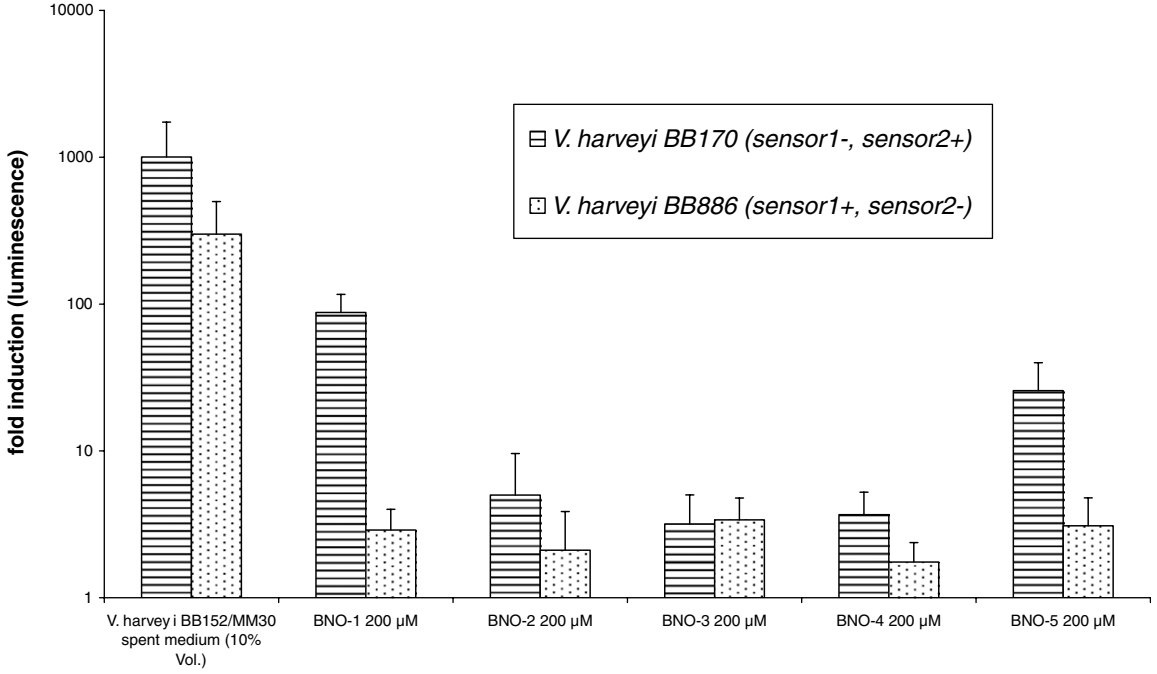

Figure 3. Screening of bioluminescence effect of BNO-1 to BNO-5 on *V. harveyi* BB170 (sensor-1^-, sensor-2^+) and *V. harveyi* BB886 (sensor-1^+, sensor-2^-). Each screened compound was added at the beginning of bacterial growth at a concentration of 200 μM. Sample without any compound served as negative control. Fold induction above the endogenous level of luminescence expressed by *V. harveyi* BB170 or BB886 was determined at the minimum luminescence time point of the negative control (after 3–4 h). Sample with 10% v/v spent medium of *V. harveyi* BB152 (AI-1^-, AI-2^+) served as positive control for *V. harveyi* BB170 and sample with 10% v/v spent medium of *V. harveyi* MM30 (AI-1^+, AI-2^-) served as positive control for *V. harveyi* BB886.

trations were below the MIC (Table 2). Therefore, we continued to explore the influence of these two compounds on the bioluminescence of *V. harveyi*. The induction effect of BNO-1 on the bioluminescence of *V. harveyi* BB170 (sensor-1$^-$, sensor-2$^+$) was positively dependent on BNO-1 concentrations in the range of 0–600 μM. At higher concentrations, the bioluminescence response was stable (Fig. 4). Under the same conditions, the effect of BNO-1 on *V. harveyi* strain BB886 (sensor-1$^+$, sensor-2$^-$) was much lower. BNO-5 was less effective than BNO-1 in induction of bioluminescence in *V. harveyi* BB170. Similar to BNO-1 also BNO-5 had relatively little effect on the mutant strain lacking sensor 2 (Fig. 5).

3.3. The combined effect of BNO-1 and BNO-5 on bioluminescence

To examine the mutual effect of *V. harveyi* autoinducers and the BNOs, we tested the combined influence of spent media containing AI-1 or AI-2 and BNO-1 or BNO-5 on *V. harveyi* MM77 (sensor-1$^+$, sensor-2$^+$; AI-1$^-$, AI-2$^-$).

A strong effect was evident when 800 μM of BNO-1 or BNO-5 and 0.4% v/v of *V. harveyi* BB152 spent medium containing AI-2 were used (Fig. 6). There was no effect of the same concentrations of BNO-1 or BNO-5 with 10% of *V. harveyi* MM30 spent medium containing AI-1. There was also no effect of these concentrations of BNO-1, BNO-5 or these spent media alone on bioluminescence, and these fold induction values were considered to be in the background area of the bioluminescence assay (fold induction of 10 and below).

Similar results of BNO-1 and BNO-5 with *V. harveyi* AI-2 were obtained when the same combinations were added together to *V. harveyi* MM77, 3 h after the beginning of growth, although the fold induction values were smaller (Fig. 7). To determine whether there was a carryover or mutual effect of those agents on each other, we have also added the second agent 3 h after the first (Fig. 7). The strongest effect was observed using 800 μM of BNO and 0.4% v/v of spent media containing AI-2. The effect was similar whether both agents were added at the beginning of growth period, 3 h after beginning of growth or when one agent was added after the other (Figs. 6 and 7).

We have also tested the mutual effect of BNO-1 or BNO-5 at concentration of 800 μM together with synthetic and chemically defined DPD at various concentrations ranging from 0.0002 to 200 nM. A strong positive dose response effect was also obtained after 7 h of growth, by BNO-1 or BNO-5 with DPD, at concentra-

Table 2. Minimal inhibitory concentration (MIC) of the different oxazaborolidines for *V. harveyi* BB170, BB886 and MM77

	V. harveyi BB170	*V. harveyi* BB886	*V. harveyi* MM77
BNO-1	20–40	20–40	20–40
BNO-2	150	5–10	10–20
BNO-3	120–150	120–150	120–150
BNO-4	10–25	10–25	10–25
BNO-5	150	10–30	10–30

Values are in mM.

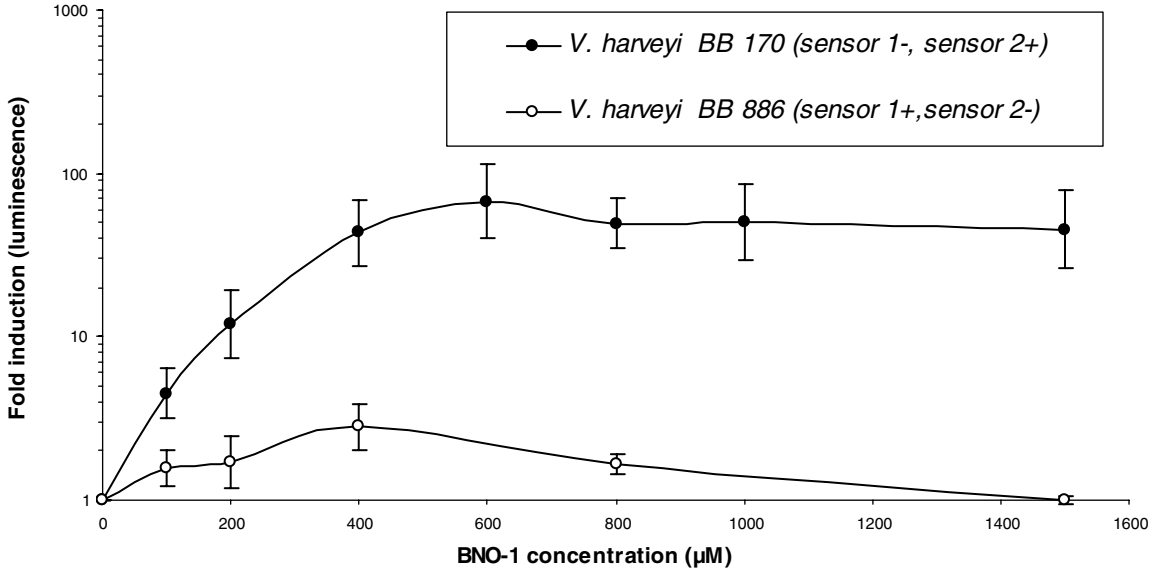

Figure 4. Effect of BNO-1 on induced bioluminescence of *V. harveyi* BB170 (sensor-1$^-$, sensor-2$^+$) and *V. harveyi* BB886 (sensor-1$^+$, sensor-2$^-$). BNO-1 was added at the beginning of bacterial growth to various concentrations, ranging from 0 to 1500 μM. Sample without any compound served as negative control. Fold induction above the endogenous level of luminescence expressed by *V. harveyi* BB170 or BB886 was determined at the minimum luminescence time point of the negative control (after 3–4 h). Sample with 10% v/v spent medium of *V. harveyi* BB152 (AI-1$^-$, AI-2$^+$) served as positive control for *V. harveyi* BB170 and sample with 10% v/v spent medium of *V. harveyi* MM30 (AI-1$^+$, AI-2$^-$) served as positive control for *V. harveyi* BB886.

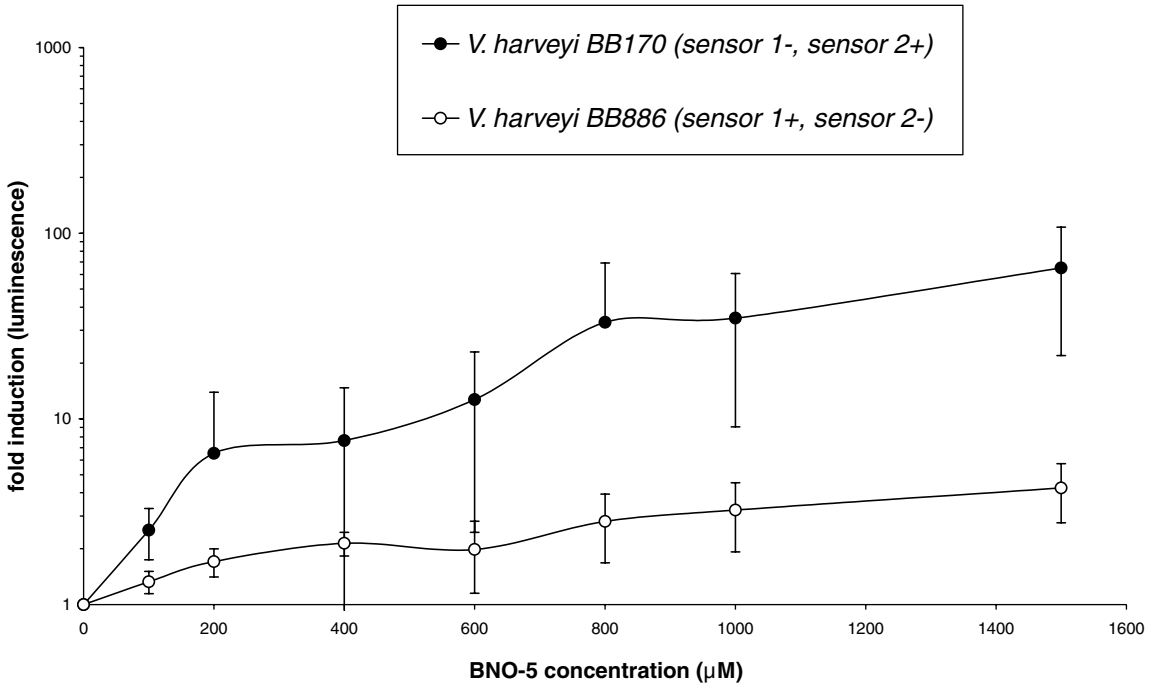

Figure 5. Effect of BNO-5 on induced bioluminescence of *V. harveyi* BB170 (sensor-1$^-$, sensor-2$^+$) and *V. harveyi* BB886 (sensor-1$^+$, sensor-2$^-$). BNO-5 was added at the beginning of bacterial growth in various concentrations, ranging from 0 to 1500 μM. Sample without any compound served as negative control. Fold induction above the endogenous level of luminescence expressed by *V. harveyi* BB170 or BB886 was determined at the minimum luminescence time point of the negative control (after 3–4 h). Sample with 10% v/v spent medium of *V. harveyi* BB152 (AI-1$^-$, AI-2$^+$) served as positive control for *V. harveyi* BB170 and sample with 10% v/v spent medium of *V. harveyi* MM30 (AI-1$^+$, AI-2$^-$) served as positive control for *V. harveyi* BB886.

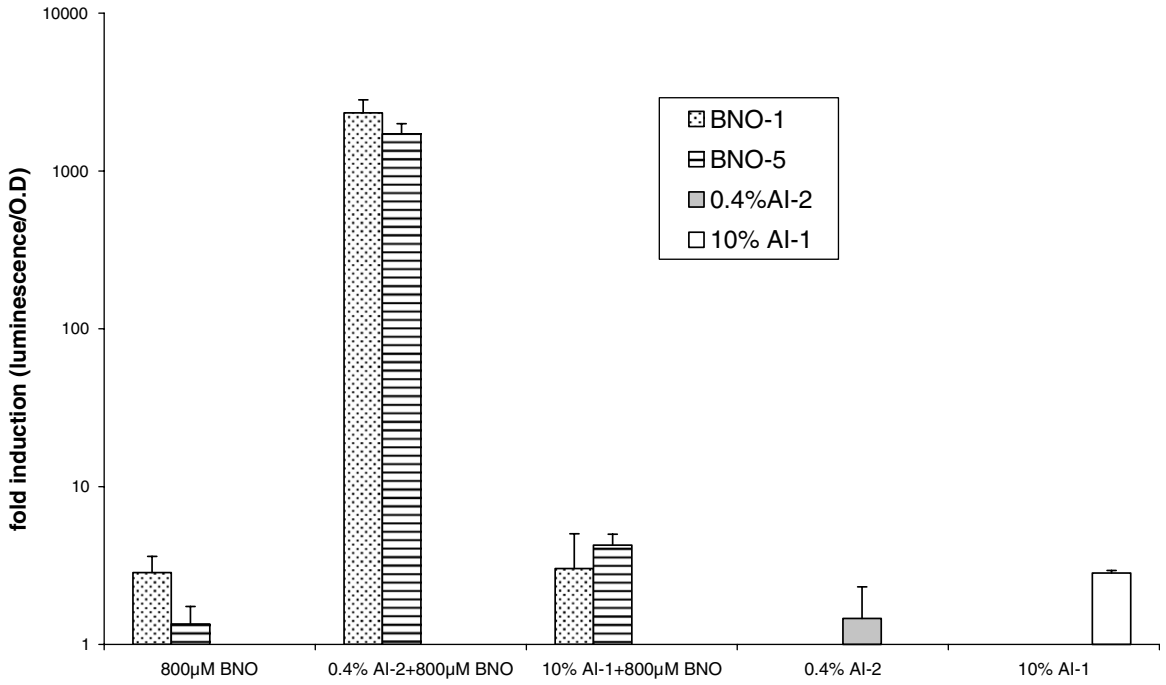

Figure 6. Mutual effect of BNO-1, BNO-5 and spent medium containing AI-1 or AI-2 on induced bioluminescence of *V. harveyi* MM77 (AI-1$^-$, AI-2$^-$). Bacteria were grown for approximately 7 h. *V. harveyi* BB152 (AI-1$^-$, AI-2$^+$) or *V. harveyi* MM30 (AI-1$^+$, AI-2$^-$) spent medium and BNO-1 or BNO-5 were added to the reaction medium immediately at the beginning of the bacterial growth. Sample without any compound or spent medium served as negative control. Fold induction above non specific luminescence background was determined at the end of bacterial growth. Constant oxazaborolidine concentration of 800 μM was used, while spent media (represented in graph as 'AI-1' or 'AI-2') are presented as percentage from each sample volume (200 μl).

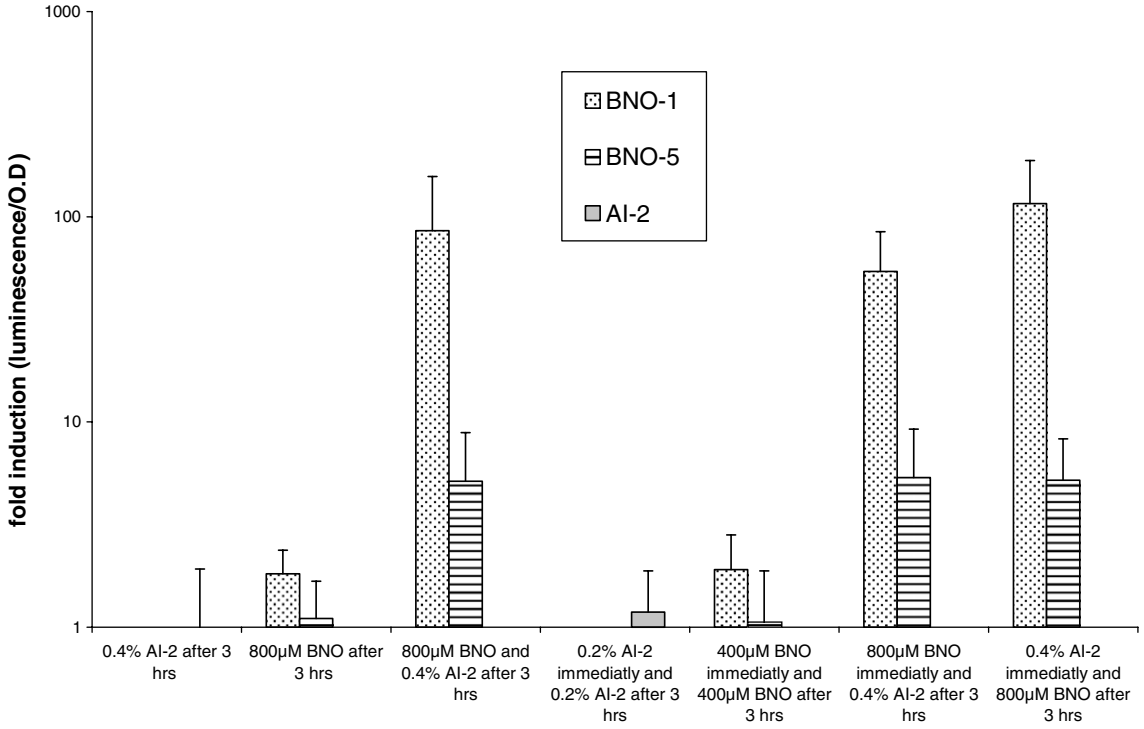

Figure 7. Mutual effect of BNO-1 or BNO-5 and AI-2 on induced bioluminescence of *V. harveyi* MM77 (AI-1$^-$, AI-2$^-$). Bacteria were grown for approximately 7 h. *V. harveyi* BB152 (AI-1$^-$, AI-2$^+$) spent medium and BNO-1 or BNO-5 were added together to the reaction medium 3 h after the beginning of the bacterial growth or one agent immediately upon the beginning of the bacterial growth and the other agent 3 h later. Sample without any compound or spent medium served as negative control. Fold induction above non specific luminescence background was determined at the end of bacterial growth. Constant oxazaborolidine concentration of 800 μM was used, while *V. harveyi* BB152 spent medium (represented in graph as 'AI-2') is presented as percentage from each sample volume (200 μl).

tions of 0.02–20 nM on *V. harveyi* MM77, lacking AI-1 and AI-2 (Fig. 8).

4. Discussion

Quorum sensing has been studied by several means, such as genes involved in quorum sensing, enzymatic inhibition in the metabolic pathway of AI synthesis or at various points in the signal transduction pathway .[1,21] Only after identification of the AI-2 molecule by Chen et al.,[8] an alternative approach, enabling evaluation of the structural requirements of AI-2, was available. Clearly, DPD in its cyclic form and furanosyl borate diester (AI-2) are potential prototypes for analog design due to their pivotal role as the final products of the AI-2 synthesis cascade.

The atom boron is considered a pivotal functional part of the AI-2 molecule.[8,9] We synthesized oxazaborolidine derivatives which chemically resemble the structure of AI-2 (Fig. 1). Five oxazaborolidine derivatives were tested on the bioluminescence of *V. harveyi*. The oxazaborolidine derivatives, BNO-1 and BNO-5 most strongly induced the bioluminescence of the reporter strain *V. harveyi* BB170 (Fig. 3). This finding prompted us to further investigate a potential mechanism of action of this induction.

Using a mutant strain of *V. harveyi*, lacking sensor 1 (BB170), we found that the presence of sensor 1 is not essential for triggering maximal bioluminescence by both BNO-1 and BNO-5 (Figs. 3–5). Supplementing spent medium containing AI-1 together with the BNOs to *V. harveyi* MM77, lacking AI-1 and AI-2 also did not evoke bioluminescence as did with AI-2. The findings that neither BNO-1 nor BNO-5 enhanced or inhibited bioluminescence induced by AI-1 in a *V. harveyi* mutant lacking sensor 2 (BB886) further indicates the specificity of the reaction, demonstrating that BNOs act via the AI-2, and not by the AI-1 receptors (Figs. 3–5). In addition, the inability of BNOs solely to induce bioluminescence in *V. harveyi* MM77 (Sensor1$^+$, sensor2$^+$, AI-1$^-$, AI-2$^-$), indicates that BNO1 or BNO-5 cannot fully substitute AI-2 (Figs. 6–8). The effect of the BNOs is therefore a co-agonistic effect where the biological outcome is dependent on the presence of BNO-1 or BNO-5 and AI-2 or pre-AI-2 (DPD) and sensor 2.

BNOs, in the presence of exogenous AI-2, evoked a significant level of bioluminescence. A similar trend was observed when the agents were introduced in the beginning of incubation period of the bacteria or 3 h later or when the sequence of introducing BNOs and AI-2 was altered. These indicate that the effect is non-time dependent and the sequence of exposure of the bacteria to either BNOs or AI-2 is not relevant to the induction of bioluminescence.

The use of structural bacterial AI analogs provides a unique means of manipulating and studying quorum sens-

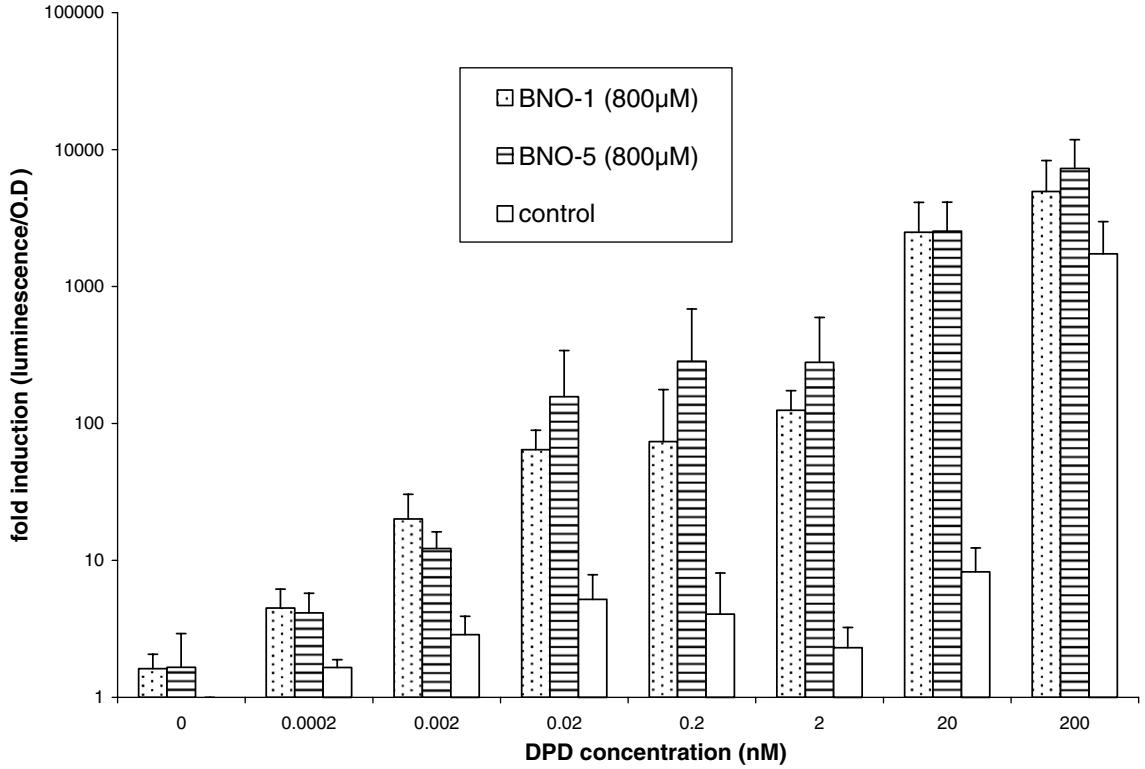

Figure 8. Mutual effect of BNO-1 or BNO-5 and synthetic DPD (pre-AI-2) at different concentrations on induced bioluminescence of *V. harveyi* MM77 (AI-1$^-$, AI-2$^-$). Bacteria were grown for approximately 7 h. Synthetic DPD was added, at various concentrations, to the reaction medium immediately upon the beginning of the bacterial growth together with BNO-1 or BNO-5. Sample without any compound served as negative control. Fold induction above non specific luminescence background was determined at the end of bacterial growth. Constant oxazaborolidine concentration of 800 μM was added. DPD concentrations are in nM.

ing.[5,21,33] The gene *luxS* is found among many types of bacteria and products of LuxS are homologs and are tightly related to bacterial AIs.[36,41] AI-2 agonists and antagonists can affect quorum sensing of a wide range of bacteria, although AI-2-like signals, from various bacterial species, are not identical.[27]

The unexpected finding by Chen et al.[8] that *V. harveyi* AI-2 is a furanosyl borate diester has highlighted boron as a cardinal structural element of AI-2,[9] although it was recently shown that boron is not essential in all bacterial quorum sensing.[23,27] The oxazaborolidines and *V. harveyi* AI-2 are hydrated complexes of heterocyclic boron. Both contain a negatively charged tetra-coordinated boron atom that has the ability to form hydrogen bonds. However, the boron atom in the BNOs is a part of the oxazaborolidine ring, whereas in *V. harveyi* AI-2 it is part of a dioxaborolane ring that does not contain nitrogen and has no aromatic substitute. Both chelation of boron and the position of the hydroxyl moieties in *V. harveyi* AI-2 are critical for activation of sensor-2.[20,32] In addition, BNO-1 and BNO-5 activity indicates that the presence of a five-membered heterocyclic ring containing tetrahedral boron bearing a hydroxyl group is sufficient for specific interaction with *V. harveyi* AI-2 signal transduction, despite the fact that this structure is only slightly reminiscent of *V. harveyi* AI-2. The finding that BNO-1 and BNO-5 induce bioluminescence in *V. harveyi*, but BNO-2, BNO-3, BNO-4 do not, may indicate that the methyl on the nitrogen is an important factor in activation of quorum sensing in *V. harveyi* and that the butyl or phenyl substitutes on the boron are of less importance.

While BNO-1 and BNO-5 share the same amino alcohol moiety, different groups are attached to boron. In BNO-1, the aromatic phenyl group is attached to boron. In BNO-5, the alkyl *n*-butyl group is attached. The fact that the two compounds substantially differ in activity may, therefore, indicate that the aromatic group must interact favorably at the active site, affecting the AI-2 cascade.

We have investigated the hydrated complexes of tested oxazaborolidines and found them to be very stable to hydrolysis in the pH range 5–13, meaning that no free boronate (boronic acid) is released from these compounds as was determined by ^{11}B NMR.[18] In a separate study (data not shown), we demonstrated that BNO-1 does not exchange with $B(OH)_3$. This indicates that an exchange with *V. harveyi* AI-2 is also highly unlikely, and that under the conditions of the above experiment, BNO-1 remains intact. This is reasonable since boronates are stronger Lewis acids than boric acid.

The use of synthetic bacterial AI analogs allows better insight into the quorum sensing cascade. We propose that derivatives of oxazaborolidines may mimic *V. harveyi* AI-2 activity. Based on this study, a full scale screening of more oxazaborolidine derivatives should re-

veal substances with stronger agonistic activity, opening up new possibilities for studies on the structure–function relationships of *V. harveyi* AI-2.

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