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Investigation of quorum sensing inhibition activity of some boron compounds

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ARTICLE INFO

Article History:
Received November 13, 2024
Accepted March 1, 2025
Available online March 31, 2025

Research Article

DOI: 10.30728/boron.1584819

Keywords:
Boron compounds
Quorum sensing inhibition
Violacein reduction

ABSTRACT

Boron is one of the important elements for organisms, especially for plants and animals. The importance of boron for humanity is increasing, and its uses are parallelly expanding. In this study, the quorum-sensing inhibition effects of five different boron compounds were investigated. The quorum-sensing inhibition assay was performed on CV026 and CV12472 using the broth dilution method. Considering the results of this study, it was determined that the highest violacein inhibition activity was provided by sodium tetraborate with 74.46%. Based on the quorum sensing activity, it was revealed that the minimum inhibitory concentration (MIC) value of boron oxide and sodium tetraborate provided the highest inhibition rate 95.15%. When the anti-quorum sensing activity data obtained from the study are examined, it was seen that these boron compounds showed high activity and should be supported by different tests such as animal experiments.

1. Introduction

Boron is an important element for organisms [1]. It is mostly acquired from plant-based boron compounds and boric acid in water. Studies show that boron is essential for certain animals, helping with cell growth and development [2]. Lack of boron can negatively affect bone health, brain function, cholesterol levels, and immune response. Animal studies have also shown that boron can protect against liver damage. Recently, research has focused more on boron's potential in cancer treatment, its antimicrobial properties, its use in drug delivery, and how it interacts with proteins and other molecules. When animals consume boron, it turns into boric acid in the small intestine and is quickly eliminated in urine, without much change. However, small amounts may build up in tissues like bones and the spleen [3]. Boron is often included in supplements and other nutrients like vitamin D, calcium, and magnesium. Some forms of boron, like boric acid and borax, raise concerns because they may accumulate in tissues over time. New forms, like boron esters, are not yet well understood, and current research is working to address this gap. Boron plays several roles in the body, including helping cell signaling, working as a co-factor for certain enzymes, aiding in electron transfer, and help maintaining cell structure. While boron's necessity in animal cells isn't fully proven, future studies may confirm this. It is also believed that boron could have contributed to evolution by forming complexes with certain organic molecules [4]. Boron can be used in pharmaceutical drug design as it has the potential to facilitate biological activities [5]. Although numerous products are produced from boron, the most common natural compounds of boron are various forms of borates. Boron compounds are also used individually across multiple chemical processes. Boron compounds have different uses in pharmaceuticals and other fields such as cosmetics, adhesives, insecticides, formicides, rodenticides, and plant nutritional compounds [6].

Quorum sensing (QS) is a connection system used especially by bacteria and fungi. Quorum-quenching (QQ) disrupts QS signaling and affects how different species interact. Bacteria use QQ to fight other species, while eukaryotes use it to defend against pathogens. QQ can happen in various ways: By stopping the production of signaling molecules, breaking them down, preventing them from binding to receptors, or blocking the expression of target genes. Inhibiting signal molecule production can act by disrupting key enzymes like acyl carrier protein or S-adenosylmethionine synthase, or by preclusion of the Lux system, a key QS system in Gram-negative bacteria [7].

Recently, research has focused more on the potential of boron in cancer treatment, wound healing effects, antimicrobial properties, use in drug delivery, and how it interacts with proteins and other molecules [10, 16]. Moreover, it is seen in the literature that there are studies investigating the cytotoxic [8], antimicrobial, antioxidant, antibiofilm, and neuroprotective [9-11], therapeutic effects [12], and enzyme inhibition [13] of boron compounds and boron derivatives.

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When the present studies in the literature were examined, it was determined that boron compounds have different activities, especially antimicrobial and antioxidant activity. Still, there is no data on anti-quorum sensing and violacein inhibition, especially considering the compounds examined in the study. From this point of view, this study, it was aimed to realize the quorum sensing performance of 5 different boron compounds (disodium octoborate, sodium pentaborate, boron oxide, sodium tetraborate, and boric acid).

2. Materials and Methods

2.1. Materials

2.1.1. Microbial strains

Three microorganisms were used for this study. These are *Pseudomonas aeruginosa* PA01, *Chromobacterium violaceum* CV12472, and *Chromobacterium violaceum* CV026. The density of the bacteria was adjusted to 0.5 McFarland and the test medium was Mueller-Hinton Broth (MHB).

2.2. Methods

2.2.1. Establishing of minimal inhibitory concentration (MIC)

200 µL of cell suspension was inoculated with different final concentrations of boron compounds (20, 10, 5, 2.5, 1.25, 0.625 mg/mL) in 96-well plates. The microplates were incubated at 37°C for 24 hours after inoculation.

2.2.2. Violacein inhibition test over C. violaceum CV12472

Firstly, the MIC values of the compounds were determined to establish the violacein inhibition. The MIC value was based on the broth dilution method and recorded as the lowest concentration at which no growth was observed [14]. Inhibition percentages against *C. violaceim* ATCC CV12472 at MIC and sub-MIC concentrations were then determined. Briefly, 10 µL of CV12472 was added to 180 µL of LB broth containing microplates, and 10 µL of compounds at MIC and sub-MIC concentrations were added. Then the plates were incubated at 30°C for 24 h. The reduction of violacein pigment was measured at 600 nm on a microplate reader. The study was carried out in triplicate. Violacein percentage inhibition was calculated by using Equation 1 [15].

$$\text{Violacein inhibition } (\%) = \frac{\text{OD 600 control} - \text{OD 600 sample}}{\text{OD 600 control}} \times 100 \quad \textbf{(1)}$$

2.2.3. Quorum sensing inhibition assay using C. violaceum CV026

The anti-quorum sensing test was carried out by using a method from a previous study [16]. First, 100 µL of an overnight culture of CV026 bacteria and 20 µL of C6HSL (a signaling molecule) were added to warm soft top agar (5 mL). The mixture was then spread on LB agar plates. After the agar solidified, small wells (5 mm) were made in the plates, and 50 µL of a filter-sterilized extract solution was added to each well at a concentration below the MIC. The plates were incubated at 30°C for 3 days. The experiment was conducted in three repetitions. Quorum sensing inhibition was calculated as a percentage using Equation 2.

$$\text{QS Inhibition } (\%) = \frac{\text{OD 600 control} - \text{OD 600 sample}}{\text{OD 600 control}} \times 100 \quad \textbf{(2)}$$

2.2.4. Statistical analysis

STATISTICA software (StatSoft Inc.,USA.) was used to calculate statistically significant differences between groups. One-way analysis of variance (ANOVA) followed by post-hoc Tukey's test was used to determine differences in measured parameters. All analyses were performed at a significance level of $p < 0.05$.

3. Results and Discussion

When the potential of boron compounds against test bacteria for anti-quorum sensing activity was examined, the MIC value was detected against CV026. The lowest MIC value was revealed by disodium octoborate with <0.625 mg/ml. Furthermore, sodium pentaborate, boron oxide, and boric acid also exhibited good activity against CV026 with a MIC value of 0.625 mg/ml (Table 1).

Table 1. MIC values of boron compounds

	Minimal Inhibitory Concentration (mg/mL)		
	C. violacaeum (CV026)	*C. violacaeum* (CV12472)	*P. aeruginosa* (PA01)
Disodium Octoborate	<0.625	2.5	1.25
Sodium Pentaborate	0.625	2.5	2.50
Boron Oxide	0.625	2.5	1.25
Sodium Tetraborate	1.250	5.0	2.50
Boric Acid	0.625	5.0	2.50

Numerous studies have been conducted on the antimicrobial activity of borons, which is highly effective against different microorganisms [17, 18]. Baygar et al. [19] investigated the biological activities of potassium metaborate in their study and revealed that it showed high activity in terms of antioxidative, antimicrobial, and anti-biofilm properties. In another study, the antimicrobial, antifibrinolytic, enzyme inhibitory, and wound-healing properties of zinc borate were examined, and it was declared that it could be used effectively in wound healing and can prevent wound infection [20]. However, there are limited or no studies on anti-quorum sensing and violacein inhibition of boron compounds. C. violaceum is a Gram (-) bacterium that is detected in different environments such as human skin, soil, and water. Bacteremia and abscesses are serious infections they cause. It is also

resistant to many antibiotics. The bacteria secrete a purple pigment called violacein, which has antibacterial effects. The expression of this pigment is controlled by quorum sensing, a bacterial communication process that regulates several functions, including antibiotic production and biofilm formation [21]. When the results of violacein inhibition were analyzed, it was found that all MIC and sub-MIC concentrations had activity. In addition, it was observed that all compounds inhibited violacein production even at the MIC/32 level (Figure 1).

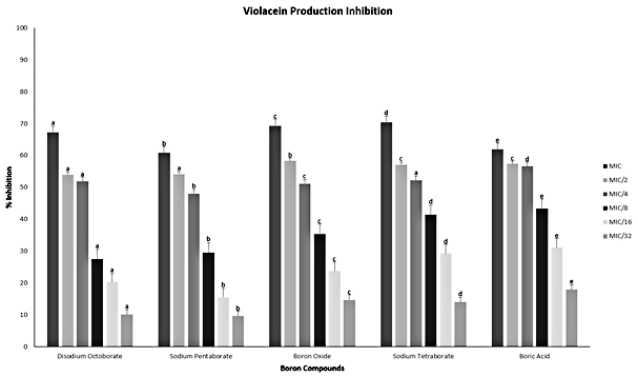

Figure 1. Violacein production inhibition of boron compounds. Data are presented as mean ± scanning electron microscope (SEM) of three replicates. Different letters above the error bars indicate significant differences between treatments ($p < 0.05$)

QS is a cellular signaling complex that lets bacteria adapt to the environment through cooperative and coordinated metabolic regulation. QS is particularly well characterized in Gram-negative bacteria and triggers the synthesis of various defense molecules (extracellular hydrolytic enzymes, virulence factors, and biofilms). This process involves synthesizing and releasing into the environment signaling molecules (such as AHLs) that affect transcription and translation [22-25]. According to the anti-quorum sensing activity results obtained from the study, boron compounds showed inhibition between 37-95% (Figure 2).

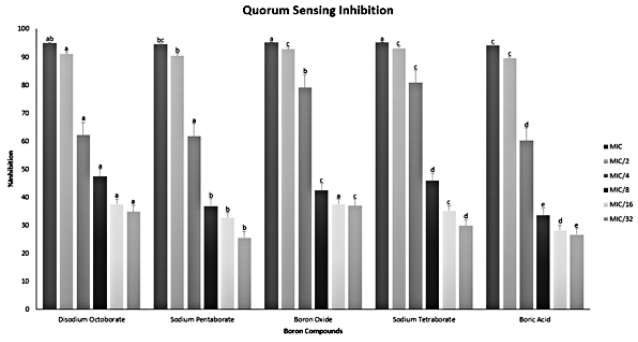

Figure 2. Quorum sensing inhibition of boron compounds. Data are presented as mean ± SEM of three replicates. Different letters above the error bars indicate significant differences between treatments ($p < 0.05$)

In the literature, it was reported that boron compounds showed high activity against antioxidants, enzyme inhibition, antibiofilm, and cancer cell lines [26, 27]. Therefore, the inhibitory potential of these compounds on the quorum sensing mechanism is parallel with our study results. This reveals that boron compounds can disrupt the quorum-sensing mechanism that provides cellular communication and reduces the cellular defense and virulence factor.

Our current knowledge has now revealed that boron compounds have a wide range of uses. Sevim et al. [28] investigated the anti-inflammation potential of boric acid and borax and reported that potential therapeutic effects were achieved in improving tissue damage even at low doses. Temel et al. [29] investigated the antimicrobial, antioxidant, cytotoxic and enzyme inhibition activities of boric acid and its derivatives. They emphasized that the new compounds showed a dominant potential in terms of antioxidants did not show cytotoxic effects, and showed antimicrobial activity of 6.50 mg/mL against E. coli. Another study revealed the wound-healing potential of boron [30]. Another study focused on the use of boron-containing compounds for triggering the immune system [31]. In another similar study, the antimicrobial, enzyme inhibition, and anti-cancer activity of quercetin-boronate esters were determined, and based on the antimicrobial activity, they showed superior glucosidase enzyme inhibition and exhibited significant anti-cancer activity with an MIC of 32.5 µg/mL against E. faecalis. These compounds demonstrated superior glucidase enzyme inhibition and exhibited significant anti-cancer activity, reducing pancreatic cell viability at 50 µM [32]. However, studies on anti-quorum sensing activity have been very limited, and this issue needs to be focused on. The inhibition of this mechanism, which is of great importance in bacterial communication and virulence, can be achieved using natural products, particularly boron compounds that are essential for organisms. This approach can not only prevent the activation of bacterial resistance development mechanisms but can also reduce the costs associated with synthetic product production. Moreover, the gap in the literature on this subject will be filled to some extent. According to the results, the quorum-sensing inhibition exhibited by the compounds is remarkable (Figure 3). Figure 3 shows that the compounds showed quorum-sensing inhibition even at the MIC/32.

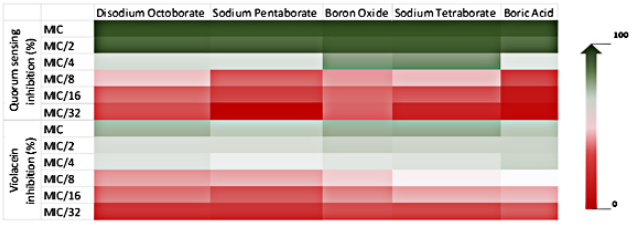

Figure 3. Heatmap of boron compounds of inhibition (%) quorum sensing and violacein production

4. Conclusions

This study reveals the potential of boron compounds to inhibit quorum-sensing mechanisms. Studies have shown that boron-containing molecules can interfere

with the quorum-sensing signaling mechanisms of gram-negative and gram-positive bacteria, thereby reducing pathogenic effects. In particular, boron compounds were found to disrupt bacterial communication and inhibit biofilm formation by interacting with quorum-sensing signaling molecules (e.g., AHL or AI-2). However, the specific effects of these compounds vary depending on bacterial species, concentrations used, and environmental factors. The findings indicate the potential of boron compounds to be evaluated as new anti-QS agents. However, extensive biochemical and pharmacological studies are required to develop these compounds as effective, safe, and specific antibacterial agents. Future research on the anti-QS activity of boron compounds may provide important contributions to developing new therapeutic strategies, especially against pathogens of clinical importance. Based on this study, further studies can be designed to understand the biological activities of boron-containing compounds.

5. Conflict of Interest

The authors declare no conflict of interest.

6. Author Contribution Statement

Özgür Ceylan: Sample and data collection, conceptualization, data curation, organization, analysis, methodology.

Aysel Uğur: Supervision, project administration.

Kutbettin Arslan: Data analysis, writing original draft, writing review, and editing.

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