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In Vitro Antibacterial Activity Evaluation of (2Z,3R,6S)-4-Hydrazono-3,6-dimethyl-2-(3-methylbutylidene)octahydrobenzofuran-3-ol

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ABSTRACT

The antibacterial activity of (2Z,3R,6S)-4-hydrazono-3,6-dimethyl-2-(3-methylbutylidene) octahydrobenzofuran-3-ol on Bacillus subtilis and Staphylococcus aureus were studied. The in vitro antibacterial circle and minimum inhibitory concentration (MIC) were used to evaluate its antibacterial effect. The results showed that the compound antibacterial circles (at 1 μg/mL) on Bacillus subtilis and Staphylococcus aureus were 19 ± 1.12 mm and 13 ± 0.94 mm; MIC of Bacillus subtilis was 0.0166 mg/mL. The novel compound has a strong antibacterial activity in vitro, especially on Bacillus subtilis.

Keywords: Antibacterial activity; (2Z,3R,6S)-4-Hydrazono-3,6-dimethyl-2-(3-methylbutylidene) octahydrobenzofuran-3-ol; *Bacillus subtilis*; *Staphylococcus aureus*

INTRODUCTION

Bacillus subtilis and *staphylococcus aureus* belong to a common Gram-positive bacterium [1-3]. *Bacillus subtilis* has two growth stages: spore sleep period and reproductive growth period. It has strong adaptability to the environment, and can also survive in high temperature, acid and alkali polar environment. Once the environment becomes suitable for growth, the spores will automatically enter the reproductive growth phase, and the spores will re-grow into *Bacillus subtilis* [4,5]. Both Gram-positive bacteria can cause infection in humans [6,7]. Among them, *Staphylococcus aureus* can cause diseases such as pneumonia and pericarditis [8]. So in this study, antibacterial activity of a new compound (2Z,3R,6S)-4-hydrazono-3,6-dimethyl-2-(3-methylbutylidene) octahydro benzofuran-3-ol (compound **1**) synthesized as a proton pump inhibitor by our group recently [9] was evaluated on *Bacillus subtilis* and *Staphylococcus aureus*. The chemical structure of compound **1** is shown in Figure 1 below.

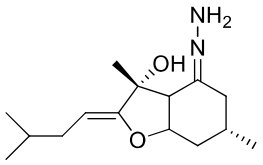

Figure 1. Chemical structure of compound 1

MATERIALS AND METHODS

Preparation of Bacterial Solution

The slanted surface of the cultured bacteria is transferred to the purification workbench, and after being placed at room temperature, the inoculating loop is used to inoculate a suitable medium, and the inoculated bacterial tube is cultured at 37℃ for 18 hours, and the medium is diluted to a certain concentration.

Determination of *In Vitro* Antibacterial Activity

The cultured bacteria were made into a certain concentration of suspension, take 1mL of the bacterial rotate the culture dish and set it on the plate. The compound is formulated into several required concentration. A piece of filter paper is picked up by a sterile forceps to be soaked in the compound solution. When the clip is taken out, it is stopped at the edge of the container for a while, the excess liquid is filtered out, and the filter paper piece is placed on the flat plate. In the center of the culture medium, press the paper piece tightly against the medium to make it seamless with the surface of the plate. A filter paper soaked in sterilized water was used as a blank control. The culture dishes were incubated at 37°C for 18 h. The diameter of antibacterial circle was measured by a vernier caliper cross method. The experiments were triply performed in parallel. The results were judged as followed: the inhibition zone <10mm was insensitive, 10mm-19mm was moderately sensitive, and ≥ 20mm was highly sensitive.

Determination of MIC

The compound was dissolved by a micro method of to prepare 1000 μg/mL drug mother liquor. On a 96-well cell culture plate, add 100 μL of 3% NaCl nutrient broth to each well of each row, and then dilute 100 μL of 1000 μg/mL drug mother liquor from the first well to the 10th well. Then the 11th wells were the drug-free control and 12th wells were blank controls. Add 100 μL of diluted 105 broth to 1-11 wells per well, add 10 μL of sterilized seawater to the 12^{th} well, mixing well, and place in a 30°C incubator for 24 hours and observe on a black background. The lowest compound concentration to completely inhibit bacterial growth in the small holes is the MIC. Each experiment was triply performed.

RESULTS AND DISCUSSION

In vitro Bacteriostatic Activity

The solution of compound **1** was diluted 10, 100, 1000, and 10,000 times to examine the diameter of the antibiotics antibacterial circle against *S. aureus* and *B. subtilis*. The results are shown in Table 1. When the compound solution is diluted 10, 100, 1000, 10000 times, the diameter of compound **1** against *B. subtilis* and *S. aureus* was 11, 19, 19, 12 mm, 15, 13, 13, 12 mm, respectively. It can be seen from Figure 2 that under the conditions of 100 times and 1000 times, the inhibition effect of the compound on *B. subtilis* is better than that of *S. aureus*, and the difference is obvious. Under the condition of 10000 times, there was almost no difference in the bacteriostatic effect between *B. subtilis* and *S. aureus*.

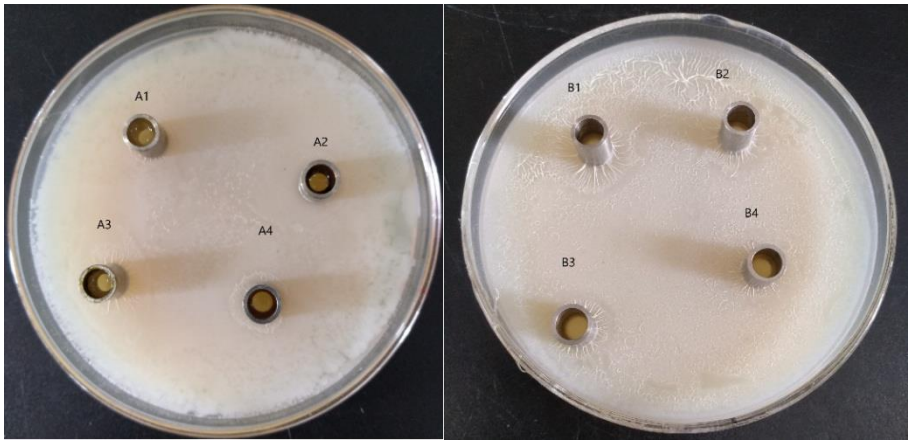

Figure 2. *In vitro* antibacterial test results of diffusion method

Note A: The inhibition effect of compound **1** on *B. subtilis*; B: the inhibition effect of compound **1** on *S. aureus*; C: the inhibition effect of Penicillin on *B. subtilis*; D: the inhibition effect of Penicillin on *S. aureus.* A1-A4 and B1-B4 are diluted 10 times, 100 times, 1000 times, and 10,000 times of compound **1** respectively. C1-C4 and D1-D4 are diluted 10 times, 100 times, 1000 times, and 10,000 times of penicillin respectively.

Table 1. Antibacterial circle size of different compound concentrations

Compound	**Compound 1**				**Penicillin**			
Concentration (μg/mL)	100	10	1	0.1	100	10	1	0.1
B. subtilis (antibacterial circle /mm)	11 ± 0.85	19 ± 1.10	19 ± 1.12	12 ± 0.86	11 ± 0.79	11 ± 0.83	11 ± 0.88	22 ± 1.25
S. aureus (antibacterial circle/mm)	15 ± 1.06	13 ± 0.89	13 ± 0.94	12 ± 0.91	11 ± 0.82	12 ± 0.95	20 ± 1.20	30 ± 2.37

MIC of Compound 1 and Penicillin for *B. subtilis*

The MIC of compound **1** and penicillin against *B. subtilis* was determined by microdilution method (Table 2). The MIC of compound **1** is at the 4th dilution (0.0166 mg/mL), which shows that compound **1** can inhibit *B. subtilis* at a low concentration.

Table 2. Determination of MIC of compounds against *B. subtilis*

Dilution gradient	**2^1**	**2^2**	**2^3**	**2^4**	**2^5**	**2^6**	**2^7**	**2^8**	**2^9**	**2^{10}**	**N**	**P**	**MIC**
Compound 1	++	++	++	—	—	—	—	—	—	—	—	++	0.0166 mg/mL
Penicillin	++	++	++	++	++	++	++	++	—	—	—	++	0.0003 mg/mL

Note: “N” negative control; “P” positive control; “-” medium clarification; “+” medium turbiditys lightly; “++” medium turbiditydis.

CONCLUSION

In this experiment, the bacteriostatic test was carried out by using the tube dish method and the microdilution method to determine the minimum inhibitory concentration (MIC). The results show that the synthesized novel proton pump inhibitor (2Z,3R,6S)-4-hydrazono-3,6-dimethyl-2- (3-methylbutylidene) octahydrobenzofuran-3-ol has the effect of inhibiting *B. subtilis* and *S. aureus* at low concentration.

ACKNOWLEDGEMENTS

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