«Силиплантом» отличается наибольшими таксационными показателями, но с сильной вариативностью значений.

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In vitro antimicrobial activity of 2,4-Diacetyl-5-hydroxy-5-methyl-3-phenyl-N-oxyethyl-1-cyclohexenylamine

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Abstract

Development of antimicrobial resistance is one of the major problems in treatment and eradication of infectious diseases. Functionally substituted organic compounds are main focus of drug development studies. We report antimicrobial properties of 2,4-Diacetyl-5-hydroxy-5-methyl-3-phenyl-N-oxyethyl-1-cyclohexenylamine against four Gram-positive bacteria and four Gram-negative bacteria. Initially, agar well diffusion method was used to evaluate antimicrobial properties of test compound at three different concentrations. Subsequently, minimum inhibitory concentration (MIC) values were determined by Resazurin assay. The test compound showed moderate to strong antimicrobial properties against Gram-negative bacteria. Gram-positive bacteria were found to be resistant against the test compound. *Escherichia coli* BDU-12 was found to be most susceptible test culture with zone of inhibition 25.3 mm and MIC 125 µg/ml while *Pseudomonas aeruginosa* BDU-49 was found to be least susceptible test culture with zone of inhibition 12.3 mm and MIC 2000 µg/ml.

Keywords: Antimicrobial resistance, Drug development, MIC, Agar well diffusion, Resazurin assay

Introduction

During last two decades, development of resistance to antimicrobial drugs is a major health problem. Drug development studies are focusing synthetic organic compounds for exploring new drugs [1]. It is thought that functionally substituted cyclohexane derivatives could act as probable antimicrobial agents of the future. This is due to the fact that functionally substituted cyclohexane derivatives possess wide range of biological properties [2]. There are very few reports regarding antibacterial activity of functionally substituted cyclohexane derivatives. Here we report the *in vitro* antibacterial properties of 2,4-Diacetyl-5-hydroxy-5-methyl-3-phenyl-N-oxyethyl-1-cyclohexenylamine against four Gram-positive bacteria and four Gram-negative bacteria.

Materials and Methods

2,4-Diacetyl-5-hydroxy-5-methyl-3-phenyl-N-oxyethyl-1-cyclohexenylamine was synthesized as described by Kriven'ko *et al.*, 2003 [3]. *In vitro* antibacterial properties were determined against four Gram-negative bacteria (*Escherichia coli* BDU-12, *Klebsiella pneumoniae* BDU-44, *Acinetobacter baumannii* BDU-32 and *Pseudomonas aeruginosa* BDU-49) and four Gram-positive bacteria (*Staphylococcus aureus* BDU-23, *Bacillus Subtilis* BDU-50, *Bacillus mesentericus* BDU-51 and *Bacillus megaterium* BDU-N2). All the test cultures

were obtained from our own collection at Department of Microbiology, Baku State University Azerbaijan. Test compound was taken from Department of organic chemistry, Baku State University Azerbaijan.

Figure 1: Structure of 2,4-Diacetyl-5-hydroxy-5-methyl-3-phenyl-N-oxyethyl-1-cyclohexenylamine [3]

Initially, standard agar well diffusion method [4] was used to determine antibacterial properties of test compound at three different concentrations i.e. 0.3%, 0.1% and 0.05%. Due to its inert nature, Dimethyl sulphoxide (DMSO) was used as solvent to dissolve the test compounds. All the test cultures were grown on Mueller-Hinton agar. Briefly, 100 μ L of 24 hour fresh broth culture (0.5 McFarland) of each bacterium was aseptically spread over agar surface. Wells with diameter 8 mm were punched aseptically in the agar plate by cork borer and 150 μ L of test compound was added in each well. Agar plates were incubated at 37°C for 24 hours. After incubation, zone of inhibition was recorded carefully. All the experiments were performed thrice and DMSO was used as control. Minimum inhibitory concentration values were determined by using Resazurin microplate assay in 96 well microtitre plates [5]. For each test bacterium, density of 24 hour fresh broth culture was adjusted to 0.5 McFarland in Mueller-Hinton medium. Serial twofold dilutions of test compound in 100 μ L of Mueller-Hinton broth were prepared. 8–2000 mg/mL range of test compound was evaluated. Sterility controls and growth controls were also maintained during experiment. Titer plate was covered and incubated at 37°C for 24 hours. After incubation, 30 μ L of resazurin dye solution was poured into each well, and the plate was again incubated for 3-4 hours. A change in color of resazurin from blue to pink indicated the growth of bacteria. Lowest concentration of compound that inhibited the change in color was defined as minimum inhibitory concentration.

Results and Discussions

The antibacterial activity of 2,4-Diacetyl-5-hydroxy-5-methyl-3-phenyl-N-oxyethyl-1-cyclohexenylamine was evaluated against four Gram-negative bacteria and four Gram-positive bacteria. Test compound demonstrated variable antimicrobial properties against different kinds of bacteria. Antimicrobial profile of test compound is shown in table 1. It is evident that test compound was more active against Gram-negative bacteria as compared to Gram-positive bacteria. Our findings are concurrent with [6], who showed that monocyclic and spirocyclic cyclohexane derivatives were more active against Gram-negative bacteria as compared to Gram-positive bacteria. The findings of [7] contradict our results, which is due to difference in structure of the test compound. All the Gram-positive bacteria were found to be resistant against the test derivative.

Results of agar well diffusion method demonstrated that *Escherichia coli* BDU-12 was most sensitive test culture with average diameter of zone of inhibition 25.3 mm at 0.3% concentration of test compound. Test compound inhibited the growth of *Escherichia coli* BDU-12 at 0.1% and 0.05% concentration. None of other test culture was found to be susceptible at 0.05% concentration. Test compound exhibited moderate antimicrobial

properties against *Klebsiella pneumoniae* BDU-44, *Acinetobacter baumannii* BDU-32 and *Pseudomonas aeruginosa* BDU-49.

Table 1.

Antibacterial profile of test compound

Test Culture	Average diameter of zone of inhibition (mm)				MIC ($\mu\text{g/mL}$)
	0.3%	0.1%	0.05%	DMSO	
<i>Escherichia coli</i>	25.3 ± 0.3	18 ± 0.0	12.7 ± 0.3	-	125
<i>Klebsiella pneumoniae</i>	17 ± 0.0	-	-	-	1000
<i>Acinetobacter baumannii</i>	19.7 ± 0.1	-	-	-	500
<i>Pseudomonas aeruginosa</i>	12.3 ± 0.4	-	-	-	2000
<i>Staphylococcus aureus</i>	-	-	-	-	NE
<i>Bacillus Subtilis</i>	-	-	-	-	NE
<i>Bacillus megaterium</i>	-	-	-	-	NE
<i>Bacillus mesentericus</i>	-	-	-	-	NE

Note: (-): inactivity, (NE): not evaluated

Results of agar well diffusion method were validated by *Resazurin microplate assay* in 96 well microtitre plates. Minimum inhibitory concentration values of test compounds showed that *Escherichia coli* BDU-12 was most susceptible test culture with MIC value of 125 $\mu\text{g/mL}$. MIC value for *Acinetobacter baumannii* BDU-32 was found to be 500 $\mu\text{g/mL}$. *Resazurin assay* showed that compound showed antimicrobial properties against *Pseudomonas aeruginosa* BDU-49 at concentration of 2000 $\mu\text{g/mL}$. It is concluded that test compound has substantial antimicrobial profile against Gram-negative bacteria. Therefore, effect of test compound on growth curve of test cultures and time kill kinetics should be determined.

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