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Short Communication

Antimicrobial Activity of Novel Functionally Substituted Monocyclic and Spirocyclic Cyclohexane Derivatives

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Authors' Contribution
MS, KG and AI designed the study and conducted the experiment. AI provided chemical compounds. AI and SU helped in writing the manuscript.

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ABSTRACT

Due to perpetual increasing antimicrobial resistance, functionally substituted alicyclic compounds are envisioned as probable antimicrobial agents of future. From screening of in house library of compounds, here we report the antimicrobial properties of three novel functionally substituted monocyclic and spirocyclic cyclohexane derivatives involving ethyl-3 (allylamino)-9,9-dimethyl- 7,11-dioxo-1,5-diphenylspiro[5.5]undec-2-en-2-carboxylate (I), ethyl-4,6-diphenyl-2- dicyanomethylene cyclohex-3-ene 1-carboxylate (II) and ethyl-4-phenyl-6-(4-chlorophenyl)-2- dicyanomethylenecyclohex-3-ene 1-carboxylate (III).Initially, these compounds were screened for *in vitro* antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi by agar well diffusion method. In second step, minimum inhibitory concentration of test compounds was determined against bacterial species by using resazurin *microplate assay*. All the tested compounds exhibited variable antimicrobial properties against various test cultures. All the compounds showed stronger antimicrobial activity against Gram-negative bacteria as compared to Gram-positive bacteria and fungi. Compound III was found to be the most effective compound. These results demonstrate the potential antimicrobial properties of mono and spiro cyclohexane derivatives and accentuate the need of *in vivo* trials for their application at clinical level.

Antimicrobial resistance is global health emergency for both humans and animals in recent times. Treatment and eradication of infectious diseases is mainly hampered by ever increasing antimicrobial resistance and shortage of new antimicrobial drugs (Shoaib and Ganbarov, 2019). Numerous microorganisms like methicillin-resistant *Staphylococcus aureus,* multidrug resistant *Escherichia coli,* drug resistant *Mycobacterium tuberculosis,*multidrug resistant *Pseudomonas aeruginosa*. pose greater challenge for clinicians for treatment of infectious diseases (Iqbal *et al*., 2016; Hussain *et al*., 2017; Li and Webster, 2018). Currently there is emergence of pan drug resistant bacteria showing resistance to almost all the available antibiotics in the market (Bielawski *et al*., 2017). Drug development studies focus on functionally substituted chemical compounds with potential antimicrobial properties and their unique mode of action to overcome antimicrobial resistance (Tsemeugne *et al*., 2018).

Functionally substituted cyclohexane derivatives possess diverse biological properties. These include anticancer activity (Sharma *et al*., 2011; Lallo *et al*., 2014; Flefel *et al*., 2014; Song *et al*., 2015), antioxidant activity (Flefel *et al*., 2014), analgesic activity (Amin *et al*., 2010) and anti-inflammatory activity (Usegilo *et al*., 2006). Antimicrobial activities of numerous cyclohexane derivatives have also been reported. Adamantyl based cyclohexane diamine derivatives showed antibacterial properties against methicillin-resistant *Staphylococcus aureus* and *Mycobacterium tuberculosis* (Beena *et al*., 2014). Cyclohexane diamine derivatives exhibited considerable antimicrobial properties against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis* (Kumar *et al*., 2011). Dibenzyl-cyclohexane-1, 2-diamine derivatives

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demonstrated excellent antifungal properties against *Candida albicans*, *Candida glabrata* and *Geotrichum candidum* (Sharma *et al*., 2011). Keeping in mind the broad range of antimicrobial properties of cyclohexane derivatives, we report antimicrobial potential of newly synthesized functionally substituted monocyclic and spirocyclic cyclohexane derivatives in present study.

Materials and methods

Functionally substituted monocyclic and spirocyclic cyclohexane derivatives; ethyl-3(allylamino)-9,9-dimethyl- 7,11-dioxo-1,5-diphenylspiro[5.5]undec-2-en-2-carboxylate (I), ethyl-4,6-diphenyl-2- dicyanomethylene cyclohex-3-ene 1-carboxylate (II) and ethyl-4-phenyl-6-(4-chlorophenyl)-2- dicyanomethylenecyclohex-3-ene 1-carboxylate (III) were obtained from Department of Organic Chemistry, Baku State University Azerbaijan. Structure of these compounds is shown in Figure 1. Test compounds were evaluated for in vitro antibacterial and antifungal activity against Gram-positive bacteria (*Staphylococcus aureus BDU-23, Bacillus subtilis BDU-50, Bacillus mesentericus BDU-51* and *Bacillus megaterium BDU-N2*), Gram-negative bacteria (*Escherichia coli* BDU-12, *Klebsiella pneumoniae* BDU-44, *Acinetobacter baumannii* BDU-32 and *Pseudomonas aeruginosa* BDU-49) and fungi (*Candida tropicalis BDU LK30, Candida pelliculosa BDU KT55* and *Candida pseudotropicalis BDU MA88*). All the test cultures were obtained from our own collection at Department of Microbiology, Baku State University Azerbaijan.

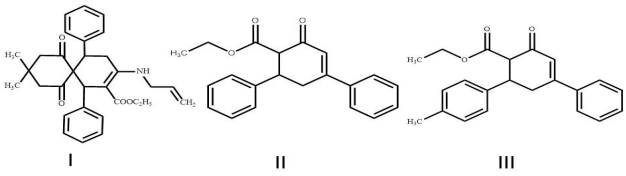

Fig. 1. Structure of compounds used in this study (Ismiev *et al*., 2018; Magerramov *et al*., 2014).

Test compounds were dissolved in Dimethyl sulphoxide (DMSO) and three different concentrations of test compounds were prepared; 0.3%, 0.1% and 0.05%. Standard agar well diffusion assay as described by (Balouiri *et al*., 2016) was used to determine diameter of zone of inhibition. Mueller-Hinton agar was used for antibacterial activity and sabouraud dextrose agar was used for antifungal screening. Briefly, 100 µL of 24 hour fresh broth culture (0.5 McFarland) of each test culture was aseptically spread over agar surface. Wells with diameter 8 mm were punched aseptically in the agar plate and 150 µL of test compound was poured in each well. Agar plates were incubated at 37°C for 24 hours for bacteria and 30°C for 72 hours for fungi.Diametre of zone of inhibition was measured.All the experiments were performed in triplicates and DMSO was used as control.

Resazurin microplate assay was used to determine minimum inhibitory concentration (MIC) as described by Palomino *et al*. (2002). Briefly, bacterial inoculum was prepared from 24 hour fresh broth culture and density was adjusted to 1×10^5 colony forming units per mL. The resazurin microtiter assay (REMA) plate method was performed in Mueller-Hinton broth. Serial twofold dilutions of each test compound in 100 µL of medium were prepared. The range of concentrations tested was 8–2000 mg/mL. Sterility controls and growth controls with no test compound were also included. Titer plate was covered and incubated at 37°C for 24 hours. After incubation, 30 µL of 0.01% resazurin 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 indicates the growth of bacteria. Lowest concentration of compound that inhibited the change in color was defined as MIC.

Results and discussion

The antimicrobial activity of functionally substituted monocyclic and spirocyclic cyclohexane derivatives were determined against four Gram-negative bacteria, four Gram-positive bacteria and three fungi. Test compounds showed better antimicrobial activity against Gram-negative bacteria as compared to Gram-positive bacteria. These results contradict the findings of Urzua *et al*. (2008), who reported that benzofuran cyclohexane-5-carboxylate derivatives have better activity against Gram-positive bacteria. This difference in the findings can be attributed to different nature of functional group in cyclohexane. In agar well diffusion assay, all the tested compounds exhibited remarkable antimicrobial properties at concentration of 0.3% (Fig. 2). Moderate to weak antibacterial activity was observed against some of pathogen at concentration of 0.1% and 0.05% (Table I). *Candida pelliculosa* was most resistant strain as none of tested compound inhibited its growth. *Candida tropicalis* and *Candida pseudotropicalis* were found to be sensitive at 0.3% concentration, showed resistance at 0.1% and 0.05% concentration. These findings are supported by Sharma *et al.* (2011) who demonstrated antifungal activity of cyclohexane diamine derivatives against *Candida* sp. Among Gram-positive bacteria, *Staphylococcus aureus* was more sensitive as compared to *Bacillus species* which coincides with findings of Beena *et al*. (2014) who reported moderate antibacterial activity of adamantyl based cyclohexane derivatives against *Staphylococcus aureus*. *Acinetobacter baumannii* was most sensitive bacteria with zone of inhibition 27.3 mm, 25.3 mm and 24 mm for compounds I, II and III respectively.

Table I. Average diameter of zone of inhibition (mm) and Minimum inhibitory concentrations (µg/mL).

Test Culture	Diameter of zone of inhibition (mm)							Minimum inhibitory concentrations (µg/mL)		
	I		II		III		DMSO	I	II	III
	0.1%	0.05%	0.1%	0.05%	0.1%	0.05%				
Escherichia coli	-	-	16.5±0.2	13.4±0.3	16±0.2	13±0.4	-	1000	500	250
Klebsiella pneumoniae	-	-	17.7±0.3	12.7±0.6	15±0.5	12±0.3	-	500	500	250
Acinetobacter baumannii	25±0.8	14±0.3	22.3±0.5	-	20.3±0.4	16±0.3	-	500	250	125
Pseudomonas aeruginosa	-	-	15.7±0.3	12.7±0.2	18±0.3	14.3±0.6	-	1000	1000	500
Staphylococcus aureus	15.3±0.3	12.3±0.3	-	-	15.3±0.1	13.3±0.4	-	2000	-	500
Bacillus subtilis	-	-	-	-	-	-	-	2000	500	1000
Bacillus megaterium	14.7±0.1	12.5±0.1	-	-	12±0.2	-	-	-	-	1000
Bacillus mesentericus	-	-	-	-	15±0.2	-	-	-	-	1000
Candida tropicalis	-	-	-	-	-	-	-	NA	NA	NA
Candida pelliculosa	-	-	-	-	-	-	-	NA	NA	NA
Candida pseudotropicalis	18.7±0.2	12.7±0.1	-	-	-	-	-	NA	NA	NA

(-): Inactivity; NA: not available.

Monocyclic cyclohexane derivatives showed better antimicrobial activity as compared to spirocyclic derivative. Compound III was found to be most potent antimicrobial agent at all the three concentrations. This can be attributed to lipophilicity of the compounds. Antimicrobial activity increases with increase in number of hydrophobic groups (Urzua *et al*., 2008). Results of agar well diffusion assay were validated by resazurin microplate assay (Table I).

From Gram-negative bacteria, MIC values were found to be ≤1000 µg/mL. Compound III showed least MIC value (125µg/mL and 250µg/mL) against *Acinetobacter baumannii* and *Escherichia coli* respectively, which demonstrated maximum activity of this compound. Bogdanov *et al*. (2007) and Nief *et al*. (2017) also reported that *Escherichia coli* was most sensitive bacteria against spirobenzo pyran and phenyl cyclohexane derivatives. From Gram-positive bacteria, MIC values were found to be between 1000-2000 *µg/mL*. Compound III showed strong antimicrobial activity, while compound II and I exhibited moderate to weak antimicrobial activities. Keeping in view the antimicrobial potential of functionally substituted cyclohexane derivatives, further synthesis and exploration of these compounds or their hybrid analogues as broad spectrum antimicrobial drugs should be envisioned.

Conclusion

Functionally substituted monocyclic and spirocyclic cyclohexane derivatives exhibited remarkable antimicrobial properties against various test cultures. Gram-Negative bacteria were found to be more sensitive to these compounds as compared to Gram-Positive bacteria. Compound III showed strong antibacterial activity against *Escherichia coli* and *Acinetobacter baumannii*. Test compounds demonstratedweak to moderate antifungal activity. Findings depict that these substancescan act as potential future antimicrobial agents.

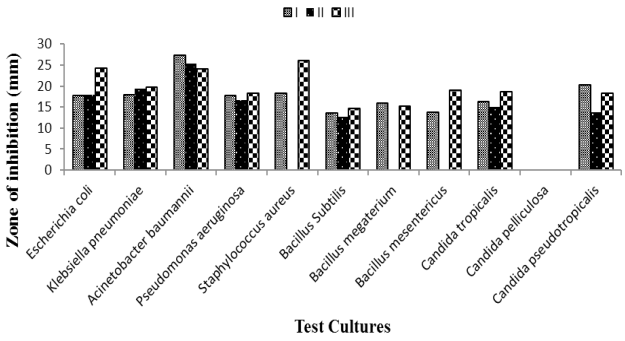

Fig. 2. Average zone of inhibition (mm) at 0.3% concentration.

Statement of conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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