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Short Communication

Two Synthetic Organic Compounds Containing Phosphorus against Pathogenic Microorganism

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ABSTRACT

In accordance with the global action plan on antimicrobial resistance established by the WHO, and with the aim of obtaining better therapeutic agents more effective than conventional antibiotics, we evaluated the antimicrobial activity of two synthetic organic compounds containing phosphate, i.e. (((cyanomethyl) (ethoxy) phosphoryl) oxy) zinc (II) chloride (compound I) and (Z)-(1-(3-(dichlorophosphoryl)-3-methyl-4-oxooxetan- 2-ylidene) ethyl) phosphonic dichloride (compound II), against ten pathogenic microorganisms including four Gram-negative bacteria (*Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginisa*), three Gram-positive bacteria (*Bacillus mesentericus, Bacillus subtilis* and *Staphylococcus aureus*) and three yeasts (*Candida albicans, Candida guilliermondii* and *Candida tropicalis*). Agar well diffusion method was applied to estimate zone of inhibition, and the double dilution method of compounds was used to determine the minimum inhibitory concentration (MIC) of both test compound. The results obtained showed an excellent antimicrobial potential of compounds against test cultures with inhibition zones ranging from 34.2 mm to 39.3 mm for compound I, and from 35.5 mm to 41.2 mm for compound II against Gram-negative and Gram-positive bacteria. The diameters of inhibition zones for antifungal activity varied from 26.3 mm to 28.0 mm for compound I, and from 30.3 mm to 31.0 mm for compound II against the *Candida* species. The MIC values showed that the genus *Candida* is very sensitive to both test compounds as compared to Gram-negative and Gram-positive bacteria.

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Authors' Contribution
KGG, GB and VI designed the study and did statistical analysis.
NU, KGG, GB and VI performed laboratory experiments and wrote the manuscript. MF and MS conducted data analysis, manuscript review and writing.

Key words
Antimicrobial activity, Pathogenic microorganisms, Organophosphates, Agar diffusion method, MIC

Infectious diseases are among the leading causes of death worldwide (WHO, 2017) and approximately 700,000 people die per annum from drug resistant infections (Francesca *et al*., 2015). People infected by antimicrobial resistant pathogens have their immune systems compromised and death can occur at any time and within a short time (Michele *et al*., 2015). Alongside these alarming health consequences, drug resistance has a significant economic impact (WHO, 2017). Indeed, the economic burden caused by antimicrobial resistance will reach 100,000 billion US dollars in 2050 (Michele *et al*., 2015; O'Neill, 2016). If nothing is done to control this global public health scourge (Renzo and Maurizio, 2020; Zhao *et al*., 2022) it could cause a health crisis more serious than COVID-19 (Shobhit and Dharmendra, 2023). Thus, the World Health Organization (WHO) has shown the emergency and necessity of global efforts and collaboration to contain resistance to antimicrobials (Francesca *et al*., 2015), because microorganisms have no borders and move freely, and no country can solve the problem of multi-drug resistance alone (O'Neill, 2016). Functionally substituted organic compounds are being investigated as likely new antimicrobials in future (Shoaib and Ganbarov, 2019). This is due to their unique mechanism of action which is not countered by drug resistant microbes (Shoaib *et al*., 2020). Keeping in mind this scenario, we carried out our research on antimicrobial potential of synthetic organic compounds containing phosphate because the development of new drugs and vaccines remains a priority to tackle drug resistant pathogens (Shikhaliyev *et al*., 2023). Furthermore, organophosphates have been widely studied and continue to be investigated due to their various biological properties. They have shown their effectiveness in the field of health (Fiore, 2018; Jolanta *et al*., 2013), in industry (Johnson and Hils, 2013; Percy *et al*., 2019) and in agriculture (Chandran *et al*., 2019). These chemical

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compounds are easy to synthesize, more toxic against insects (Daisley *et al*., 2018) and are biodegradable, which allows environmental protection (Rim *et al*., 2022).

In the current study we examined the antimicrobial activity of two synthetic organic compounds containing phosphate i.e. (((cyanomethyl) (ethoxy) phosphoryl) oxy) zinc (II) chloride (compound I) and (Z)-(1-(3-(dichlorophosphoryl)-3-methyl-4-oxooxetan-2-ylidene) ethyl) phosphonic dichloride (compound II), against ten pathogenic microorganisms including four Gram-negative bacteria, three Gram-positive bacteria and three *Candida* species. The minimum inhibitory concentrations (MIC) of both compounds were determined to highlight the most sensitive test cultures.

Materials and methods

The structures of test compounds used to evaluate the antimicrobial activity are illustrated in the Figure 1. These compounds were obtained from Department of Organic Chemistry, Baku State University (BSU), Azerbaijan.

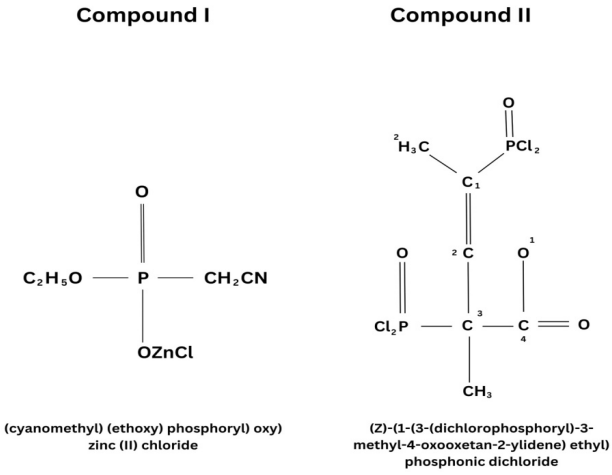

Fig. 1. Structure of test compounds.

The pathogenic microorganisms against which these compounds were tested were taken from our own repository at BSU Azerbaijan. Test cultures included *Acinetobacter baumannii* BDU-32, *Escherichia coli* BDU-12, *Klebsiella pneumoniae* BDU-44, *Pseudomonas aeruginosa* BDU-49, *Bacillus mesentericus, Bacillus subtilis, Staphylococcus aureus* BDU-23, *Candida albicans, Candida guilliermondii* and *Candida tropicalis.*

Agar well diffusion method was used to find out zone of inhibition for 0.3% concentration of test compounds (Gaoussou *et al*., 2023). Test compounds were dissolved in dimethyl sulph oxide (DMSO), because it has no antimicrobial activity. We dissolved 0.3 µg of test compound in 1 mL of DMSO to prepare 0.3% concentration of each compound. Bacteria were grown on nutrient agar and fungi were grown on sabouraud dextrose agar. 150 µL of 24 h fresh broth culture of test microorganisms (0.5 McFarland) was aseptically spread on agar surface. Two wells were made aseptically in agar plate by sterile tips, and wells were labeled for test compounds. 150 µL of each compound was added in respective well. Agar plates were incubated at 37°C for bacteria and at 30°C for fungi for 24 h. After incubation, the diameter of inhibition zones was measured carefully. Procedure was performed four times to find mean values.

Minimum inhibitory concentration (MIC) of test compounds were determined using double dilution method as mentioned by Balouiri *et al*. (2016) for different dilutions of compounds. Different concentrations of test compounds ranging from 1000 µg/mL to 7.8 µg/mL were used to evaluate MIC. The smallest concentration of test compound that inhibited the growth of microorganisms was considered as MIC for that particular microorganism (Andrei *et al*., 2022).

Table I. Antimicrobial activity of test compounds by agar well diffusion method.

Test cultures	Diameter of inhibition zone (mm), M±m	
	Compound I	Compound II
Acinetobacter baumannii	37.0±2.2	40.5±2.4
Escherichia coli	36.0±2.1	38.7±2.2
Klebsiella pneumoniae	36.8±2.1	38.2±2.2
Pseudomonas aeruginosa	34.2±2.0	38.0±2.2
Bacillus mesentericus	34.8±2.0	35.5±2.0
Bacillus subtilis	34.2±2.0	38.2±2.2
Staphylococcus aureus	39.3±2.3	41.2±2.4
Candida albicans	26.8±1.0	30.3±1.4
Candida guilliermondii	26.3±1.0	31.0±1.4
Candida tropicalis	28.0±1.2	30.8±1.4

Results and discussion

The diameters of zones of inhibition of two organophosphates are shown in the Table I. At 0.3% concentration, test compounds exhibited excellent antimicrobial activities against all tested microorganisms. For Gram-negative bacteria, the diameters of inhibition zones ranged from 34.2 mm to 37.0 mm for compound I, and from 38.0 mm to 40.5 mm for compound II. For Gram-positive bacteria, the diameters of inhibition zones ranged from 34.2 mm to 39.3 mm for compound I, and from 35.5 mm to 41.2 mm for compound II. For *Candida*

species, the diameters of inhibition zones varied from 26.3 mm to 28.0 mm for compound I, and from 30.3 mm to 31.0 mm for compound II. These results are better than those mentioned by Kathiriya *et al*. (2015), Rim *et al*. (2022). Furthermore, Rim *et al*. (2022) evaluated the antifungal activity of two compounds i.e. diethylhydroxyphenyl α-aminophosphonate compounds (4a-k) and diethyl phosphate diethyl α-aminophosphate derivatives (6a-k) against *Fusarium oxysporum* and *Botrytis cinerea*. The latter was more effective for inhibiting mycelium growth with a value of 87% for *Fusarium oxysporum* and 92% for *Botrytis cinerea*.

Our results clearly show the capacity of these two organic compounds to inhibit the growth of all the ten tested microorganisms, unlike certain organic compounds, which have a selective action. Functionally substituted cyclohexane derivatives are more portent against Gram-negative bacteria as compared to Gram- positive bacteria and fungi (Shoaib *et al*., 2020). The findings of Urzua *et al*. (2008) are not in agreement with our results. They reported that benzofuran derivatives were more effective against Gram- positive bacteria. This is due to difference in structure of test compounds.

The different values of the MIC varying from 62.5 µg/mL to 125 µg/mL showed that Gram-negative and Gram-positive bacteria were more sensitive as compared to fungi for tested compounds. Our findings are similar to those reported by Ismiyev *et al*. (2020). They demonstrated that diethyl esters of dicarboxylic acids are batter antibacterial agents than antifungal agents. This is thought to be due to difference in cell wall of bacteria and fungi (Shoaib *et al*., 2019). *Acinetobacter baumannii* is more sensitive to compound II as compared to compound I having MIC value of 62.5 µg/mL. *Pseudomonas aeruginosa* and *Bacillus subtilis* are more sensitive to compound I compared to compound II. *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* have the same susceptibility to both tested compounds with MIC value of 62.5 µg/mL. *Bacillus mesentericus* is equally vulnerable to both tested compounds with 125 µg/mL as MIC value. Furthermore, the organophosphates used by (Zhao *et al*., 2022) have much lower MIC values compared to our compounds. Indeed, the amino phosphonates introduced into aloe emodin and sulfonamide hybrids by (Zhao *et al*., 2022) showed remarkable antibacterial potential. Aloe emodin derivative containing dimethyl phosphate moiety showed excellent antibacterial activity against *Enterococcus faecalis, Escherichia coli* and *Acinetobacter baumannii* at low concentrations, ranging from 0.25 to 2 µg/mL, higher than tested antibiotics.

The MIC of fungi showed that *Candida albicans* and *Candida guilliermondii* are more susceptible to compound I compared to compound II. *Candida tropicalis* has the same vulnerability to both compounds with 15.6 µg/mL as MIC value. For 50 µg/mL concentration, the organophosphate derivatives tested by (Sujan *et al*., 2022) against phytopathogenic fungi including *Fusarium oxysporum* and *Cytospora mandshurica* inhibited the mycelial growth of these pathogens, and all showed superiority compared to hymexazol, the commercial fungicide. Our results contradict the reports of Ismiyev *et al*. (2019), who demonstrated that toluenesulfonyl derivatives of pyrazoles were ineffective against *Candida* species and exhibited considerable antibacterial properties.

There are numerous factors which decide the antimicrobial potential of tested synthetic organic substances. This can be attributed to chemical structure of compounds, functionally substituted groups and positions of different functional groups in the compound. Numerous functional groups impart diverse range of biological properties to organic compounds. Differences in the susceptibility of different test cultures is due to their diverse genetic make up and variation sin the structures such as cell wall and cell membranes (Shoaib *et al*., 2019).

Conclusion

The two tested compounds, (((cyanomethyl) (ethoxy) phosphoryl) oxy) zinc (II) chloride and (Z)-(1-(3-(dichlorophosphoryl)- 3-methyl-4-oxooxetan-2-ylidene) ethyl) phosphonic dichloride, inhibited the growth of all pathogenic microorganisms, with very high zone of inhibitions. However, the MIC results showed that the genus *Candida* is the most sensitive to both compounds compared to gram-negative and gram-positive bacteria. Additional and more specific studies should be carried out on these two compounds to explore their mode of action and allow their use in clinical therapy.

DECLARATIONS

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IRB approval

The study was approved by the Ethical Committee of the Faculty of Biology, Baku State University, Azerbaijan.

Statement of conflict of interest

The authors have declared no conflict of interests.

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