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ANTIMICROBIAL EFFECT OF (2-CHLORO-4-CYANO-5-ETHOXY-3,5-DIOXOPENTAN-2-YL) PHOSPHONIC ACID AGAINST PATHOGENIC BACTERIA AND FUNGI

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Abstract. The synthesized organic compound ((2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid)) was evaluated against pathogenic microorganisms using agar well diffusion method. The results obtained reveled that, the diameters of inhibition zones were ranging between 24.0-36.3 mm for gram-negative bacteria, from 28.3 mm to 34.5 mm for gram-positive bacteria and between 18.2-20.3 mm for genus *Candida*. The MIC value 62.5 µg/mL showed that among gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* were the most sensitive to the compound compared to *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus mesentericus* and *Bacillus subtilis*) had the same sensitivity to the compound with 125 µg/mL as MIC value. Among fungal strains, *Candida tropicalis* with MIC value 62.5 µg/mL was the most sensitive to the compound compared to *Candida albicans* (250 µg/mL) and *Candida guilliermondii* (125 µg/mL).

Keywords: *Antimicrobial activity, organic compound, pathogenic bacteria and fungi, Agar well diffusion method, MIC.*

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1. Introduction

The ability of microorganisms to evade the action of drugs designed to kill them or inhibit their growth is one of the greatest challenges facing the global health system. Indeed, following antibiotic therapy, bacteria either modify the target of antibiotic or prevent molecules from penetrating into the cell or evacuate the antibiotic so that it is in low quantity in bacteria through efflux pumps or deactivate the antibiotic before reaching its target (Rosales Hurtado, 2019). Unfortunately, faced to resistance mechanisms that evolve quickly, the diagnostic technologies, which can characterize infections, guide the treatment, controlling unnecessary use of antibiotics and personalizing therapeutic strategies for specific patients have not been developed (Syal *et al*., 2017). Furthermore, therapeutic approaches based on antimicrobial hybrids are current and evolving. These

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hybrids combine biological characteristics of two or more active molecules, which further strengthens the pharmacological power of the new combined molecules (Tighadouini *et al*., 2020). This combination must be reasonable, productive and effective to mitigate drug resistance (Ratrey *et al*., 2021). The present study is part of the general strategy to combat antimicrobial resistance. Thus, hybrid organic compound ((2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid) was synthesized and evaluated against ten pathogenic microorganisms.

2. Materials and methods

The organic compound (2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid was synthesized in Organic Chemistry Department of Baku State University, Azerbaijan (Ismailov *et al*., 2016). As test cultures, gram-negative bacteria: *Acinetobacter baumannii* BDU-32, *Escherichia coli* BDU-12, *Klebsiella pneumoniae* BDU-44, *Pseudomonas aeruginosa* BDU-49; gram-positive bacteria: *Bacillus mesentericus* BDU-51, *Bacillus subtilis* BDU-50, *Staphylococcus aureus* BDU-23; yeasts: *Candida albicans* BDU MI-44, *Candida guilliermondii* BDU-217 and *Candida tropicalis* BDU LK-30.

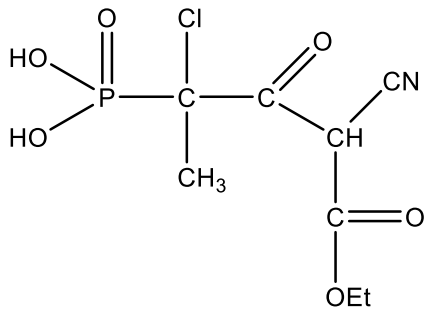

Figure 1. Structure of (2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid (Ismailov *et al*., 2016)

Agar well diffusion method

The antimicrobial activity of compound was determined by agar well diffusion method for 0.3% concentration. Dimethyl sulphoxide (DMSO) was chosen as solvent to dissolve the compound and for negative control. The pathogenic bacteria were grown on nutrient agar and fungal strains were grown on sabouraud dextrose agar (Binate *et al*., 2024). All experiments were performed four times.

Determination of MIC

The double dilution method in micro tubes Eppendorf was used to determine the Minimum Inhibitory Concentration (MIC) of compound, as described by Balouiri et al. (2016) for antibiotic dilutions. The lowest concentration of organic compound that inhibited the growth of each pathogenic microorganisms was the minimum inhibitory concentration (Bogdanov *et al*., 2022).

3. Results and discussion

The antimicrobial activity results of compound for 0.3% concentration and its minimum inhibitory concentration (MIC) are mentioned in the table.

Table 1. Antimicrobial activity results of (2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid

Microorganisms		Diameters of inhibition zones (mm), M ± m	MIC (µg/mL)
Gram negative bacteria	*Acinetobacter baumannii*	36.3±2.1	125
	Escherichia coli	27.0±1.1	62.5
	Klebsiella pneumoniae	24.0±0.9	125
	Pseudomonas aeruginosa	24.5±0.9	62.5
Gram positive bacteria	*Bacillus mesentericus*	29.0±1.2	125
	Bacillus subtilis	28.3±1.2	125
	Staphylococcus aureus	34.5±2.0	125
Fungi	*Candida albicans*	19.0±0.7	250
	Candida guilliermondii	18.2±0.7	125
	Candida tropicalis	20.3±0.8	62.5

Note: MIC (Minimum Inhibitory Concentration)

Considering the diameters of inhibition zones in gram-negative bacteria, the compound was 1.3 and 1.5 times more active against *Acinetobacter baumannii* than *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* respectively. This compound exerted the same antibacterial effect against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* In gram-positive bacteria, the compound was 1.2 times more active against *Staphylococcus aureus* than *Bacillus mesentericus* and *Bacillus subtilis*. This compound also exerted the same antibacterial effect against both *Bacillus* species. The antibacterial activity of (2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid against bacterial strains was appreciable, because it inhibited both the growth of gram-negative and gram-positive bacteria. Indeed, *Acinetobacter baumannii* was on the one hand, 1.3 times more sensitive to the compound than *Bacillus mesentericus* and *Bacillus subtilis* and on the other hand had the same sensitivity to the compound with *Staphylococcus aureus.* Furthermore, *Staphylococcus aureus* was 1.3 and 1.4 times more sensitive to the compound than *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* respectively. In fungal strains, the compound exerted the same antifungal activity against *Candida albicans, Candida guilliermondii* and *Candida tropicalis.*

Comparing antibacterial and antifungal activities of (2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid, it clearly appears that this compound was more effective against bacteria compared to fungi. The results showed that, the compound was respectively 1.4 and 2.0 times more active against *Escherichia coli, Acinetobacter baumannii* and *Staphylococcus aureus* than *Candida* species. In addition, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus mesentericus* and *Bacillus subtilis* were respectively 1.3 and 1.5 times more sensitive to the compound than *Candida* species.

According to the minimum inhibitory concentration (MIC) in gram-negative bacteria, *Acinetobacter baumannii* and *Klebsiella pneumoniae* had the same sensitivity to the compound. *Escherichia coli* and *Pseudomonas aeruginosa* had also the same sensitivity to the compound. Which means that *Escherichia coli* and *Pseudomonas aeruginosa* were 2.0 times more sensitive to the compound than *Acinetobacter baumannii* and *Klebsiella pneumoniae.* The sensitivity of gram-positive bacteria to the compound was the same despite the difference between the diameters of inhibition zones. In fungal strains, despite the same diameters of inhibition zones, *Candida tropicalis* was

2.0 and 4.0 times more sensitive to the compound than *Candida guilliermondii* and *Candida albicans*, respectively.

All these results clearly indicate that 2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl phosphonic acid was effective against bacterial and fungal strains, on the one hand. On the other hand, the diameters of inhibition zones alone do not fully confirm the sensitivity of microorganisms to antimicrobial agents. Only the minimum inhibitory concentration makes it possible to confirm the sensitivity of microorganisms to antimicrobials.

In the global strategy against antibiotic resistance, researchers have also worked on hybrid compounds. Indeed, Shoaib (2019) tested functionally substituted cyclohexane derivatives against pathogenic bacteria and fungi. The results showed that the compound was more active against gram-negative bacteria compared to gram-positive bacteria and fungi. Ismiyev et al. (2019) reported a new method for synthesis of two new toluenesulfonyl derivatives of pyrazoles annelated with polyfunctional cyclohexane ring. Structure of newly synthesized compounds were tested against bacteria and fungi. Gram-negative bacteria were more sensitive to the compound compared to gram-positive bacteria. Fungal strains were resistant to both compounds. The studies conducted by Shoaib et al. (2020) on antimicrobial properties of three novel monocyclic and functional substituted spirocyclic derivatives of cyclohexane revealed variable results. Depending on the diameters of inhibition zones, most compounds were ineffective against gram-positive bacteria and fungi. Moreover, gram-negative bacteria were generally more sensitive to compounds, especially compound III. The minimum inhibitory concentration (MIC) values were ranging between 125-2000 µg/mL. Our results are better than those found by these authors. Furthermore, Binate et al. (2024) tested antibacterial properties of three organic compounds derived from cyclohexane, furan and phosphate against seven pathogenic bacteria. The compounds were effective against pathogenic gram-negative and gram-positive bacteria. The MIC values (62.5 µg/mL) was observe against *Escherichia coli* and *Pseudomonas aeruginosa* for each organic compound. The MIC value for gram-positive bacteria was 125 µg/mL against *Bacillus subtilis* for compounds I and II. Which is in accordance with our antibacterial results.

4. Conclusion

The synthesized organic compound ((2-chloro-4-cyano-5-ethoxy-3,5-dioxopentan-2-yl) phosphonic acid)) was very effective against pathogenic bacteria and fungi. The highest diameters of inhibition zone were observed with *Acinetobacter baumannii* (36.3 mm), *Staphylococcus aureus* (34.5 mm) and *Candida tropicalis* (20.3 mm). The MIC result 62.5 µg/mL showed that *Escherichia coli* and *Pseudomonas aeruginosa* were the most sensitive among bacterial strains and *Candida tropicalis* was the most sensitive among fungal strains.

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