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ANTIMICROBIAL ACTIVITY OF HYDROPYRIDINE DERIVATIVES AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE PATHOGENIC BACTERIA

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Abstract. The effect of five organic compounds: 5-Amino-2'-oxo-2,3-dihydro-1l2-spiro[imidazo[3,2-a]pyridine-7,3'-indoline]-6,8-dicarbonitrile (I), 6'-Amino-5-bromo-2-oxo-1',2',3',4'-tetrahydro-spiro[indoline-3,8'-pyrido[1,2-a]pyrimidine]-7',9'-dicarbonitrile (II), 3'-Acetyl-6'-amino-5-bromo -2'-methyl-2-oxospiro[indoline-3,4'-pyran]-5'-carbonitrile (III), 6'-Amino-2-oxo-1',2',3',4'-tetra-hydrospiro[indoline-3,8'-pyrido[1,2-a]pyrimidine]-7',9'-dicarbonitrile (IV), Ethyl 2'-amino-6'-(chloromethyl)-3'-cyano-2-oxospiro[indoline-3,4'-pyran]-5'-carboxylate (V) on the growth of gram-negative (*Acinetobacter baumannii* BDU-32, *Escherichia coli* BDU-12, *Klebsiella pneumoniae* BDU-44 and *Pseudomonas aeruginosa* BDU-49) and gram-positive (*Bacillus subtilis* BDU-50, *Bacillus mesentericus* BDU-51, *Staphylococcus aureus* BDU-23) pathogenic bacteria was studied. It has been shown that all tested organic compounds have different degrees of antibacterial activity. *P. aeruginosa* and *B. mesentericus* showed high sensitivity to all tested compounds; *E. coli* and *B. subtilis* showed high sensitivity to compounds III and V; S. aureus - to compounds II, III, IV and V. K. pneumonia showed the same low sensitivity to all tested compounds. However, compounds II and III, which had a low minimum inhibitory concentration, turned out to be the most effective.

Keywords: *Antimicrobial activity, organic compounds, gram-positive pathogenic bacteria, gram-negative pathogenic bacteria.*

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1. Introduction

Antimicrobial resistance (AMR) has become one of the leading public health challenges of this century, threatening the successful prevention and treatment of an ever-increasing number of infections caused by bacteria, fungi, viruses and parasites that have developed resistance to commonly used medications. The problem of AMR is especially critical when it comes to bacterial resistance to antibiotics. Over the past several decades, bacteria responsible for common or severe infections have developed resistance to each new antibiotic (Aghapour *et al*., 2019; Prestinaci *et al*., 2015). Various areas of modern medicine rely on the availability of powerful antibiotics: cancer chemotherapy, organ transplants, hip replacement surgeries, intensive care for premature infants and numerous other medical procedures would not be feasible without effective antibiotics. Given this reality, taking action to prevent the looming global

healthcare crisis is essential (Aghapour *et al*., 2019; Mali & Sapkal, 2015). Drug - resistant bacteria make it difficult to treat and recover from infectious diseases. As a result, there is an urgent need to develop new antibiotics to stay ahead of bacterial resistance (Jose *et al*., 2014; Yoneyama & Katsumata, 2006).

Many organic chemical compounds and their derivatives are being evaluated and investigated as an antimicrobial agent. These compounds show considerable potential as antibacterial, antifungal and anti-inflammatory medicines (Binate & Ganbarov, 2023; Duruskari *et al*., 2018; Ganbarov *et al*., 2024; Israyilova *et al*., 2022; Mehrabani *et al*., 2020; Naghiyev *et al*., 2022; Shikhaliyev *et al*., 2019).

Newly synthesized organic compounds, namely: 5-Amino-2'-oxo-2,3-dihydro-1,2-spiro[imidazo[3,2-a]pyridine-7,3'-indoline]-6,8-dicarbonitrile (I); 6'-Amino-5-bromo-2-oxo-1',2',3',4'-tetrahydrospiro[indoline-3,8'-pyrido[1,2-alpyrimidine]-7',9'-dicarbonitrile(II);3'-Acetyl- -6'-amino-5-bromo-2'-methyl-2-oxospiro[indoline-3,4'-pyran]-5'-carbonitrile(III);6'-Amino-2oxo -1',2',3',4'-tetrahydrospiro[indoline-3,8'-pyrido[1,2-a]pyrimidine]-7',9'-dicarbo-nitrile (IV); Ethyl 2'-amino-6'-(chloromethyl)-3'-cyano-2-oxospiro[indoline-3,4'-pyran]-5'-carboxylate (V) (Magerramov *et al*., 2018; Naghiyev *et al*., 2022) could be potential antimicrobial agents.

The aim of our study is to evaluate the antibacterial properties of the above five (I-V) organic compounds against gram-negative and gram-positive pathogenic bacteria.

2. Material and methods

Organic chemical compounds (I-V) were studied for their antibacterial activity at a concentration of 0.1%, using the agar well diffusion method (Balouiri *et al*., 2016). Nutrient agar (Liofilchem, Italy) was used to determine the *in vitro* antibacterial properties, which were assessed against three Gram-positive bacteria: *Bacillus subtilis* BDU-50, *Bacillus mesentericus* BDU-51, *Staphylococcus aureus* BDU-23 and four Gram-negative bacteria: *Acinetobacter baumannii* BDU-32, *Escherichia coli* BDU-12, *Klebsiella pneumoniae* BDU-44 and *Pseudomonas aeruginosa* BDU-49. All test cultures were obtained from the collection of Baku State University. Dimethyl sulfoxide (DMSO) was used as a solvent to dissolve the test compounds. 100 µl of a 24-hour fresh culture suspension (0.5 McFarland) of each test bacterium were aseptically spread over the surface of the nutrient agar. Wells of 8 mm in diameter were aseptically punched in the agar plate and 150 µl of the test compound solution were poured into each well. Antimicrobial activity was assessed by measuring the inhibition zone after 24 h of incubation at 37°C. The diameters of the inhibition zones were measured in mm. All experiments were performed in quadruplicate and DMSO was used as a control substance.

The minimum inhibitory concentration (MIC) was determined using the two-fold dilution method (Andrews, 2001; CLSI, 2018). All experimental data were statistically processed (Kobzar, 2006).

3. Results and discussion

The effect of five organic compounds on the growth of gram-positive and gram-negative pathogenic bacteria was studied. It was shown that all tested organic compounds suppressed bacterial growth to varying degrees (Table 1). Compound I exhibited high antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*

and *B. mesentericus* and low activity against *Escherichia coli* and *Staphylococcus aureus*. The highest activity was observed against *Pseudomonas aeruginosa*, where growth suppression was 1.5 and 1.6 times greater, compared to activity against *Escherichia coli* and *Staphylococcus aureus*.

Compound II demonstrated the greatest growth inhibition against *Pseudomonas aeruginosa, B. mesentericus* and *Staphylococcus aureus* and the least activity against *Escherichia coli* and *Bacillus subtilis*. Antimicrobial activity against the former was 1.7 and 1.8 times greater than that of the latter.

Compound III demonstrated significant growth inhibition against *Escherichia coli*, *Pseudomonas aeruginosa*, *B. mesentericus*, *Bacillus subtilis* and *Staphylococcus aureus*, with antimicrobial activity 1.3-1.5 times greater than that of *Klebsiella pneumonia*.

Compound IV showed significant growth inhibition against *Pseudomonas aeruginosa* and *B. mesentericus*, where the antimicrobial activity was 1.3-1.4 times greater than that of *Escherichia coli, Klebsiella pneumoniae* and *Bacillus subtilis*.

Compound V showed significant growth inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the least against *Klebsiella pneumoniae*. The former had antimicrobial activity 1.3 and 1.5 times greater than that of the latter.

Table 1. Antibacterial activity of hydropyridine derivatives against bacteria

Microorganisms		**№ compounds and diameter of zone of inhibition (mm)**				
		I	**II**	**III**	**IV**	**V**
Gram-negative bacteria	*Acinetobacter baumannii*	16.0±0.1	16.0±0.5	17.5±0.5	16.5±0.4	17.0±0.1
	Escherichia coli	11.0±0.2	21.0±0.7	20.0±0.1	14.5±0.3	18.0±0.4
	Klebsiella pneumoniae	14.5±0.3	14.5±0.4	14.0±0.5	14.0±0.4	15.8±0.4
	Pseudomonas aeruginosa	18.0±0.7	19.3±0.8	21.5±0.9	19.5±0.8	24.0±0.3
Gram-positive bacteria	*Bacillus mesentericus*	17.0±0.5	19.3±0.6	20.5±0.8	19.0±0.8	18.2±0.7
	Bacillus subtilis	17.6±0.4	11.0±0.1	20.0±0.4	14.5±0.3	18.0±0.4
	Staphylococcus aureus	12.2±0.5	18.8±0.7	18.3±0.6	17.5±0.4	20.5±0.8

Note: I - 5-Amino-2'-oxo-2,3-dihydro-1,2-spiro[imidazo[3,2-a]pyridine-7,3'-indoline]-6,8-dicarbonitrile;
II - 6'-Amino-5-bromo-2-oxo-1',2',3',4'-tetrahydrospiro[indoline-3,8'-pyrido[1,2-a]pyrimidine]-7',9'-dicarbonitrile;
III - 3'-Acetyl-6'-amino-5-bromo-2'-methyl-2-oxospiro[indoline-3,4'-pyran]-5'-carbonitrile;
IV - 6'-Amino-2-oxo-1',2',3',4'-tetrahydrospiro[indoline-3,8'-pyrido[1,2-a]pyrimidine]-7',9'-dicarbonitrile;
V - Ethyl 2'-amino-6'-(chloromethyl)-3'-cyano-2-oxospiro[indoline-3,4'-pyran]-5'-carboxylate

Acinetobacter baumannii and *Klebsiella pneumoniae* showed similar sensitivity to all tested compounds (Table 1). *Escherichia coli* showed the highest sensitivity to compounds III and V and the lowest sensitivity to compounds I and II, where the antibacterial activity of the former was 1.6 and 1.8 times greater than that of the latter. *Pseudomonas aeruginosa* and *B. mesentericus* showed high sensitivity to all tested compounds. *Bacillus subtilis* showed high sensitivity to compounds I, III and V, where the antibacterial activity was 1.6-1.8 times greater than that of compounds II and IV. *Staphylococcus aureus* showed high sensitivity towards II, III, IV and V compounds, whose antimicrobial activity was 1.4-1.7 times greater than that of I.

In order to identify the effectiveness of the tested compounds, their minimum inhibitory concentration (MIC) was determined. The data obtained are presented in

Table 2. It was shown that compounds I and IV had a high MIC (1000 μg/ml) towards all the tested bacteria. Compounds II and III had a MIC of 500 μg/ml towards *Acinetobacter baumannii, Klebsiella pneumoniae, Bacillus subtilis* and *Staphylococcus aureus* and MIC was 2 times less towards *Escherichia coli* and *B. mesentericus*. Consequently, organic compounds II and III were found to be the most effective antimicrobial substances.

Table 2. Minimum inhibitory concentrations of organic compounds against bacteria

Microorganisms		**Minimum inhibitory concentrations (μg/ml)**				
		I	**II**	**III**	**IV**	**V**
Gram-negative bacteria	*Acinetobacter baumannii*	1000	500	500	1000	1000
	Escherichia coli	1000	250	250	1000	1000
	Klebsiella pneumoniae	1000	500	500	1000	500
	Pseudomonas aeruginosa	1000	1000	1000	1000	1000
Gram-positive bacteria	*Bacillus mesentericus*	1000	250	250	1000	500
	Bacillus subtilis	1000	500	500	1000	500
	Staphylococcus aureus	1000	500	500	1000	500

Note: The names of the compounds (**I, II, III, IV** and **V**) are listed in the previous table

4. Conclusion

Thus, it has been shown that all tested organic compounds have different degrees of antibacterial activity. *Pseudomonas aeruginosa* and *Bacillus mesentericus* showed high sensitivity to all tested compounds; *Escherichia coli* and *Bacillus subtilis* showed high sensitivity to compounds III and V; *Staphylococcus aureus* - to compounds II, III, IV and V. *Acinetobacter baumannii* and *Klebsiella pneumoniae* showed the same low sensitivity to all tested compounds. However, compounds II and III, which had a low minimum inhibitory concentration, turned out to be the most effective.

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