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THE DETERMINATION OF SENSITIVITY OF BACTERIA TO THE SYNTHESIZED DERIVATIVES OF POLYCARBONYL NITROGEN-CONTAINING COMPOUNDS

Abstract: The purpose of this study was to screen the antimicrobial activity of some of the synthesized compounds and to identify the compounds, active towards the test cultures of microorganisms. As a result of the tests, was revealed the antimicrobial activity of the tested substances: ethyl 6-hydroxy-6-methyl-3-(2-oxo-2-phenylethoxy)-4-phenyl-4,5,6,7-tetrahydro-1H-indazole-5-carboxylate (sample No. 1), diethyl-1-isobutyl-9-hydroxy-9-methyl-7-phenyl-1,4-diaza-spiro[4,5]decane-6,8-dicarboxylate (sample No. 2), ethyl-6-methyl-3-oxo-4-phenyl-1,3,4,5-tetrahydrobenzo[c]isoxazole-5-carboxylate (sample No. 3), diethyl-4-hydroxy-4-methyl-2-phenyl-6-(2-(4-phenylthiazol-2-yl)hydrazine)cyclohexane-1,3-dicarboxylate (sample No. 4 [1,2,3,5]).

Key words: dicarboxylate, antimicrobial activity, disk diffusion method, furacilin, nitrofungin.

Language: English

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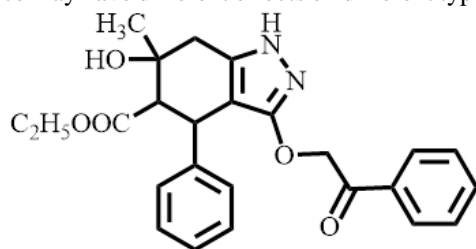
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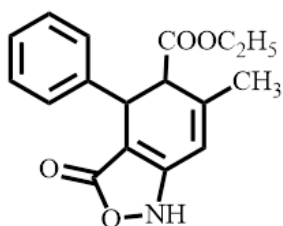
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Introduction

Modern synthetic antimicrobial agents occupy a leading place in the treatment of bacterial infections. The difficulties of treatment and prevention of infectious diseases, due to the diversity of biological forms of pathogens, the constant emergence of multi-resistant forms and new types of dangerous pathogens, determine the relevance of creating new antimicrobial agents. The need for new drugs have various reasons: the expansion of the antimicrobial spectrum, increase of activity against resistant pathogens, improvement of pharmacokinetic properties and reduction of toxicity. Organic compounds inhibit or completely stop the growth of microorganisms. Chemicals cause either microbiocidal (lead to the death of microorganisms) or microbiostatic action (stop their growth, but after the removal of these substances the growth resumes again). The nature of action depends on the dose of the substance, the time of its exposure, as well as temperature and pH. The same organic substance may have different effects on different types



sample No. 1

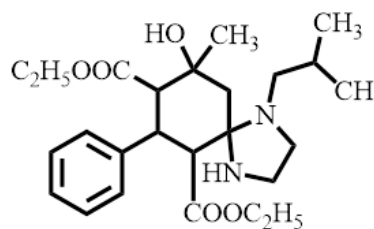


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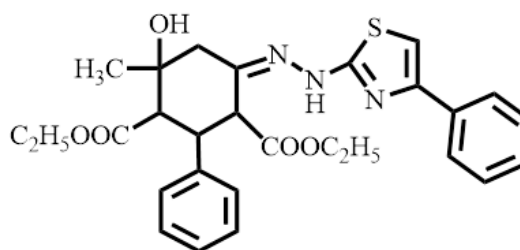
of microorganisms. Antimicrobial substances act more strongly on vegetative cells than on spores.

The sensitivity of microorganisms to chemical compounds is determined by several methods, one of which is the dilution method: bacterial strains are cultured in nutrient media, in which substances are added in certain concentrations. Nutrient media for these purposes can be both dense and liquid. The sensitivity of microorganisms to a chemical compound is assessed by the minimum concentration of the drug, at which their growth is suppressed. This is the minimum inhibitory concentration (MIC) of the drug. The method of serial dilutions determines the minimum concentration of a chemical compound that inhibits the growth of the investigated bacterial culture [6,7].

Structure of obtained compounds was researched with the help of RSA Shelldrick G.M. SHELXTL, Structure Determination Software Suite, Brüker AXS, Madison, Wisconsin [4].



sample No. 2



sample No. 4

2.Experimental part

In order to determine the sensitivity of microorganisms by a disk diffusion method, a suspension was prepared from daily cultures of microorganisms (1 billion microbial cells in 1 ml) [8]. Further, this suspension was uniformly applied to the surface of meat-peptone agar in a Petri dish. The excess liquid was removed with a pipette; then the cup is dried for 10-15 minutes in a thermostat. Sterile filter paper disks are moistened with a solution of the tested substances and placed on the surface of the prepared agar.

After cultivation in thermostat at a temperature of 37 °C for 18-24 hours, the result is obtained by measuring the diameter of the inhibition zone around the disk in millimeters. Diffusion of the tested chemical compound leads to the formation of an

inhibition zone of the growth of microorganisms around the disks. As a result, the diameter of the inhibition zones for the substances were: (sample No. 1) *S.aureus* -15 mm, *E.coli* - 15 mm, *Ps.aeruginosa* - 15 mm, *C.albicans* - 20 mm; (sample No. 2) *S. aureus* - 15 mm, *E.coli* - 18 mm, *P. aeruginosa* - 18 mm, *C.albicans* - 18 mm; (sample No. 3) *S.aureus* - 15 mm, *E.coli* 17 mm, *P. aeruginosa* - 18 mm, *C.albicans* - 18 mm; (sample No. 4) *S.aureus* -10 mm, *E.coli* 17 mm, *P.aeruginosa* - 17 mm, *C.albicans* - 18 mm.

In order to determine the sensitivity of microorganisms by the serial dilution method, the tested substances were taken in the amount of a 1% alcohol solution. The studied dilutions were in the following concentrations: 1:100, 1:200, 1:400, 1:800. As representative of Gram-positive bacteria was used *S. aureus*, Gram-negative – *E. coli* and *P. aeruginosa*,

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fungi – yeast fungus of the genus *Candida*. MPA pH 7.2-7.4 was used as a nutrient medium for bacterial strains, and Saburo medium was used for *Candida*.

3.Results and Discussion

Table 1. Antimicrobial effect of the new synthesized compounds

Test culture	Exposure time (min)	Tested compounds															
		(XII) (№ 1)				(III) (№ 2)				(XVII) (№3)				(XXII) (№4)			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
<i>S.aureus</i>	10	-	-	-	+	-	-	-	+	-	-	+	+	-	-	+	+
	20	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
	40	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
	60	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
<i>P.aeruginosa</i>	10	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	20	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+
	40	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+
	60	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+
<i>E.coli</i>	10	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+
	20	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	+
	40	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+
	60	-	-	+	+	-	-	+	+	-	-	-	+	-	-	-	+
<i>C.albicans</i>	10	-	-	+	+	-	-	-	+	-	-	+	+	-	-	+	+
	20	-	-	-	+	-	-	-	+	-	-	+	+	-	-	+	+
	40	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+
	60	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+

Note: The dilution ratio is designated as 1 (1: 100), 2 (1: 200), 3 (1: 400), 4 (1: 800)

Microbial loading in all trials was 1 billion microbial cells per 1 ml, from which one drop was added to each tube. Streaking was carried out every 10, 20, 40, 60 minutes of exposure. As a result it was found that the test samples 2 and 4 have strong antimicrobial effect. As seen from Table 1 the Sample No. 4 at dilution of 1: 400 demonstrate effect on *E.*

coli in 10 minutes, *P. aeruginosa* in 20 minutes, *Candida* in 40 minutes. Sample # 2 kills *S. aureus* in 20 minutes even at dilution of 1:800.

For a comparative study of the activity of the tested substances, well-known drugs (ethyl alcohol, rivanol, furacilin, nitrofungin) were taken as controls.

Table 2. Antimicrobial activity of control substances

Tested culture	Exposure time (min)	Control drugs															
		Ethanol				Furacilin		Nitrofungin									
		1	2	3	4	1	2	1	2	3	4	1	2	3	4		
<i>S.aureus</i>	10	-	+	+	+	-	+	+	+								
	20	-	+	+	+	-	+	+	+								
	40	-	+	+	+	-	+	+	+								
	60	-	+	+	+	-	+	+	+								
<i>P.aeruginosa</i>	10	+	+	+	+	+	+	+	+								
	20	-	+	+	+	+	+	+	+								
	40	-	+	+	+	+	+	+	+								
	60	-	+	+	+	+	+	+	+								
<i>E.coli</i>	10	+	+	+	+	-	+	+	+								
	20	-	+	+	+	-	+	+	+								
	40	-	+	+	+	-	+	+	+								
	60	-	+	+	+	-	+	+	+								

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<i>C.albicans</i>	10	+	+	+	+	+	+	+	+	+	+	+	+
	20	+	+	+	+	+	+	+	+	+	+	+	+
	40	-	+	+	+	+	+	+	+	-	+	+	+
	60	-	+	+	+	+	+	+	+	-	+	+	+

Note: the dilution ratio: 1 (1: 100), 2 (1: 200), 3 (1: 400), 4 (1: 800)

The results of the control experiments are given in Table 2. As it can be seen from the table, the antimicrobial effect of the three drugs, used in medicine, is much less than the effect of the synthesized compounds. The long latent period of the antimicrobial action can be explained by the unequal number of membranes, surrounding those active centers, with which interact the tested compounds. The increase in the effectiveness of the action of drugs with dilution proves good permeability through the cell membrane. The most important is that, regardless

of the content of the various functional groups, they are effective against all microorganisms. The obtained data show that the bactericidal effect of the synthesized compounds is associated with their inhibitory effect on the formation of the cell wall of microorganisms, protein denaturation, impaired permeability of the cytoplasmic membrane, and inhibition of the enzymes important for the vital activity of bacteria. The results show that the synthesized compounds can be used as bactericidal and fungicidal preparations.

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