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Results of Microbiological Investigation of Cyclohexane Derivatives Containing Nitrogen and Sulfur Atoms

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Abstract

In the world, there are currently has developed a quite difficult situation with the use of antibiotics in infectious diseases treatment. According to the fact, clinical isolates of the bacteria resistant against most of antibiotics in many cases. The treatment of diseases, caused by even opportunistic strains such as S. aureus, E. coli, etc., becomes more difficult. Antibiotics are used strictly for a purpose with a certain doze and duration in order to prevent the emergence and spread of resistance. For example, in the treatment of intestinal infections, antibiotic treatment is justified only in 20% of cases. Another serious problem with some antibiotics - is their low bioavailability (for instance, tsefeksim - 40-50% of bioavailability) and high toxicity (fluoroquinolones - hepatotoxicity, nephrotoxicity and ototoxicity).

Keywords: S aureus; E coli;S enteridis; Ps aeruginosa; Candida albicans; cyclohexanones; heterocyclic compounds.

1. Introduction

The ability of the number of chemicals to suppress activity of microorganisms is known from an extreme antiquity. Use of chemicals is a basis of a method of antiseptics (Joseph Lister in 1867 offered)[1].

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Efficiency depends on the concentration of chemical substances and the time of contact with the germ. Chemicals are showing a static effect and can inhibit the growth and reproduction of microorganisms. The mechanism of action of different drugs are not the same and may be associated with protein denaturation, interruption insight plasmatic membrane, inhibition of enzymes important for the life of micro-organisms [2].

In recent years, among organic compounds has been identified active substances possessing antiviral and antibacterial activity in developing sensitive cells. From active organic compounds can be mentioned guanidine and its derivatives, a number of amino acids, enzymes, lipids, and other compounds.

In 1964 according to the Lwoff A offer, effect of the specified active agents is connected with existence of NH3[3] group in them. The analysis of action of a guanidine showed influence them (specific effects) on fermentable proteins (RNA – a replicate, etc.) participating in the replications of a virus and preventing by that to association of monomers of enzyme leading to violation of its tertiary structure.

In recent years researches on antivirus and antibacterial screening as abroad and in various regions of the neighboring countries and at us in Azerbaijan are actively conducted [4].

Considering not less complex problems with bacterial flora namely in respect of factors of an antibiotic stability of bacterial flora, and also a number of side effects of antibiotics, researches and on antibacterial screening of chemical compounds are conducted [5,6].

As we know from literature substituted β-cycloketoles possess biological activity. So, 5-hydroxy-3,5-dimethyl-2,4 diethoxycarbonylcyclohexanon- it is recommended as the calming and sleeping medicine, and also acetyl substituted ketoles show antimicrobial activity[7,8]. 3-Azabidicyclononanes are potential antimicrobial agents.

Derivatives of the above substances have psycholeptic, gipoklemicheskoy, sedative activity. Thus 3-acetyl-4-hydroxy-4,9-dimethyl-2-m-nitrophenyl-7-aza-8-oxabicyclo [4.3.0] 6,9-nonadiene inhibited 5 kinds of bacteria : *Staphylococcus aureus , Bacillus subtilis), Eschirichia coli, Proteus vulgaris, Pseudomonosovalis.*

Other compounds, that have derived from the β-ketols, have antimicrobial action. In particular, we have synthesized and studied the following 4 substances [9,10]. On the basis of the above compounds, the synthesis of more branched structures of heterocyclic series has performed, which contain a thiazole, phenylhydrazinetiosemikarbazidny fragments, as well as various derivatives contain nitrogen-containing group on a single and double bond. Nitrogen atoms and their arrangement play a special role in the substances by the double bond and cycle. We may suppose the existence of an intramolecular hydrogen bond in each of these substances due to a free pair of electrons on nitrogen atoms that may form N...H type hydrogen bond because of a certain location to the hydroxyl group.Thus, at considering the test 1, we can assume the action of mesomeric effect and thereby intensifying the nitrogen atom in the indazole ring. If we look at the second test then we may suppose that it has less activity than the test 1 due to absence of an electronegative sulfur atom. Test 3 is a good product for further synthesis on a nitrogen atom located at the NH2 group. Test 4 differs from the other three tests by itsclear framework and a nitrogen atom located next to the hydroxyl group is an active center.

Considering everything above stated, we undertook methodical approach to approbation and a hygienic assessment of four new synthesized preparations of rather bacterial flora.

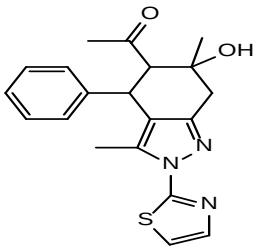

Figure1: Sample 1(1-(-6-hydroxy-3,6-dimethyl-4-phenyl-2-(thiazol-2-yl)-4,5,6,7-tetrahydro-2H-indazol-5-yl) ethan-1-one)

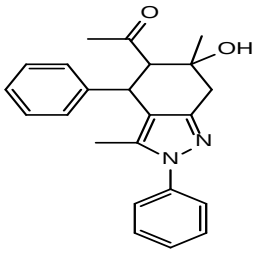

Figure 2: Sample 2(1- (6-hydroxy-3,6-dimethyl-2,4-diphenyl-4,5,6,7-tetrahydro-2H-indazol-5-yl) ethan-1-one)

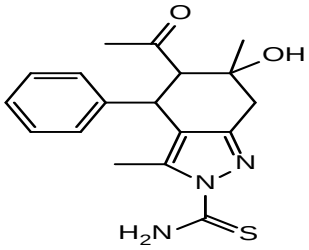

Figure 3: Sample 3 5-Acetyl-6-hydroxy-3,6-dimethyl-4-phenyl-4,5,6,7-tetrahydro-2H-indazole-2-carbothioamide

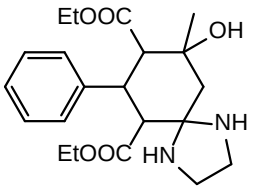

Figure 4: Sample 3(diethyl-9-hydroxy-9-methyl-7-phenyl-1,4-diazaspiro [4.5] decane-6,8dikarboksilat)

Purpose were screening researches on studying of biological activity of the chemical compounds synthesized by us relatively the gram-positive and the gram-negative of bacteria on the example of S aureus, E coli, S enteritis and Ps aeruginosa.

2. Materials and methods

In work were investigated and used both museum strains of cultures, and isolates of the allocated cultures from water, foodstuff, and also hospital strains

1) S aureus ATCCR 25923

2) E coli ATCCR 25922

3) S enteritis ATCCR27853

4) Ps aeruginosa ATCCR13076

5) Candida albicans ATCCR 10231

Test of I was warmed slightly up on a water bath at a temperature 60z before full dissolution. Examinees of connection were dissolved in the organic CHLOROFORM pure solvent (Czech Republic) in concentration of 1 mg/ml.

Equal volumes of microbial flora (on 1ml) accumulated on Petri's cups with the selective environment for each activator, using standard inoculum, Mack corresponding on density 0,5 standards. Farlanda

The slightly opened cups dried in boxing at the room temperature within 10-15 min. Then from the studied samples disks prepared. In advance moistened in chloroform solution with concentration 1mg/ml, also put sensitivity, using the disco – a diffusion method of determination of antimicrobial efficiency. After incubation the thermostat at 37 ˚ within 24 hours observed change of cultural properties of microorganisms and carried out processing of the received results.

3. Results and discussion

The control cups with nutrient medium placed with test compounds, but without applying a pure culture and vice versa with the bacterial microflora of any kind of microorganisms, but without testing of new synthetic drugs to study the effect on the biochemical properties, mobility and respiration of microorganisms.

As is known there are two types of E. coli bacterias : one of them are mobile and have flagellas, others are fixed.

E. coli is aerobic, capable to utilize complex organic compounds; it grows well on all mediums at the alkalescent reaction and at optimal t 37 C. Growth and reproduction of bacteria are possible with large fluctuations both the PH pf medium and temperature regime.

E. coli has the enzymatic properties as museum strains and isolates, which extracted from foodstuffs, it may benoted that E. coli produces multiple saccharolytic enzymes, which decomposing various carbohydrates: glucose, lactose, sucrose, maltose, etc.; and form acetic acid, propionic acid, lactic acid and other. With regard to Salmonella, they cleave glucose and form H2S, but not lactose, sucrose and indole.

Nevertheless these two microorganism g (-) are bacillus and belong to Enterobacteriaceaefamilia, but they show a different biological activity and behave differently with a tested substance.

Studing all properties of E. coli and Salmonellenterica and observing a suppression zone of body height concerning our synthesized connection, we may suggest that existence of a fragment of a thiacinder cycle in test of 1 and a fragment of a diazospirocyclic framework in test of IV is bound to the interaction of the examiner of connection not only with a microorganism, but also to sugar of nutrient medium. Splitting of lactose and sucrose in the first case and splitting of glucose and Mannitum with formation of H2S in the second case. Observing from here ill-defined bactericidal properties in tests of I and IV in the first case and in tests of II and III in the second respectively (see the table).

As a result of a research it was revealed that all 4 tests show the expressed bactericidal properties to g (+) bacteria – S. aureus.

All tests have a growth inhibition of 80-90% on the museum strains. All tests on S. aureus isolates, which isolated from foodstuff, proved differently: I test of 3,7 cm; II test of 3,3 cm; III test of 3,2 cm; IV test of 3,9 cm respectively.

Ps. aeruginosa g (-) is a bacillus - a polymorphic gram-negative bacterium, mobile, monotrih, but sometimes there are cells with 2 or more polar flagella.

Pseudomonas aeruginosa is an obligate aerobe, which ferments glucose to produce an acid, slightly produces H2S, liquefies gelatin. If with respect to Mr. E. Coli (-) I sample bacillus and IV did not show the activity sensitive, and tests II and III show 40-30% growth inhibition, respectively, then with respect to such a microorganism as Ps aeruginosa a sample I - 80%; II - 60%; III - 50%; IV - 85%activity of growth inhibition. Tests I and IV were very active in first case, tests II and III were weak in the second case on example, of E. coli.

In practical Microbiology in the detection of E coli isolates from foodstuff and water, as suplementa use broad-spectrum antibiotics, such as vancomycin (VANKO SUPP Biomerieux Franca) to inhibit accompanying microflora growth. The fact that the obtained tests 1 and 4 showed a rather high activity against all tested bacterial strains, except E. coli ATCCR 25922 (0%), and sufficiently low activity against isolates of E. coli (test 1 - 10% test 4 - 12%), it allows us assume term use of these compounds as additives during the determination of microbiological test strain E. coli bacteria as growth of other strains is considerably suppressed and an isolation of E. coli will be much easier.

Then testes 2 and 3 showed high activity against S. aureus g (+) cocci and also showed activity against g (-) Ps aeruginosa bacillus, which gives us reason to continue the modification of the compounds pharmacophore groups that can be attached to the active centers of these compounds. Also of interest is the action testes 1 and 4 E. coli ATCCR 25922, which is a standard for expression studies and low activity with respect to the bacterium isolates (10% and 12%, respectively) indicating that mutation of the cases occurring in the isolates, exposed to different environmental influences, this makes our strain sensitive to compounds 1 and 4. It is possible that a mutationoccurs in a certain chain molecule or molecular structures existing modified bacteria that are sensitive

to the effects of derivatives β- Ketola.

4. Conclusion

Our research reveals a relationship between bactericidal action of synthesized substances and their structure and composition.

The obtained data shows that the bactericidal effect of drugs is associated with their inhibitory effect on the formation of the cell wall of microorganisms, denaturation of the protein, violation of the permeability of the cytoplasmic membrane, with inhibition of enzymes that are important for the life of bacteria.

Table № 1: The results of the research of the biological activity of the synthesized combinations

Name of microorganisms Established strains	Test combinations	Growth suppression zones	Name of microorganismsisolates	Percentage **%**
G (-) rods E. coliATCC® 25922	Test 1	-	E. coli 0,5 cm	0 – 10 %
	Test 2	1,9 cm	1,6 cm	40 – 36 %
	Test 3	1,3 cm	1,4 cm	30 – 32 %
	Test 4	-	0,6 cm	0 – 12 %
S. enterica (enteridis) ATCC® 13076	Test 1	1,9 cm	S. enterica2,1 cm	40 – 43%
	Test 2	1,3 cm	1,4 cm	30 – 32 %
	Test3	1,1 cm	0,8 cm	20 – 17 %
	Test 4	2,7 cm	2,5 cm	60 – 58 %
Ps. aeruginosa ATCC® 27853	Test 1	3,6 cm	Ps. aeruginosa 3,5 cm	80 – 78 %
	Test 2	2,7 cm	2,5 cm	60 – 64 %
	Test 3	2,4 cm	2,6 cm	50 – 56 %
	Test 4	3,9 cm	3,7 cm	85 – 82 %
G (+) coccus S. aureus ATCC®25923	Test 1	4,0 cm	S. aureus 3,7 cm	90 – 82 %
	Test 2	3,6 cm	3,3 cm	80 – 73 %
	Test 3	3,6 cm	3,2 cm	80 – 70 %
	Test 4	4,0 cm	3,9 cm	90 – 85 %
Yeast fungi CandidaalbicansATCC® 10231	Test 5	1,6 cm	Candidaalbicans 1,9 cm	37 – 40 %
	Test 6	2,4 cm	2,5 cm	50 – 53 %
	Test 7	2,6 cm	2,8 cm	55 – 57 %

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