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Antimicrobial and toxic properties of macrocyclic and acyclic onium derivatives of uracil

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Author's abstract of thesis for the degree of Candidate of Biological Science

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The work was implemented at the laboratory of chemical and biological investigations of Federal State Budgetary Institution of Science "A.E. Arbuzov Institute of Organic and Physical Chemistry of Kazan Scientific Center of Russian Academy of Science"

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Thesis defense will take place on October «29» 2015 at 13:00 on the meeting of the Thesis Council D 212.081.08 at FSAEI HPE "Kazan (Volga region) Federal University" at the address: 420055, Kazan, Karl Marx str., 74, at the hall of the Academic Council meetings (auditorium 205).

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Author's abstract sent «25» September 2015

Scientific secretary of the Thesis Council,
Doctor of Biological Science  Z.I. Abramova

Timeliness of the investigation topic and the level of its development. Nowadays, more than 20 thousands of natural substances are described and hundreds of thousands of individual chemical compounds with antimicrobial properties are synthesized, which influence pathogens of various nature. However, a wide application of these drugs leads to a number of effects complicating the possibility of its rational use. They are: allergic reactions, serious toxic effect on the body, and development of drug resistance of pathogens to antimicrobial preparations being used.

In this connection the investigations dedicated to purposeful search of high-efficient low-toxic drugs with a broad spectrum of activity among new promising antimicrobial agents become of great importance.

Pyrimidine derivatives have been attracting attention of chemists-synthetics as well as that of specialists of adjacent areas for a long time due to a significant variety of their useful properties. A number of drugs on the basis of the pyrimidine derivatives are used in medical practice at present. They are applied as pharmaceuticals with antimicrobial, antiphlogistic, anticancer, regenerating and antimalarial properties (Машковский [Mashkovsky], 2012; Hugo et al., 1987).

The compounds containing onium group have been arousing great interest of the researchers in the area of chemotherapy for a long time. Today they have wide applications: therapeutic antiseptics of local pyoinflammatory processes, prophylactic antiseptics for the intact skin before operations, antiseptics for mucous membranes; preservation of eye drops, injection solutions, tooth-pastes, cosmetic products, etc. Typical representatives of onium derivatives are: benzalkonium chloride, benzalkonium propionate, mecetronium methyl sulfate. Benzalkonium chloride has a broad spectrum of antimicrobial action in respect of a number of pathogens and forms a part of many famous drugs: pharmatex, benatex, septolete (Опарин [Oparin], 2003; РЛС Энциклопедия лекарств [RD Encyclopedia of drugs], 2012).

Antimicrobial activity of the onium compounds is a subject of a great number of publications (Корочкина и др. [Korochkina et al.], 2010; Корочкина и др. [Korochkina et al.], 2011; Кудрявцев и др.[Kudryavtsev et al.], 2011; Миргородская и др. [Mirgorodskaya et al.], 2011; Zhiltsova et al., 2012; Mirgorodskaya et al., 2014; Pashirova et al., 2015; Kenawy, 2007). However, there are practically no works dedicated to studies of antimicrobial activity of macrocyclic and acyclic onium derivatives of uracil.

In this respect, the search of new original drugs with broad spectrum of antimicrobial activity and low toxicity to mammals in the series of macrocyclic and acyclic onium derivatives of uracil is of high importance.

Consequently, the **purpose** of the given work is the investigation of antimicrobial and toxic properties of new macrocyclic and acyclic onium derivatives of uracil.

The main objectives of the study:

1. To characterize the antimicrobial activity macrocyclic and acyclic onium derivatives of uracil.

2. To analyze the relation “structure – activity” in the series of the compounds being investigated.

3. To study the influence of onium derivatives of uracil on the activity of enzymes of dehydrogenases and lipases, as well as on the biosynthesis of biomarker ribosomal proteins *Escherichia coli F 50.*

4. To estimate acute toxicity on laboratory mammals (mice, intraperitoneally).

5. To investigate genotoxic and hemolytic properties macrocyclic and acyclic onium derivatives of uracil.

6. To give a comparative estimation of the potential of practical application of macrocyclic and acyclic onium derivatives of uracil as antimicrobial agents.

Scientific novelty. For the first time, antimicrobial activity of new chemical compounds has been studied, peculiarities of molecular structure have been determined, which provide low values of MIC (minimal inhibitory concentration) at the level of the known drugs (ciprofloxacin, ofloxacin, clotrimazole, amphotericin B, ketoconazole) and moderate values of toxicity on laboratory mice. The investigated compounds are not hemolytic agents. The Ames test has shown the absence of their genotoxicity. Macrocyclic and acyclic onium derivatives of uracil have been proposed to development as antimicrobial preparations.

Practical significance of the work. The main practical achievement of the given work is determining in the series of macrocyclic and acyclic onium derivatives of uracil the compounds with high antimicrobial activity at the level of known standards. Macrocyclic and acyclic onium derivatives of uracil demonstrate selective properties against gram-positive bacteria. Acyclic onium derivatives have turned out to be less specific and have shown high antimicrobial activity as against gram-positive and gram-negative bacteria, so against fungi. The results identified in conjunction with the data, indicating that the compounds studied have no mutagenic and hemolytic properties and demonstrate moderate toxic properties in relation to laboratory mice, open a prospect of using them to treat bacterial and fungal infections of humans and animals.

Hypothesis to be defended

1. Among 37 investigated compounds, the optimal combination of targeted antimicrobial and side toxic properties (acute toxicity, hemolytic activity, genotoxicity) appears in the series of acyclic derivatives of alloxazin.

2. In relation to gram-positive bacteria macrocyclic and acyclic onium derivatives of uracil demonstrate antimicrobial activity at the level of ciprofloxacin and ofloxacin (MIC = 0.2-3.9 mg/l); in relation to gram-negative bacteria the derivatives of hinzelin-2,4-dione and alloxazine are the most active (MIC = 3.1-31.3 mg/l); in relation to fungi the acyclic derivatives of alloxazine and uracil are the most active (MIC = 0.39 – 3.9 mg/l).

3. Acyclic derivatives of alloxazine cause inhibition of the activity of dehydrogenases and lipases *S. aureus 209-P* and *C. albicans 885*-653 by (38-82%); all compounds studied

affect protein biosynthesis in cells *E. coli F 50*.

Connection of the work with scientific programs and the author's contribution into the investigation. The work has been implemented at the laboratory of chemical and biological investigations of A.E. Arbuzov Institute of Organic and Physical Chemistry of Kazan Scientific Center of Russian Academy of Science. It is a part of the investigations according to the direction of the Institute on state, budget themes "Synthesis and study of the structure, chemical and biological properties of microcyclic compounds containing pyrimidine and triazine fragments (№ of state registration 0120.0005796); "Functionalization of chelate and microcyclic compounds containing N-heteroaromatic and carbocyclic fragment, in order to give them practically useful properties" (№ of state registration 0120.0503489). The work is supported by the programs OCSM RAS "Chemistry and physicochemistry of supramolecular systems and atomic clusters", "Biomolecular and medical chemistry", "Medical chemistry", by the program of the Presidium of RAS "Development of methods of obtaining chemical substances and creation of new materials", by Federal target programs "Investigations and developments in priority directions of developing the scientific and technological complex of Russia on 2007-2012" (state contract № 02.513.12.0018), "Scientific and scientific-pedagogical personnel of innovative Russia" (agreement № 8432) and by the grants Russian Fund of Fundamental Investigations: № 07-03-00392-a "Amphiphilic macrocyclic compounds containing pyrimidine fragments: synthesis, catalysis, self-organization", № 10-03-00365-a "Synthesis and properties of cryptan-like and nanoscale pyrimidinophanes".

Work approbation. The main results of the work were reported and discussed in the collection of articles "New drugs: progress and perspectives" (Ufa: Gilem, 2005); in the collection of articles "Chemistry and biological activity of synthetic and natural compounds, Nitrogen-containing heterocycles" (Moscow, 2006); on International symposium "Advances in synthetic and medicinal chemistry" (St. Petersburg, 2007); scientific conference "Organic chemistry for medicine" (Orhimed – 2008) (Moscow, 2008); scientific and practical conference "Biologically active substances: fundamental and applied issues of obtaining and application" (the Crimea, Ukraine, 2009); scientific and practical conference Biologically active substances: fundamental and applied issues of obtaining and application" (the Crimea, Ukraine, 2011); 47thInternational Conference on Medicinal Chemistry "Drug discovery and selection" (Lyon, France, 2011); International Congresson Organic Chemistry (Kazan, Russian Federation, 2011); 17thEuropean Symposiumon Organic Chemistry "ESOC 2011" (Hersonissos, Crete, Greece, 2011); on the first Russian conference on medical chemistry (MedChem Russia – 2013), (Moscow, 2013).

Publications. On the thesis topic six works have been published, including three articles in international journals and three articles in Russian journals, which answer the requirements of State Commission for Academic Degrees and Titles.

Structure and volume of the thesis. The thesis consists of standard sections, they are: introduction, literature review, description of the materials and methods of the investigations, investigation results, their discussion, conclusions and references. The work is presented on 107 pages, contains 12 tables, 27 figures and the list of 116 references including Russian and foreign authors.

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CONTENT OF THE WORK

1. Materials and methods

1.1. Compounds and test-objects used in the experiments

As standards we used drugs of commercial origin (antibiotics substances of ciprofloxacin, ofloxacin, streptomycin, gramicidin S and antifungal agents of clotrimazole, ketoconazole, amphotericin B) (Sigma). Triethylmethylammonium bromide (a simple analog of the compounds being investigated, which has been studied in all test-systems as a model compound, containing the same specific pharmacophores (decile radical and onium group)), 22 representatives of macrocyclic onium derivatives of uracil, 8 acyclic onium derivatives of uracil, 4 representatives of acyclic onium derivatives of hinzelin-2,4-dione and 3 representatives of acyclic onium derivatives of alloxazin have been synthesized at the laboratory of chemistry of nucleotide bases of A.E. Arbuzov Institute of Organic and Physical Chemistry of Kazan Scientific Center of Russian Academy of Science.

Biological objects

Strains of the test microorganisms, used in the work, are listed in Table 1.

Table 1.

№	Strains of bacteria	Strains of microscopic fungi
1	*Staphylococcus aureus ATCC 209p*	*Aspergillus niger BKMF-1119*
2	*Bacillus cereus ATCC 8035*	*Trichophyton mentagrophytes var. gypseum 1773*
3	*Escherichia coli CDC F-50*	*Candida albicans ВКПГу-401/NCTC 885-653*
4	*Pseudomonas aeruginosa ATCC 9027*	
5	*Enterococcus faecalis ATCC 8043*	
6	*Salmonella typhimurium TA 100*	

The bacteria have been obtained from the All-Russian collection of microorganisms strains State Scientific and research institute on standardization and quality control of drugs n.a. L.A. Tarasevich, Moscow, the fungi have been obtained from the All-Russian collection of pathogenic fungi (ARCPF), St.-Petersburg.

To cultivate test microorganisms standard nutrient media have been used: Hottinger Broth for the bacteria and Sabouraud's medium for fungi.

1.2. Determination of antimicrobial activity of macrocyclic and acyclic onium derivatives of uracil

Antimicrobial and antifungal activities have been determined by the method of serial dilution in liquid nutrient media, identifying the concentration of the compound being studied, under which total inhibition of test microorganism growth has been observed. This concentration (mg/l) has been marked as MIC (NCCLS, 2000; NCCLS, 1998).

1.3. Impact of macrocyclic and acyclic onium derivatives of uracil on biochemical processes of microorganisms

1.3.1. Dehydrogenase activity of *S. aureus 209p* and *C. albicans 885-653* has been determined in anaerobic conditions according to the time of decolorization of methylene blue by Thunberg method (Rajvaidya, 2006).

1.3.2. Lipase activity *S. aureus 209p* and *C. albicans 885-653* has been studied by the method based on titrimetric determination of free fatty acids generated as a result of hydrolysis of lipids (Ota's and Yamada's modified method) (Kashmiri, 2006; Macedo, 1997). In the experiments, we used suspensions of the cultures *S. aureus 209p* and *C. albicans 885-653* containing 1×10^9 cfu/ml.

1.3.3. Spectra of the biomarker ribosomal protein *Escherichia coli F-50* have been detected with MALDI-TOF mass spectrometer Autoflex («Bruker Daltonics», Germany). As a control sample, as well as an external calibrator a standard set of proteins of the firm «Bruker Daltonics» (Germany) has been used. To obtain each mass spectrum, we have used

from 1400 to 2000 impulses of the laser with of radiant flux power, fixed at minimal threshold value that has been sufficient for desorption-ionization of the sample. The mass spectrometer parameters have been optimized for the m/z range from 3000 to 100000 Da. For each sample, we have registered a spectrum obtained in the result of summation of 10 single spectra (1400-2000 laser impulses). For the detection, we have used the software FlexControl 2.4 (Build 38), while to process and analyze mass spectra – FlexAnalysis 2.4 (Build 11) of the firm «Bruker Daltonics» (Germany).

The suspension of the cells of *E.coli F-50* (1×10^9 cfu/ml) has been inhibited within 24 hours with the solution of the compounds being studied in effective concentrations. After that, mass spectra of ribosomal proteins of the given culture have been obtained. The results have been estimated according to decrease or total disappearance of spectral peaks.

As a positive control, we used samples containing a standard inhibitor of the protein synthesis, that is, the antibiotic streptomycin.

1.4. Estimation of compounds acute toxicity

The test on estimation of acute toxicity has been carried out on laboratory mammals: white outbred mice of both sexes with the body weight 19.0±2.0 g. The animals have been kept on a standard diet under natural lightning at temperature of 20±2°C (Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ [Reference book on experimental (preclinical) study of new pharmacological substances], 2005).

The initial toxicological estimation of the compounds (in the framework of the flow screening) at intraperitoneal method of injection of water solutions (or with addition of 0.2 % of tween-80) has been carried out during acute experiments on mice of both sexes with the body weight 19.0±2.0 g. After that, during the next 72 hours, we have been observing the mice state, and registering the intoxication symptoms. The toxicity was estimate indicator LD50, that is, a dose (in μm), under which lethal outcome of 50 % of animals has been observed. To determine LD50, each compound has been injected to four groups of mice (6 animals for each dose; n=24). The conversion of doses from μm to mg/kg has been made according to the formula:

$$[\text{mg/kg}] = (\mu\text{m} \times \text{molecular mass}) / 1000$$

All the compounds studied have been ranked in accordance with the classification of acute toxicity of substances when injecting into peritoneal cavity of an animal (Измеров и др. [Izmerov et al.], 1977).

1.5. Estimation of hemolytic activity of macrocyclic and acyclic onium derivatives of uracil

The estimation of hemolytic action of the compounds being investigated was carried out according to Russian National Standard of International Standards Organization 10993.4-99. The method is based on the comparison of density of the solution of the substance being studied with blood with the optical density of blood at 100 % hemolysis.

For the investigation 10 % suspension of human erythrocytes (I «+») and those of the sheep.

1.6. Determination of genotoxicity of macrocyclic and acyclic onium derivatives of uracil

Drugs ability to induce gene mutations in cells was determined with Ames test without metabolic activation according to reversion of the test-strain *Salmonella typhimurium TA 100* which is histidine auxotrophic to prototrophy (Ames et al., 1973; Maron, Ames, 1983).

1.7. Statistical analysis of the results obtained was carried out using the programs Microsoft Office Excel 2007. The accuracy of differences of average values was estimated using Student's coefficient ($P \leq 0.05$). The interrelation of series of data was determined with the help of the correlation coefficient. Table and graphical data contain average values and a standard error.

2. INVESTIGATION RESULTS

2.1. Determination of antimicrobial action of the compounds being investigated

2.1.1. Antimicrobial activity of macrocyclic onium derivatives of uracil

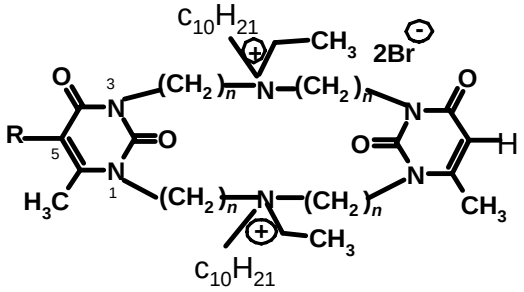


Fig. 1. General formula of macrocyclic onium derivatives of uracil (**M**)

Table 2. Antimicrobial activity of macrocyclic onium derivatives of uracil

№	n	R	Minimal Inhibitory Concentration (MIC) (mg/l)							
			Gram-positive			Gram-negative		Fungi		
			S. aureus	*B. cereus*	*E. faecalis*	*E. coli*	*P. aeru ginosa*	*A. niger*	*T. menta gro phytes*	***C. albi cans***
(4)	5	H	**0.97** ±0,09	1.95 ±0.19	500 ±49	62.5 ±5.8	500 ±49	250 ±25	125 ±12	125 ±12
(5)	6	H	**0.97** ±0.09	1.95 ±0.18	156.0 ±16	62.5 ±6.2	500 ±49	250 ±24	125 ±12	125 ±12
(6)	5	All	**0.24** ±0.02	3.9 ±0.4	390 ±39	15.6 ±1.5	250 ±25	250 ±24	6.25 ±0.62	**1.9** ±0.2
Triethyldecyl ammonium bromide			2.5 ±0.2	50 ±4.5	—	156 ±12	—	—	500 ±41	62.5 ±4.9
Ciprofloxacin			**0.25** ±0.02	0.25 ±0.02	3.9 ±0.4	0.50 ±0.05	0.50 ±0.05			
Ofloxacin			**0.97** ±0.09	1.5 ±0.1	15.6 ±1.5	0.50 ±0.05	3.1 ±0.3			
Ketonazole									**3.9** ±0.4	**3.9** ±0.4

(—) MIC >500 mg/l, All – allyl radical (CH_2=CH-CH_2)

Antimicrobial activity of macrocyclic onium derivatives of uracil was observed within the limits (0.24–500) mg/l. Table 2 presents the values of MIC of the most active macrocyclic derivatives of uracil. Compound **6,** containing allyl radical in the 5th position of the uracil fragment, showed antibacterial activity against *S. aureus 209 P* on ciprofloxacin level (MIC – 0.24 mg/l). Antifungal activity of the given compound against *C. albicans 885–653* is twice higher than the value of activity of the antifungal drug ketonazole. Compounds **4** and **5** with unsubstituted 5th position in the uracil fragment turned out be specific to gram-positive bacteria (*S. aureus 209 P* and *B. cereus 8035),* antimicrobial activity was exhibited on the level of the antibiotic ofloxacin (MIC – 0.97 mg/l). High values of the activity were not indicated against *E. faecalis 8043* and gram-negative bacteria (MIC 156–500 mg/l).

2.1.2. Antimicrobial activity of acyclic onium derivatives of uracil

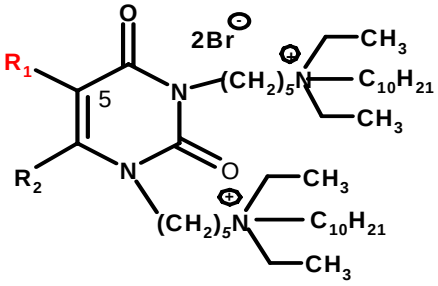


Fig. 2. General formula of acyclic onium derivatives of uracil (**A**)

Table 3. Antimicrobial activity of acyclic onium derivatives of uracil

№	R_1	R_2	Minimal Inhibitory Concentration (MIC) (mg/l)							
			Gram-positive			Gram-negative		Fungi		
			S. aureus	*B. cereus*	*E. faecalis*	*E. coli*	*P. aeru ginosa*	*A. niger*	*T. enta- gro phytes*	***C. albi cans***
(7)	NO_2	H	**0.97** ±0.09	15.6 ±1.5	125 ±12	15.6 ±1.6	—	—	31.3 ±3.0	**0.97** ±0.09
(8)	CH_3	H	**0.97** ±0.09	6.3 ±0.6	125 ±12	15.6 ±1.5	500 ±49	125 ±12	12.5 ±12	**0.97** ±0.09
(9)	**$C_{10}H_{21}$**	**CH_3**	**0.97** ±0.09	**0.50** ±0.05	125 ±12	62.5 ±6.2	250 ±25	500 ±50	62.5 ±6.0	**0.97** ±0.09
Triethyldecyl ammonium bromide			2.5 ±0.2	50 ±4.5	—	156 ±12	—	—	500 ±41	62.5 ±4.9
Ofloxacin			**0.97** ±0.09	**1.5** ±0.1	15.6 ±1.5	0.50 ±0.05	3.1 ±0.3			
Amphotericin B								20.0 ±1.9	3.1 ±3.0	**0.97** ±0.09

(—) MIC >500 mg/l

Antimicrobial activity of acyclic onium derivatives of uracil was observed in the concentration range (0.5–500) mg/l. Compounds **7, 8** and **9** showed antibacterial activity against *S. aureus 209 P* and *B. cereus 8035* on ofloxacin level, while antifungal activity was exhibited against *C. albicans 885-653* at the level of the drug amphotericin B (MIC –

0.97 mg/l). Compound **9**, containing decyl radical ($C_{10}H_{21}$) in the 5th position of the uracil fragment, appeared to be the most active. The value of the activities against *E. faecalis 8043* and gram-negative bacteria is much lower, as well as in case of macrocyclic derivatives (Table 3).

2.1.3. In the series of acyclic onium derivatives of hinzelin-2,4-dione, compound **12** was obtained, which had a high antibacterial activity against gram-positive bacteria, including ***E. faecalis.*** MIC values were exhibited on ciprofloxacin level.

Compound **12** is less active against gram-negative bacteria, but the highest antibacterial activity was observed against *P. aeruginosa 9027* (MIC – 31.3 mg/l) in comparison with macrocyclic and acyclic onium derivatives of uracil. However, antifungal properties of hinzelin-2,4-dione derivatives yield significantly to the compounds described above (Table 4).

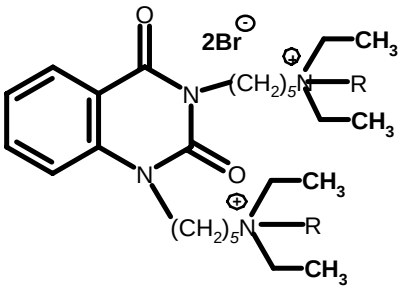


Fig .3. General formula acyclic onium derivatives of hinzelin-2,4-dione (**H**)

Table 4. Antimicrobial activity of acyclic onium derivatives of hinzelin-2,4-dione

№	R	Minimal Inhibitory Concentration (MIC) (mg/l)							
		Gram-positive			Gram-negative		Fungi		
		S. aureus	***B. cereus***	***E. faecalis***	*E. coli*	*P. aeru-ginosa*	*A. niger*	*T. menta-gro phytes*	*C. albi cans*
(10)	$C_{16}H_{33}$	250 ±25	500 ±49	—	—	—	—	—	—
(11)	C_5H_{11}	31.3 ±3.0	125 ±12	500 ±49	—	—	—	62.5 ±6.1	62.5 ±6.2
(12)	**$C_{10}H_{21}$**	**0.20 ±0.02**	1.95 ±0.19	**3.9 ±0.4**	15.6 ±1.5	31.3 ±3.0	250 ±25	31.3 ±3.0	31.3 ±3.1
Triethyldecyl ammonium bromide		2.5 ±0.2	50 ±4.5	—	156 ±12	—	—	500 ±41	62.5 ±4.9
Ciprofloxacin		**0.25 ±0.02**	0.50 ±0.05	**3.9 ±0.4**	0.50 ±0.04	0.50 ±0.05	-	-	-
Ketoconazole		-	-	-	-	-	-	3.9 ±0.4	3.9 ±0.4

(—) MIC >500 mg/l

2.1.4. Acyclic onium derivatives of alloxazin, as well as acyclic onium derivatives of uracil showed high antimicrobial activity against gram-positive bacteria and fungi (Table 5).

Antibacterial activity of compounds **14** and **15** against *S. aureus 209 P* and *B. cereus 8035* was exhibited on the level of ciprofloxacin and ofloxacin. Compound **14** showed the highest antifungal activity among all compounds investigated. Against *C. albicans 885-653,* the activity of this compound was exhibited on clotrimazole level, while its activity against *T. mentagrophytes 1773* was 8 times higher than the MIC value of the known antifungal agent.

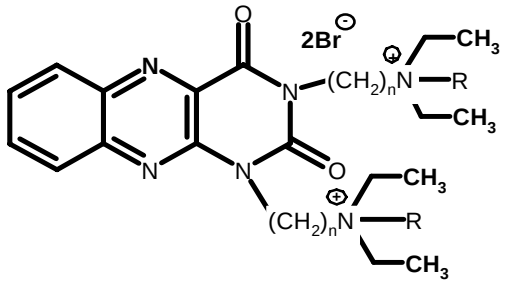


Fig.4. General formula acyclic onium derivatives of alloxazin (**AL**)

Table 5. Antimicrobial activity of acyclic onium derivatives of alloxazin

№	n	R	Minimal Inhibitory Concentration (MIC) (mg/l)							
			Gram-positive			Gram-negative		Fungi		
			S. aureus	***B. cereus***	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A. niger*	***T. mentagrophytes***	***C. albicans***
(13)	5	C_2H_5	—	—	—	—	—	—	—	—
(14)	5	$\mathbf{C_{10}H_{21}}$	**0.24** ±0.02	**0.24** ±0.02	500 ±49	3.1 ±0.3	125 ±12	125 ±12	**0.39** ±0.04	**0.39** ±0.03
(15)	6	$\mathbf{C_{10}H_{21}}$	**0.97** ±0.09	**1.5** ±0.14	—	12.5 ±1.2	500 ±48	—	1.9 ±0.1	1.9 ±0.1
Triethyldecyl ammonium bromide			2.5 ±0.2	50 ±4.5	—	156 ±12	—	—	500 ±41	62.5 ±4.9
Ciprofloxacin			**0.25** ±0.02	**0.25** ±0.02	3.9 ±0.4	0.50 ±0.05	0.50 ±0.05	-	-	-
Ofloxacin			**0.97** ±0.09	**1.5** ±0.1	15.6 ±1.5	0.50 ±0.05	3.1 ±0.3	-	-	-
Clotrimazole			-	-	-	-	-	-	**3.1 ±0.3**	**0.39 ±0.03**

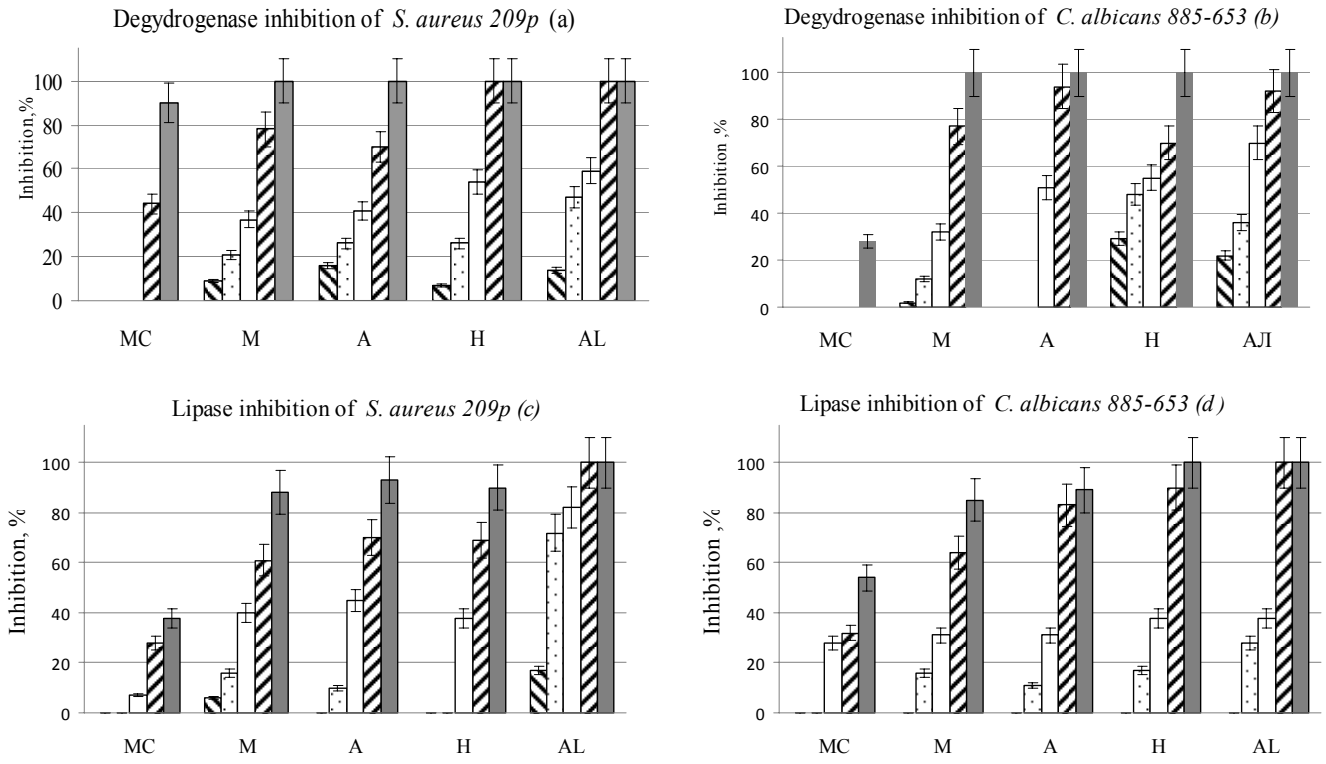
(—) MIC >500 mg/l

2.2. Estimation of the influence of macrocyclic and acyclic onium derivatives of uracil on biochemical processes of microorganisms

2.2.1. Effect of the compounds being studied on dehydrogenases and lipases of *S. aureus 209-P* and *C. albicans 885-653*

In contrast to triethylmethylammonium bromide, all investigated compounds begin inhibiting dehydrogenase activity of *S. aureus 209-P* at the range of low concentrations 0.05-5 mg/l (Fig. 5*a*). Alloxazin derivatives in concentration 0.5 mg/l inhibit the enzyme

action on 47 %, while in concentration 5 mg/l – on 60 %. Dehydrogenase activity of *C. albicans 885-653* is most strongly inhibited by the derivatives of hinzelin-2,4-dione and alloxazin (Fig. 5*b*). In concentration 0.05 mg/l, the percentage of inhibition was 29 % and 22 %, in concentration 0.5 mg/l – 48 % and 36 %, while in concentration 5 mg/l – 55 % and 70 % respectively. Triethyldecyl ammonium bromide inhibits insignificantly dehydrogenase activity of *C. albicans 885-653* only in high concentration 500 mg/l. Lipase activity of *S. aureus 209-P* is most actively inhibited by the derivatives of alloxazin (in concentration 0.5 mg/l – on 72 %, 5 mg/l – on 82 %) (Fig. 5c). The rest of compounds are less active against of this enzyme. On lipase activity of *C. albicans 885-653,* the derivatives of alloxazin have the strongest impact (in concentration 5 mg/l – inhibition is 38 %) (Fig. 5d).



Concentrations of compounds: ■ 500 mg/l ▨ 50 mg/l □ 5 mg/l ⬚ 0.5 mg/l ▧ 0.05 mg/l

Fig. 5. Effect of the compounds being studied on dehydrogenase and lipase activity of *S. aureus 209-P* and *C. albicans 885-653.* **MC** – model compound (Triethyldecyl ammonium bromide); **M** – macrocyclic onium derivative of uracil; **A** – acyclic onium derivative of uracil; **H** – acyclic onium derivative of hinzelin-2,4-dione; **AL** – acyclic onium derivative of alloxazin.

2.2.2. Influence of macrocyclic and acyclic onium derivatives of uracil on the synthesis of biomarker ribosomal proteins *Escherichia coli F 50*

Using the method of MALDI-TOF mass spectrometry we obtained mass spectra of ribosomal proteins *E. coli F-50* with molecular mass of 9064, 9229, 9533 and 9735 Da (Fig. 6 A).

After streptomycin affected the ribosomal proteins *E. coli F-50* in MIC – 25 mg/l, the analysis of their mass spectra showed that the 1st peak, corresponding to protein S12 (mass

9064 Da**),** almost completely disappears (Fig. 6 B).

Further the influence of representatives of each series of the investigated compounds in MIC on biosynthesis of ribosomal proteins *E. coli F-50* was studied (Fig. 7 A). Triethyldecyl ammonium bromide (Fig.7 B) and acyclic onium derivative of uracil (compound **9**) (Fig. 7 C), as well as streptomycin had a strong effect on the spectrum of ribosomal protein S12, so as on the 4th ribosomal (9735 Da). The derivatives of hinzelin-2,4-dione and alloxazin contribute to the reduction of the spectra of the 2nd and 3rd ribosomal proteins with molecular masses of 9229, 9533 (compounds **12, 14**) (Fig. 7 D, E).

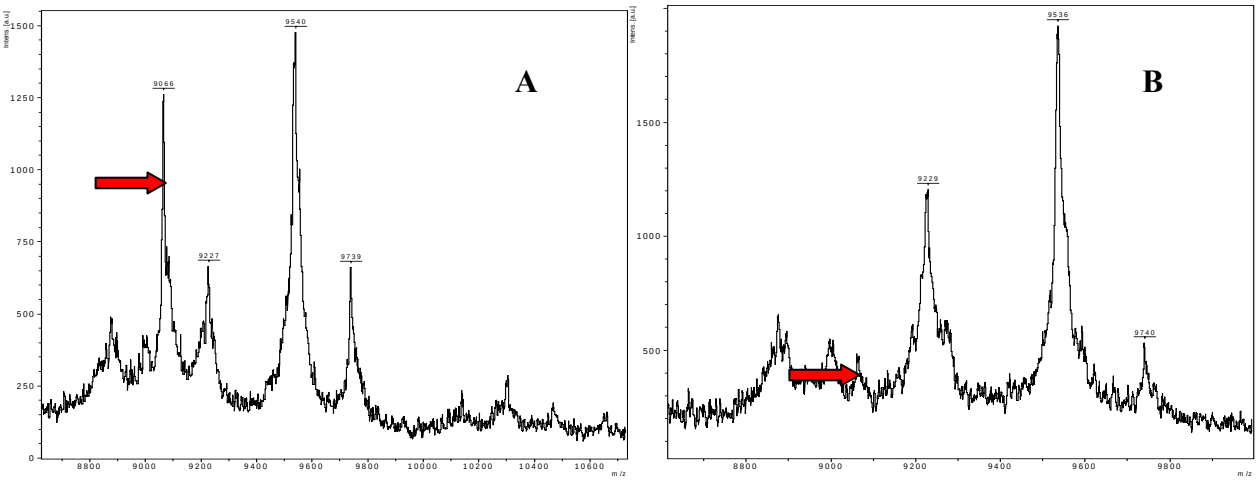


Fig. 6. Influence of the antibiotic streptomycin on biosynthesis of biomarker ribosomal proteins *Escherichia coli F* 50: A) control – mass spectra of ribosomal proteins *E. coli F-50* with molecular masses of 9064, 9229, 9533 and 9735 Da; B) streptomycin effect in MIC – 25 mg/l on biosynthesis of ribosomal proteins *E. coli F-50*).

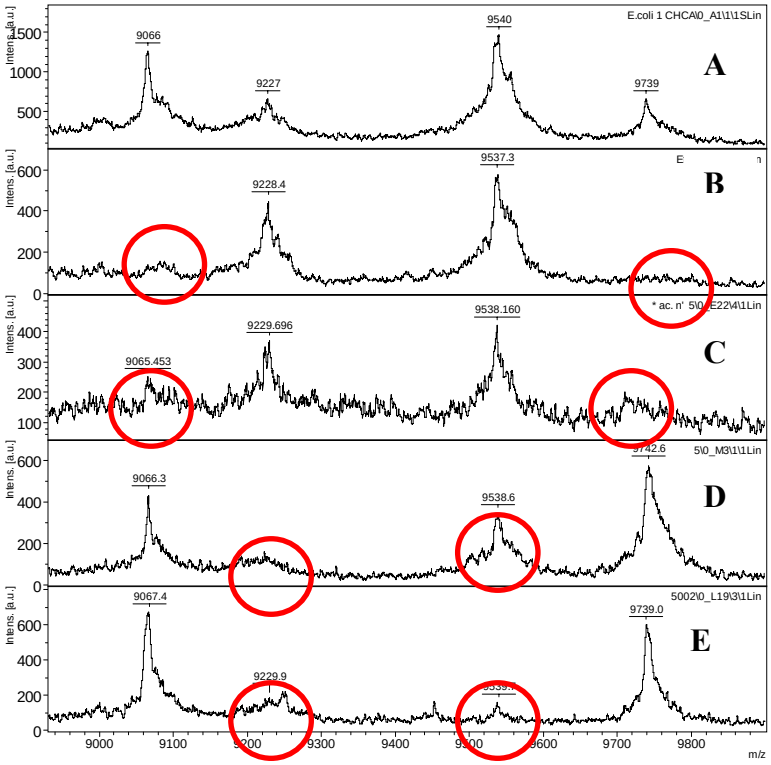


Fig.7. Influence of macrocyclic and acyclic onium derivatives of uracil on biosynthesis of biomarker ribosomal proteins *Escherichia coli F 50*: A) control – mass spectra of ribosomal proteins *E. coli F-50*; B) influence of triethyldecyl ammonium bromide; C) influence of acyclic onium derivative of uracil (compound **9**); D) influence of the derivative of hinzelin-2,4-dione (compound **12**); E) influence of the derivative of alloxazin (compound 1**4**).

The effect of macrocyclic onium derivative of uracil with the highest antimicrobial activity (compound **6**) leads to significant reduction of the spectrum of the 3rd ribosomal protein with molecular mass of 9533 Da (Fig. 8).

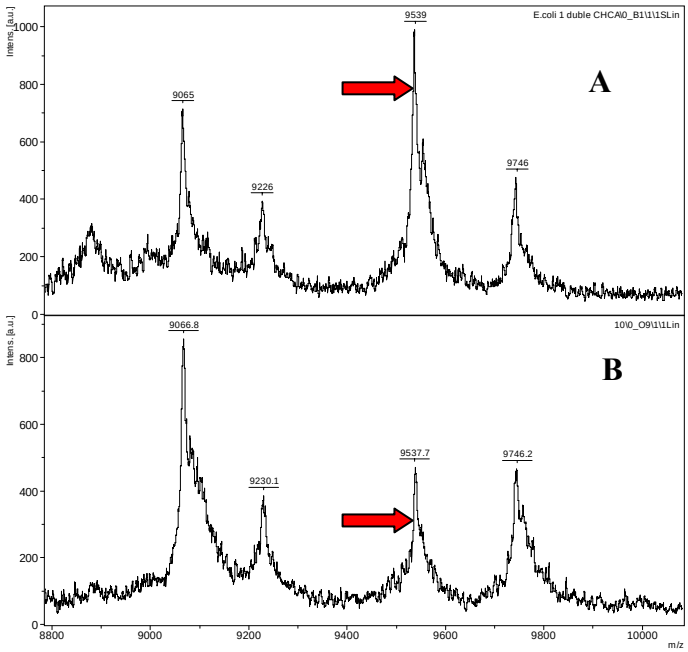


Fig. 8. Influence of macrocyclic onium derivative of uracil on biosynthesis of biomarker ribosomal proteins *E. coli F-50:* A) control – mass spectra of ribosomal proteins *E. coli F-50*; B) influence of macrocyclic onium derivative of uracil (**6**).

2.3. Study of acute toxicity of macrocyclic and acyclic onium derivatives of uracil

The estimation of acute toxicity was carried out for the compounds with high antimicrobial activity (Table 6). All the compounds tested fall into the category of "moderately toxic" (III class of danger according to the degree of the impact on the body).

Table 6. Acute toxicity of the compounds investigated

№ of the compound	LD_{50}; mg/kg, intraperitoneally, mice
M-4	18.8 (16.3÷21.6)
M-5	18.8 (16.3÷21.6)
M-6	22.6 (19.9÷25.4)
A-7	19.8 (17.3÷22.5)
A-9	17.5 (15.6÷19.8)
H-12	18.6 (16.1÷22.5)
AL-14	20.9 (17.6÷24.7)
AL-15	19.2 (14.9÷23.1)
Triethyldecyl ammonium bromide	28.3 (22.9÷34.8)
Gramicidin S	28.0 (25.4÷31.5)

M **(4–6)** – macrocyclic onium derivatives of uracil; A **(7, 9)** – acyclic onium derivatives of uracil; H **(12)** acyclic onium derivative of hinzelin-2,4-dione; AL (**14, 15**) – acyclic onium derivatives of alloxazin.

2.4. Estimation of hemolytic action of macrocyclic and acyclic onium derivatives of uracil

From each series of the compounds being investigated, we selected a representative with the highest antimicrobial activity to estimate hemolytic action. The compounds were studied in minimal concentrations causing inhibition of growth of bacteria and fungi. According to Russian National Standard of International Standards Organization 10993 4-99, if hemolysis is ≥ 2 %, the substance is considered to be hemolytically active. All the compounds tested do not have hemolytic action in the concentrations studied (Fig. 9).

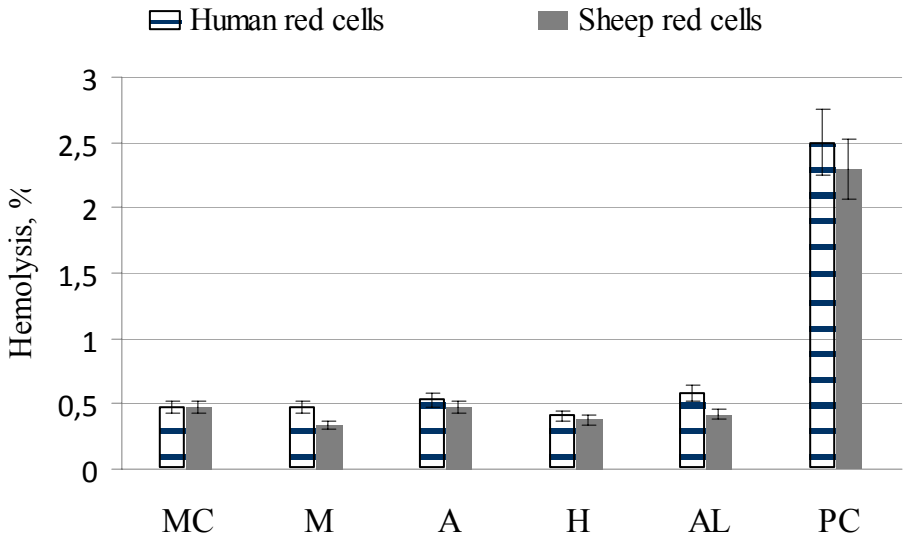


Fig. 9. Hemolytic action of macrocyclic and acyclic onium derivatives of uracil.
Compounds concentrations (mg/l): Triethyldecyl ammonium bromide (**MC**) – 62.5; macrocyclic onium derivative of uracil (**M 6**) – 15.6; acyclic onium derivative of uracil (**A 9**) – 0.97; derivative of hinzelin-2,4-dione (**H 12**) – 31.3; derivative of alloxazin (**AL14**) – 0.39, (**PC**) – positive control – 0.5 % NaCl.

2.5. Genotoxic properties of macrocyclic and acyclic onium derivatives of uracil

To estimate mutagenic effect, we used the same concentrations of the compounds being studied as when determining hemolysis. In the Ames test without metabolic activation it was shown that the number of His^+ reverants being induced by onium compounds didn't exceed spontaneous background of mutation (Fig. 10).

As the number of colonies-reverants in experimental and control samples differ by less than 2.5 times, it can be considered that macrocyclic and acyclic onium derivatives of uracil do not have mutagenic activity in the concentrations being studied.

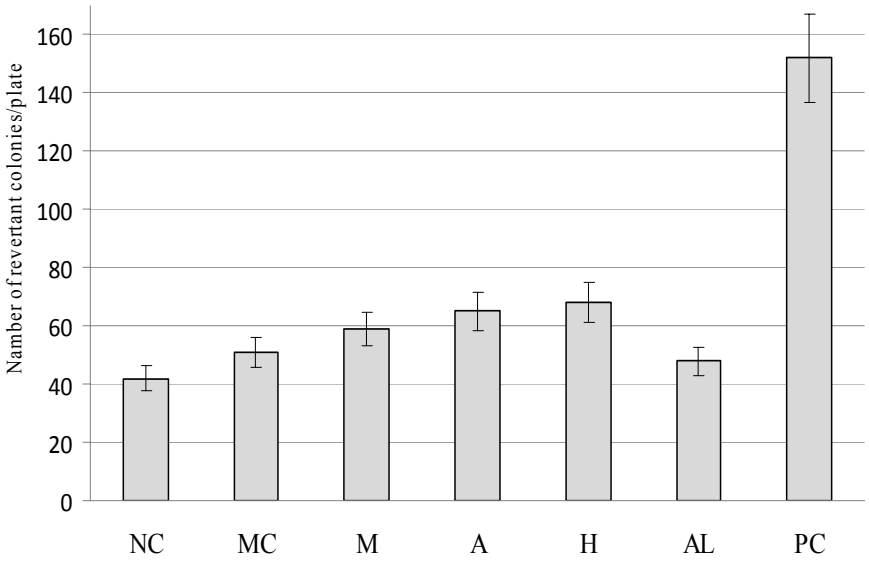


Fig. 10. Estimation of mutagenic effect of macrocyclic and acyclic onium derivatives of uracil. Compounds concentrations (mg/l): Triethyldecyl ammonium bromide (**MC**) – 62.5; macrocyclic onium derivative of uracil (**M 6**) – 15.6; acyclic onium derivative of uracil (**A 9**) – 0.97; derivative of hinzelin-2,4-dione (**H 12**) – 31,3; derivative of alloxazin (**AL 14)** – 0.39; **PC** – positive control (Na-azide); **C** – control (spontaneous background of mutation).

3. DISCUSSION OF THE RESULTS

3.1. Antimicrobial activity of macrocyclic and acyclic onium derivatives of uracil

Macrocyclic onium derivatives of uracil are narrowly specific against gram-positive bacteria *S. aureus 209-P* and *Bacillus cereus 8035.* Such selectivity of action is apparently connected with tree-dimensional structure of their molecules, which correspondence to three-dimensional structure of the biotarget can provide possibility of selective and strong attachment. Large conformational homogeneity of microcyclic derivatives, in comparison to acyclic compounds, makes it possible to reach a certain biotarget without nonspecific losses. A lot of natural antibiotics and toxins, having a complicated cyclic structure, act in such way.

It is obvious that antimicrobial activity of microcyclic derivatives is connected with the presence of pyrimidine fragment in their molecules. Apparently, in this case microcyclic derivatives of nucleotide bases act as nonspecific fragment, which intensifies antimicrobial action of specific pharmacophore (onium group containing lipophilic decyl radical) and even gives it new properties (selectivity in respect of gram-positive bacteria).

Acyclic onium derivatives of uracil, as well as macrocyclic compounds, have high antibacterial activity against gram-positive bacteria. Moreover in respect of *E. faecalis 8043* antimicrobial activity of the majority of acyclic derivatives is 3 times higher than the most active macrocycle has.

It was determined that acyclic onium derivatives of uracil appeared to be more active against fungi and gram-negative bacteria. Such changes of antimicrobial properties in direction of broadening the spectrum of action, possibly, are connected with lower conformational homogeneity of acyclic derivatives of uracil in comparison to macrocyclic

derivatives.

Transfer to condensed derivatives of uracil, containing hinzelin-2,4-dione fragment, leads to appearance of selectivity in respect of bacteria and reduction of antifungal activity.

Introduction of alloxazin fragment allowed obtaining a compound with the highest antimicrobial activity and broad spectrum of activity.

Model compound (triethyldecyl ammonium bromide), which is a classical surface-active substance, is significantly less active than all the compounds investigated.

Thus, the influence of substituents nature at uracil fragment on antimicrobial activity is obvious, and the data obtained allow speaking about specific mechanism of action, which is determined directly by uracil fragment and structural peculiarities of the compound, not only by their solubilizing ability, which is observed in case of classical surface-active substances.

The analysis of the relation "structure – activity" showed that the main structural factors influencing the antimicrobial activity of the compounds studied are: 1) presence of decyl radical in onium group composition; 2) penta- and hexamethylene chain between nitrogen atoms of onium group and N-heterocycle.

3.2. Estimation of influence of macrocyclic and acyclic onium derivatives of uracil on biochemical processes of microorganisms

3.2.1. Influence of macrocyclic and acyclic onium derivatives of uracil on the activity of dehydrogenases and lipases *S. aureus 209-P* and *C. albicans 885-653.*

In view of the fact that metabolism in microorganisms cells is connected with enzymes activity and none of biochemical reactions takes place without them, it is obvious that the investigation of influence of various substances (including drugs) on cells metabolic processes is connected with the study of their impact on enzymatic processes.

The experiments were carried out that made it possible to estimate the influence of the compounds being studied on such important enzymes in a cell as dehydrogenases and lipases, because the change of activity of the given enzymes reflects the dynamics of development of pathologic processes occurring in the organism. A lot of researchers note the inhibition of the activity of dehydrogenases and lipases of microorganisms by various antimicrobial and chemotherapeutic drugs (Очерки по микробиологии [Essays in Microbiology], 2011–2014), (Plewig, 1993).

We carried out estimation of the influence of the compounds being studied on dehydrogenases and lipases *S. aureus 209-P* and *C. albicans 885–653.* The choice of tester strains was connected with the fact that these microorganisms appeared to be the most sensitive to all the compounds studied.

The results obtained showed that in contrast to triethyldecyl ammonium bromide all the compounds studied inhibit actively enough dehydrogenases *S. aureus 209-P* and *C. albicans 885–653.*

On the bases of these data it can be suggested that the action mechanism of the compounds studied is connected with inhibition of enzyme systems of the respiratory chain

of microorganisms at early stages of interaction with cellular targets, which disrupts normal flow of the synthesis of vital compounds in the microorganism cell.

Lipases *S. aureus 209-P* and *C. albicans 885–653* inhibit the derivatives of alloxazin most of all. The rest compounds are less active in respect of these enzymes. The influence of these series of compounds on microorganisms probably causes the disturbance of metabolic processes in cells connected with the action of lipolytic enzymes.

3.2.2. Influence of macrocyclic and acyclic onium derivatives of uracil on synthesis of biomarker ribosomal proteins *Escherichia coli F 50*

The use MALDI-TOF mass spectrometry method made it possible to give quite an exact illustration of the action mechanism of the known antibiotic streptomycin. The disappearance of the peak of S12 protein is in all probability an evidence of the disturbance of its synthesis in *E . coli F 50* cells.

Having obtained a clear picture of the known antibiotic effect on the synthesis of ribosomal proteins *E. coli F 50*, we made an attempt to study the influence of macrocyclic and acyclic onium derivatives of uracil on biosynthesis of biomarker ribosomal proteins *E. coli F 50* using MALDI-TOF mass spectrometry method. The analysis of the results obtained has made it possible to suggest that the action mechanism of the compounds studied is connected with the disturbance of protein synthesis in a bacterial cell.

3.3. Estimation of acute toxicity, hemolytic action and genotoxic properties macrocyclic and acyclic onium derivatives of uracil

The results obtained speak that all the compounds tested are related to the category of “moderately toxic” (III class of danger according to the degree of impact on the body). Toxic properties of the compounds being investigated are revealed on the level of the known antibiotic Gramicidin S, but in contrast to it onium derivative of uracil do not hemolytic action. Genotoxic properties have not been revealed in the Ames test among the series of macrocyclic and acyclic onium derivatives of uracil.

Thus, the analysis of the results obtained showed that the compounds studied can be recommended for the work with living objects.

AFTERWORD

Macrocyclic and acyclic onium derivatives of uracil exhibited high antimicrobial activity on the level of the known drugs: ciprofloxacin, ofloxacin, clotrimazole, ketoconazole, amphotericin B. The connection between the structure of the compounds studied and their antimicrobial activity was determined. In the series of macrocyclic onium derivatives of uracil the compounds narrowly specific in respect of gram-positive bacteria were identified. Acyclic onium derivatives of uracil appeared to be less specific in respect of microorganisms used in the experiments and exhibited quite high antifungal activity on the level of widely used drugs: clotrimazole, ketoconazole and amphotericin B. The data on the study of the effect of macrocyclic and acyclic onium derivatives of uracil on biochemical processes of microorganisms have made it possible to suggest that the action mechanism of

these compounds is connected with inhibition of such important enzymes in a cell as dehydrogenases and lipases and protein biosynthesis. During the experiments it has been determined that all the compounds tested are related to the substance category of "moderately toxic", do not exhibit hemolytic and mutagenic properties and can be applied for the work with living objects.

Further investigations aimed at more thorough study of the action mechanism and new pharmacological properties will help develop a strategy for creating a new class of biologically active substances.

CONCLUSIONS

1. Against gram-positive bacteria, macrocyclic and acyclic onium derivatives of uracil exhibit antimicrobial activity on the level of ciprofloxacinand ofloxacin (MIC = 0.2-3.9 mg/l); against gram-negative bacteria, acyclic derivatives of hinzelin-2,4-dione and alloxazin are the most active (MIC = 3.1-31.3 mg/l); against fungi, acyclic derivatives of alloxazin and uracil are the most active, which antifungal activity is shown on the level of ketoconazole, clotrimazole and amphotericin B (MIC = 0.39-3.9 mg/l).

2. The analysis of the relation "structure – activity" showed that macrocyclic onium derivatives of uracil exhibit selective properties against gram-positive bacteria. Acyclic onium derivatives appeared to be less specific and exhibited high antimicrobial activity as against gram-positive and gram-negative bacteria, so against fungi.

The main structural factors, influencing antimicrobial activity of the compounds studied, are: 1) presence of decyl radical in composition of onium groups; 2) penta- and hexamethylene chains between nitrogen atoms of onium group and N-heterocycle.

3. Maximal inhibition of the activity of dehydrogenases and lipases is reached under the action of alloxazin derivatives: of dehydrogenase activity of *S. aureus 209-p* is inhibited on 60 %, *Candida albicans 885*-653 – on 70 %, lipase activity of *S. aureus 209-p* is inhibited on 82 %, *C. albicans 885*-653 – on 38 %. All the compounds studied influence protein biosynthesis in the cells of *E. coli F 50*.

4. Macrocyclic and acyclic onium derivatives of uracil are related to the substance category of "moderately toxic" (mice, intraperitoneally *in vivo*).

5. The compounds studied do not exhibit hemolytic and genotoxic properties *in vitro*.

6. Macrocyclic and acyclic onium derivatives of uracil can be recommended for development as antimicrobial drugs, which are selective against gram-positive bacteria (macrocyclic derivatives), and have a broader spectrum of action (acyclic derivatives of alloxazin and uracil).

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