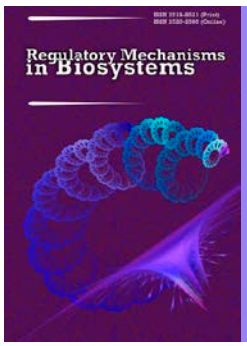

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Determination of antimicrobial activity of some 1,2,4-triazole derivatives

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We carried out MIC of the derivatives of 1,2,4-triazole II (4-((5-nitrofuran-2-yl)methyleneamino)-1-propyl-4H-1,2,4-triazolium bromide) and I (N-((5-nitrofuran-2-yl)methylene)-4H-4-amino-1,2,4-triazolidium chloride) against *Escherichia coli* ATCC 3912/4 and *E. coli* k88ad, *Staphylococcus aureus* ATCC 25923 and *S. aureus* k99, *Klebsiella pneumonia* k56 and *Salmonella typhimurium* 144, *S. enteritidis*. All test cultures were sensitive to compound II at concentrations of 1,25–0,039 μg/ml. Similar MIC (0,039 μg/ml) of compounds II and I were set for *E. coli* k88a and *S. aureus* k99 test cultures – 0,156 μg/ml. Only *S. aureus* ATCC 25923 and *K. pneumonia* k56 had sensitivity to ceftriaxone (MIC = 0,097 μg/ml). Antiviral activity of Trifuzol (piperidine 2-[5-(furan-2-il)-4-phenil-1,2,4-triazol-3-ilthio]acetate) and avistim (morpholines 3-(4-pyridyl)-1,2,4-triazolil-5-thioacetate) against the chicken infectious bronchitis virus (VIB) strain 4/91 was characterized by a decrease in mortality and pathological changes of chicken embryos (CE) which were induced by the virus. Death of infected CE provoked by the strain 4/91 of VIB in dilution 10^{-3} occurred at 57.1%. The reduction in the percentage of deaths of CE infected by the virus in dilution 10^{-3} in the presence of Avistim was 28.6%, and with Trifuzol 14.3%. The use of avistim and Trifuzol compounds reduced VIB infectious activity when it was cultivated in CE, reducing the titre of the virus (strain 4/91) by 3 lg EID 50 cm^{-3}.

Keywords: derivatives of 1,2,4-triazole; Trifuzol; Avistim; antibacterial activity; antiviral activity

Introduction

The compounds of 1,2,4-triazol-5 and 3-nitro-1,2,4-triazole-5 were obtained at the beginning of the 20th century, and considered as effective compounds in decreasing of the influence of many diseases (Gehlen et al., 1964). The typical species in a range of compounds of 1,2,4-triazole are 5-amino-1,2,4-triazole-3-carboxylic and 5-amino-1,2,4-triasole-3-iloacetic acid. The first of these was known at the end of the 19th century. Dyes, plant protection products and antivirals (ribavirin) were synthesized in industry on its basis (Abo-Bakr, 2014).

Derivatives of 1,2,4-triazoles are used in industry, agriculture, veterinary and humane medicine. Among a large number of various agents that have antibacterial, antifungal and antiviral effects, these compounds deserve special attention in veterinary practice (Parchenko, 2011; Pattan et al., 2012; Zoumpoulakis et al., 2012).

The analysis of scientific and technical studies in recent years has shown that the nucleus of 1,2,4-triazole is a structural fragment of antifungal drugs (Fluconazole, Itraconazole), antidepressant (Trazodone, Alprazolam), hepatoprotective, wound healing and antiviral (Thiotriazolin) effects (Plech Tomaz et al., 2013).

Currently 1,2,4-triazoles attract interest in antimicrobial chemotherapy due to their high spectrum of biological activity. The emergence of new pathogens and the difficult problem of resistance give rise to the need for new antimicrobial agents with greater selectivity and low accidental effects (Jyoti Sinha et al., 2017).

Among the compounds that have antibacterial activity, special attention should be paid to 2-pyrazolines, 1,3,4-oxadiazoles, glycosides, salicylates, quinolines, amino acids (glycopeptides), isatins, oxoindoles and triazoles. The spectrum of use of morpholines 2-[5-(pyridine-4-il)-1,2,4-triazole-3-ilthio]acetate for the treatment and prevention of poultry diseases is very wide. The main indicators for the successful use of any drug in the poultry sector are activation of the general resistance, increase of preservation, growth and development of poultry post-hatching, reduction of the influence of various stress factors, fortification of the immune response to vaccines, prevention of the sickness rate of the poultry in the context of threat of an infectious outbreak (Alrawashdeh, 2008). Some compounds of the triazolin group have a high level of biological activity (Mavrova et al., 2009). So, the cytotoxicity of 21 compounds against thymocytes was proved (Truchlinski et al., 2006), when the turkeys were given 1,2,4-triazole derivatives in comparison with garlic extract and echinovite, there was an increase of leukocytes number, cellular immunity the percentage of phagocytic activity of neutrophils by 2 times. Ognik (2009) has determined the positive effect of 5-oxo-1,2,4-triazole on 11-week-old turkey organisms at the dose of 30 μg/kg of body weight per day and established the antioxidant activity of this compound.

Some strains of microorganisms, in the presence of Lozeval, received resistance to it longer than to other drugs (Ampicillin, Levomytsetin). Studying the stability of *S. aureus*, *E. coli* and *Salmonella*, it was established that even after the 20th passage, high sensitivity to Lozeval remains, however this has emerged to a much lesser extent to other antibiotics (Onischiuk, 2004).

The minimum inhibitory concentration of compound 3f(4-{[(E)-(3-Phenyl-1H-pyridazol-4-yl)methylidene]-amino}-4H-1,2,4-triazole-3-thiol) against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* is 3 times lower than the concentration of Ceftriaxon of 1.6125 mg/mL (Malladi et al., 2017).

New synthesized compounds – derivatives of 1,2,4-triazole have had a variable inhibitory effect on Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration of (MIC) compounds: 3-(4-chlorophenyl)-6-(p-tolyl)-3,3a-dihydro-2H-pyrazolo [30,40:4,5] thiazolo[3,2-b][1,2,4]triazole(4b); 3-(3-nitrophenyl)-6-(p-tolyl)-3,3a-dihydro-2H-pyrazolo [30,40:4,5] thiazolo[3,2-b][1,2,4]triazole(4c); 3-(4-nitrophenyl)-6-(p-tolyl)-3,3a-dihydro-2H-pyrazolo [30,40:4,5] thiazolo [3,2-b][1,2,4]triazole(4d); 3-(4-bromophenyl)-6-(p-tolyl)-3,3a-dihydro-

2H-pyrazolo [30,40:4,5] thiazolo[3,2-b][1,2,4]triazole(4h); 3-(3-nitrophenyl)-6-(p-tolyl)-3,3a-dihydroisoxazolo [30,40:4,5] thiazolo[3,2-b] [1,2,4] triazole(5c)3-(4-nitrophenyl)-6-(p-tolyl)-3,3a-dihydroisoxazolo [30,40:4,5] thiazolo[3,2-b][1,2,4]triazole(5d); 3-(4-bromophenyl)-6-(p-tolyl)-3,3a-dihydroisoxazolo [30,40:4,5] thiazolo[3,2-b][1,2,4]triazole(5h) was 50% lower than the concentration of Chloramphenicol and Streptomicin of 3,125 mg/ml against *B. thuringiensis*. Compounds 4b and 4d provoked the inhibition of growth of *P. aeruginosa* culture at the concentration that was 50% lower than the compared species (Seelam et al., 2016).

Bactericidal activity at a concentration of 10 mg/cm^3 was established in 8% of the studied compound. The most promising compound was 1.3.6.8-tetraazatricyclo[4.4.1.1.3.8]dodecan (AI-20, tiotropin), from which were synthesized two derivatives AI-21 (Zn-containing derivative of tetraazadigomoadamantane) and AI-22 (NH_3 retaining derivative tetraazadihydroadamantane). After 24 h of exposure, inactivation of the cultures of *S. aureus* 209P, *E. coli* K12, *E. coli* 195β, *S. enteritidis* occurred at concentrations of tetraazadigomodamantane not lower than 0,032%, 0,017%, 0,012% and 0,019%, respectively. The antimicrobial activity of the synthesized ilidenhydrazide compounds against *S. aureus* 209P, *E. coli* 675, *S. disenteriae* 1675 E, *Proteus vulgaris* 5, *B. anthracis* 1319, *P. aeruginosa* 165 detects antimicrobial activity, but does not exceed the activity of standard comparison (Danilchenko et al., 2017).

The dynamics of sensitivity of *S. aureus* and *E. coli* strains have been studied. These strains showed a high level of resistance to the preparations of the groups of aminoacid, oxoindole, triazole and glicoside despite the presence in molecules of the latest – OH-radicals and phenols, stipulating the antiseptic properties of medicinal substances of this group. Triazole M100 had a bacteriostatic effect only against *S. aureus*, whereas Trizole M129 showed antimicrobial activity only against *E. coli* strains (Thomas et al., 2012).

New alkil derivatives of 5-(furan-2-il)-4-amino-1,2,4-triazole-3-thiol and esters of 2-((5-(furan-2-il)-4-amino-1,2,4-triazol-3-il)thio)-acetate acid have been investigated in relation to antimicrobial activity. All ten compounds showed moderate activity. The most sensitive was *S. aureus* ATCC 25923 to 3-pentylthio-5-(furan-2-il)-4-amino-1,2,4-triazole, isopropyl 2-((5-(furan-2-il)-4-amino-1,2,4-triazol-3-il)thio)acetate and isobutil 2-((5-(furan-2-il)-4-amino-1,2,4-triazol-3-il)thio)acetate (Pruglo et al., 2016).

The group of antiviral drugs, based on the nucleus of 1,2,4-triazole, is quite wide. It is established that the well-known drug tiotriazolin, which is an S-derivate of 1,2,4-triasole, forwards the improvement of the clinical picture of patients with hepatitis B and C. First of all the antiviral activity of this drug is linked with its immune modulation properties. The widely-known medicinal product ribavirin is a synthetic analogue of nucleoside. *In vitro* it is active against some RNA and DNA viruses.

New derivatives of 1,2,4-triazole were created. They are different from the morpholine 5-methyl-1,2,4-triazole-3-ilthioacetate-tiotriazolin, which realized antiviral activity in inadequate degree. These derivatives reduce the infectious activity of the VIB virus, strain H – 120 for 1,8 lg, and strain Calnek 1143 of Poultry Infectious Encephalomielitis virus at 1.0 lg when cultivating in 9-day chicken embryos (CE). In this case, the compound actions affected the intensity of displaying the pathological features induced by the virus . New compounds that are derivatives from 1,2,4-triazole provide antiviral activity with low toxicity and could be used in pharmacology to create new drugs for this type of action (Bushueva et al., 2017).

Research on effectiveness of Triufuzole vaccination against viral diseases of broilers has been performed on the experimental group. This group did not receive Trifuzol. The control group received 1% Trifuzol solution at 7–9 day of bird breeding at the rate of 0,5 ml per 10 kg of live weight for 3 consecutive days. The level of protective antibodies against VIB responded to the base norm, but the vaccination index (IV) in the control group was 1.5 times higher. The average antibodies titre to the Newcastle disease virus with use of the Trifuzol was 5 $\log_2$, and the group immunity – 87.5%, which corresponds to the standard (Varynskyi et al., 2015). When piperidine 2-[5-(furan-2-il)-2H-1,2,4-triazol-3-ilthio]acetate was added to the culture medium 2 h after the virus adsorption, the largest antiviral activity for the whole long period of its reproduction was observed 120 h after the start of infection. The infectious activity decreased by more than 4.75 lg TCD_{50}/cm^3, and the compound of comparison Thiotriazolin decreased the virus to more than 2,0 lg TCD_{50}/cm^3 (the difference between them exceeded 2.5 lg). This indicates the greater antiviral action of piperidine 2-[5-(furan-2-il)-2H-1,2,4-triazole-3-ilthio] acetate compared to Thiotriazolin (Sinha, 2017).

The studied compounds reduce the intensity of pathological changes in infected chicken embryos, which have been induced by vaccine virus strains. Starting with 0.01% concentration, the compounds suppress the occurrence of pathological changes induced by viruses. Thus, the percentage of changes such as hyperemia, thickening of the chorionalantois membrane (CAM), embryonic head hemorrhage, lag in growth and development, the clay of the liver decrease during the simultaneous injection of the piperidine 2-[5-(furan-2-il)-4-phenyl-1,2,4-triazole-3-ilthio] acetate and (morpholine 2-[5-(pyridine-4-1,2,4-triazol-3-ilthio] acetate in 0.01%, 0.02% concentration. Thus, the morpholine 2-[5-(pyridin-4-il)-1,2,4-triazol-3-ilthio] acetate and piperidine 2-[5-(furan-2-il)-4-phenyl-1,2,4-triazol-3-ilthio] acetate) cause the decrease the biological activity of the viruses that affected the intensity of the manifestation of patho-anatomical fetures, induced by the virus and reduced is titre (Alrawashdeh, 2008).

The analysis of scientific sources against the synthesis and screening of biological activity of derivatives of 1,2,4-triazole determines the relevance of this direction of scientific research all over the world. Spreading of the spectrum of antimicrobial agents is associated with the emergence of resistant strains of microorganisms and the viruses' variability, especially in the poultry industry. In this regard, the aim of our research was to determine the sensitivity of bacteria, isolated from chickens, to new triazolin compounds and their antiviral activity.

Materials and methods

For bacteriological studies, blood and red bone marrow from 1-day, 10-day and 21-day Habbard cross chickens were collected. Sensitivity of microorganisms to antibiotics was carried out relative to NCCLS "Methods for the determination of susceptibility of bacteria to antimicrobial agents" (1999) and EUCAST Definitive document CLSI "Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems" (2015).

List of compounds, studied in the research:

I – N-((5-Nitrofuran-2-il)methylene)-4H-4-amino-1,2,4-triasolidium chloride (formula 1);

II – 4-((5-Nitrofuran-2-yl)methyleneamino)-1-propil-4H-1,2,4-triazolium bromide (formula 2);

Avistim – morpholines 3-(4-pyridyl)-1,2,4-triazolil-5-thioacetate (formula 3);

Trifuzol – piperidine 2-[5-(furan-2-il)-4-phenil-1,2,4-triazol-3-ilthio] acetate (formula 4);

The compounds were synthesized in Zaporizhzhya State Medical University, Ukraine.

Dimethylformamide (DMF) – N,N-Dimethylformamide (formula 5);

Ceftriaxone – [6R-[6alpha7beta(z]])-7-[[(2-Amino-4-thriazolil) methoxyminoacetyl]amino]-8-oxo-3-[[(1,2,5,6-tetrahydro-2-metil-5,6, lbjrcj-1,2,4-triasin-3-yl)tio]metil]-5-tia-1-azabicyclo[4.2.0.]octa-2-ene-2-carboxylic acid (formula 6);

Preparation of solutions and serial dilutions of the studied substances. The compounds are synthesized in Zaporizhzhya State Medical University, Ukraine.

The dilution of compounds I and II were prepared from 1:20 to 1:0.039 based on a matrix solution (dilution of compounds 1:40 in DMF) on the sterile physiological solution for 24 units of McFarland.

Ceftriaxone is a dry powder for injection of 1,000 active units (1 g), was diluted in 2 ml of meat infusion broth (MIB), and a working solution of 500 units/ml was obtained. Then dilutions were repeated, by adding 1 ml of the working solution of antibiotic with 500 units/ml to 4 ml of MIB. Thus, the working solution was obtained at a concentration of 100 units/ml. Subsequently 1 ml of MIB was added to the same solution

and received 50 units/ml (McFarland 0,5). Thus, the tested concentrations of Ceftriaxone were from 25 to 0.0488 units.

Preparation of inoculum of test cultures. Typical representatives of Gram-positive and Gram-negative bacteria were selected as test objects: *Staphylococcus aureus* ATCC 25923 and k99; *Escherichia coli* ATCC 3912/41 i k88 ad.; *Klebsiella pneumoniae* k56 and *Salmonella typhimurium* 144. For the production of inoculums of microorganism cultures, daily cultures were grown on Mueller-Hinton Agar (HiMedia Laboratories Ltd., India).

Inoculum was prepared from the daily culture of microorganisms, on MIB. The inoculum concentration was 0.5 units by MacFarland, which is 1.5×10^8 CFU/ml. The working concentration of the inoculum was 5·CFU/ml (10^6 CFU/ml).

Serial dilutions in the broth were accompanied by control of crop growth in MIB without the use of compounds I or II, Ceftriaxone and DMF.

The purity of the suspension of microorganisms was confirmed by sowing on agar, followed by Gram-staining and microscopy. The assessment of sensitivity of microorganisms to the studied compounds was carried out on the basis of a minimum inhibitory concentration (MIC). The results of the experiment were taken into account after the assessment control of the inoculums, which was at temperature 40 ºC and in the thermostat (37 ºC).

Disc-diffusion method. Disks of sterile filter paper, 5 mm diameter, were soaked in appropriate concentrations of the studied compounds I and II. The content of the studied compounds on the disk was 25 and 50 μg. Petri dishes with the Muller-Hinton agar were sown by test culture in the concentration equal to the turbid standard McFarland 0,5. After that the disks, which contained the test substances and antibiotics, were replaced on the agar surface, in accordance to the stencil. Petri dishes were maintained at room temperature for 30 minutes and placed in the thermostat at the temperature of 8 °C in an inverted position. Antibiotics: Ofloxacin, Norfloxacin, Ciprofloxacin, Levomycetin, Erythromycin, Azithromycin, Josamycin, Cefuroxime, Ceftriaxone, Cefepime, Gentamicin, Amoxicillin were control. In 24 h, the sensitivity of test-cultures to compounds I and II was determined by the diameter of retardation zone (Patel, 2013).

Studying of antiviral activity of triazolin range compounds against the virus of the infection of chicken bronchitis. Chicken embryos (CE) were incubated in a laboratory. Biological evaluation of eggs was carried out according to the recommendations of Dyadichkin (2010) and Besarabov (2010). Vaccine strain that was used in this research: the lyophilized live vaccine against chicken bronchitis infectious (Nobilis IB 4-91, virus titer10^6 EID_{50}/cm^3). The preparation of the virus from 10^{-1} to 10^{-4} was prepared in a sterile physiological solution. The determination of the antiviral activity of the compounds of Trifuzol and Avistim in concentration 1% was carried out by simultaneous infection by the strain of 4/91 VIB of chicken embryos 9-day incubation on the CAM in a dose of 0.2 cm^3. The incubation of infected and control CE were carried out in a laboratory incubator at the temperature of 37 ºC and a humidity of 65% for 4 days. Evaluation of pathological changes induced by VIB strain 4/91 and the effects of triazolin range compounds, selection of the restrictive virus material were carried out at 4 days incubation of CE and pre-cooling at the temperature of 40 ºC for 12 h. The criterion for assessing the reproduction of the virus was pathoanatomical changes in the embryo, special to the action of the virus and the titre of the virus, which was calculated by Reed and Mench and expressed in EID_{50}/cm^3 (Stefanov, 2001).

Statistical processing of the results. Statistical data were processed by a program Statistica 7.0 (StatSoft, USA) (Rebrova, 2006).

Results

Strains of *E. coli* and *S. aureus* were isolated from 1, 10 and 21-day chickens. As a result of serological typing, there were 78 enterotropic bacteria cultures, 87% of them were classified into serological variants of k88 ad; k88 ad, ab; k99. *E. coli* and *S. aureus* were detected from the blood of 1-day chickens, *E. coli* of serovariant k 88 ad were detected from the red bone marrow, whereas in the blood of 10-day old chickens – *E. coli* serovariant k 88 ad, ab and *S. aureus* k99 were detected. *E. coli* serovariants k99 were isolated from red bone marrow of 21-day old chickens. The frequency of detection of *S. aureus* – 16.2% and *E. coli* – 13.2% (Fig. 1).

Isolated strains of *E. coli* and *S. aureus* had the highest sensitivity to erythromycin (diameter of the growth inhibition zones 32 ± 3.5 mm and 25 ± 2.8 mm respectively).

Both *S. aureus* and *E. coli* were sensitive to gentamicin (diameter of the growth inhibition zones 19 ± 2.1 mm and 21 ± 2.3 mm respectively). In this case, the *S. aureus* culture had an average sensitivity to Doxycycline. Thus, microorganism cultures isolated from chickens at different incubation periods have the high sensitivity to antibiotics of the Macrolide and Aminoglycoside classes.

The evaluation of isolated *E. coli* 88 showed the resistance to Doxycycline (diameter of the growth inhibition zone 2.5 ± 0.27 mm), Josamycin (4.3 ± 0.47 mm), and Azithromycin (5.1 ± 0.56 mm). The resistance of *S. aureus* k99 was also detected to Azithromycin, Josamycin, Ceftriaxone and Ciprofloxacin. In the subsequent study we expanded the antibiotics spectrum of different groups and tested the potential antimicrobial action of new derivatives of 1,2, 4-triazole.

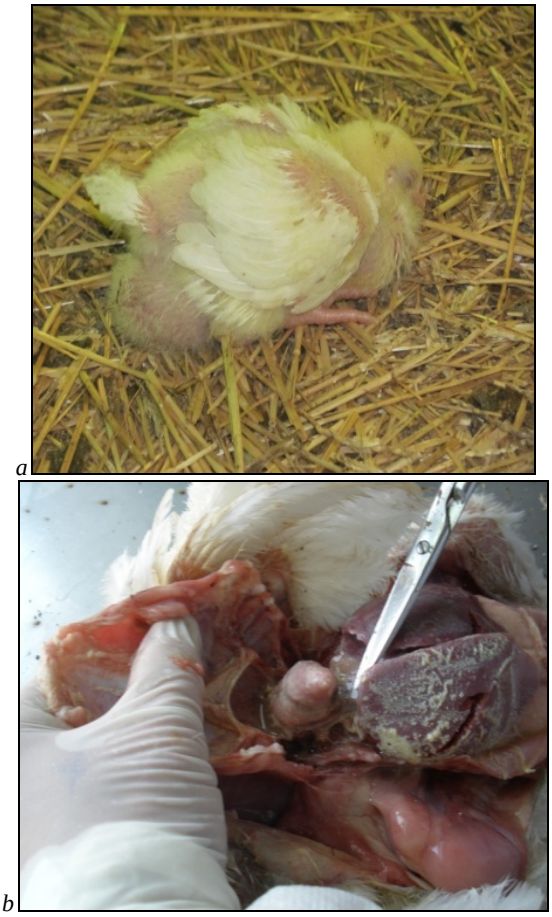


Fig. 1. Clinically sick chicken Hubbard cross (*a*) and pathological changes in chicken with coli bacillosis (*b*)

Antimicrobial activity of triazolin compounds. The sensitivity to Cefuroxim was shown by *E. coli* ATCC 3912/41 and *S. aureus* ATCC 25923. The diameter of the growth inhibition zone exceeded the norm for this antibiotic. Cultures, which were isolated from the chickens, stayed insensitive to Cefuroxim.

E. coli ATCC 3912/41 detected high sensitivity to Ceftriaxone that also exceeded the normative index. All studied cultures were highly susceptible to Gentamicin and only test-cultures were susceptible to Ciprofloxacin and Norfloxacin. The sensitivity of *S. aureus* culture, isolated both from the chickens and among the museum strains, should be noted.

Antibacterial activity of the new triazolin range compounds can be compared with the normative indices, in the case of their sensitivity to the action of Cefuroxim, Cefepim, Norfloxacin, Levomycetin, and Doxycycline in the growth delay zone, as tested and as isolated cultures (Table 1).

Table 1
Antibacterial activity of the investigated compounds compare to antibiotics (M ± m)

Drug class or subclass / antibiotic	The content of the antibiotic on the disk, μg	The diameter of the growth inhibition zone, mm			
		E. coli, 5×10^5 CFU/cm^3		*S. aureus*, 5×10^5 CFU/cm^3	
		K88 ad	ATCC O55 K59 912/41	ATCC 25923	K99
Erythromycin	15	32 ± 3.5	15.3 ± 0.5	16.2 ± 0.1	25 ± 2.77
Azithromycin	15	5.1 ± 0.56	–	–	3.6 ± 0.4
Josamycin	15	4.3 ± 0.47	–	–	2.3 ± 0.25
Cefuroksim	30	6.2 ± 0.68	19.4 ± 0.4	24.3 ± 0.2	8.5 ± 0.94
Ceftriaxone	30	7.8 ± 0.86	23.3 ± 0.5	–	4.6 ± 0.51
Cefepim	30	6.5 ± 0.72	–	–	8.9 ± 0.98
Amoxicilin	20	10.1 ± 1.12	–	–	8.6 ± 0.95
Ofloxacin	5	–	24.0 ± 0.82	–	27.6 ± 0.24
Ciprofloxacin	5	9.3 ± 1.03	34.0 ± 0.1	30.1 ± 0.7	4.9 ± 0.54
Norfloxacin	10	10 ± 1.11	22.6 ± 0.24	18.5 ± 0.3	9.6 ± 1.06
Doxycycline	30	2.5 ± 0.27	–	–	12.3 ± 1.36
Gentamicin	120	21 ± 2.33	22.0 ± 0.7	20.5 ± 0.5	19.0 ± 2.1
Levomycetin	30	–	17.8 ± 0.48	21.4 ± 0.1	27.3 ± 0.48
N-((5-Nitrofuran-2-il)methylene)-4H-4-amino-1,2,4-triazolium chloride (I)	25	15.8 ± 1.55	13.5 ± 2.14	–	0
	50	17.8 ± 1.44	17.8 ± 0.25	–	18.8 ± 1.65
4-((5-Nitrofuran-2-il)methylene-amino)-1-propyl-4H-1,2,4-triazolium bromide (II)	25	14.8 ± 1.7	13.5 ± 1.20	–	14.0 ± 1.47
	50	12.0 ± 2.16	14.8 ± 2.05	–	17.3 ± 0.47

Note: "–" – not sensitive.

The detection of sensitivity of test cultures and isolated strains of *E. coli* and *S. aureus* to new derivatives of 1,2,4-triazolo N-((5-nitrofuran-2-il)methylene)-4H-4-amino-1,2,4-triasolidium chloride (I) and 4-((5-nitrofuran-2-il)methylenemino)-1-propyl-4H-1,2,4-triazolium bromide (II), in comparison with the antibiotics of different groups, indicates their antimicrobial action. The most effective antimicrobial activity was shown by the compound I at a concentration of 50 μg against the *S. aureus* (diameter of inhibition of growth zone 18.8 ± 1.65 mm). The compound I had lower activity with respect to *E. coli* (17.8 mm), respecttively, serovariant k88 ad and ATCC 3912/41. Compound II inhibited the growth of *S. aureus* k99 (diameter of inhibition of growth zone 17.3 mm).

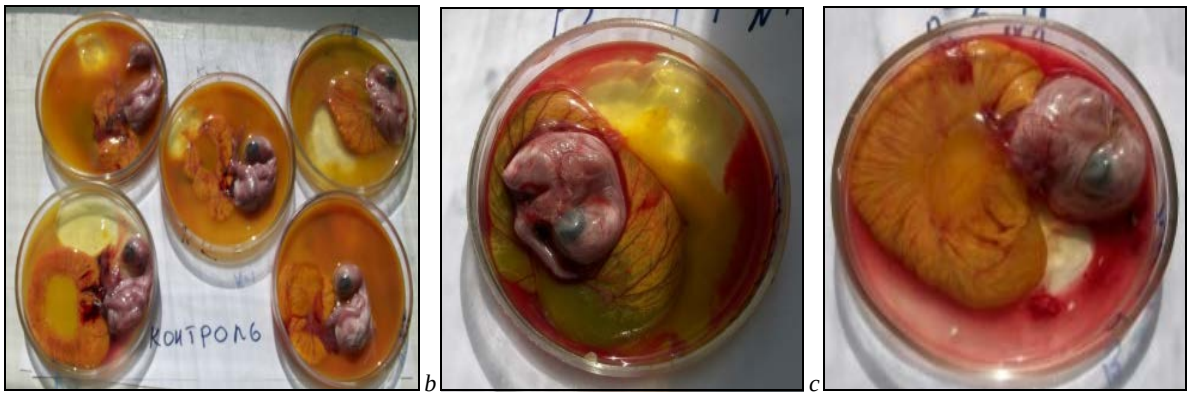
The detection of antimicrobial activity of triazolinium group compounds by the method of serial dilutions. The experimental data on effect of compounds I and II indicates the high bactericidal and bacteriostatic effects of the compound on the epizootic strains of bacteria, isolated from chicken. The minimum bactericidal concentration of compound II for *E. coli* k88 ad cultures was 0.039 μg/ml. The minimum inhibitory concentration of compound II to *S. aureus* k99 culture was 0.16 μg/ml (Table 2).

Table 2
Antibacterial activity of Triazoline compounds

Strains of microorganisms	Inhibitory concentration (MIC) interpretive criteria, μg/ml			
	I	II	Ceftriaxone	DMF
E. coli K88 ad	0.039	0.039	–	–
E. coli ATCC O55K59 3912/41	–	0.625	–	–
S. aureus K99	0.156	0.156	–	–
S. aureus ATCC 25923	–	0.039	0.097	–
K. pneumoniae K56 3534/51	–	1.250	0.097	–
S. typhimurium 144	–	0.313	–	–

Note: "–" – not sensitive.

The detection of antiviral activity of 1,2,4-triazole derivatives in relation to strain 4/91 of the infectious bronchitis chicken virus. The compounds of Trifuzol and Avistim in concentration 1% used simultaneously with infection by the VIB strain 4/91 in dilutions 10^{-1} to 10^{-4} in 9-day CE on CAM reduced infectious activity of the virus, depending on its concentration. We detected the reduction at 14.3% of the pathological symptom "the incorrect embryo position" in CE, which were infected by VIB strain 4/91 in dilution of 10^{-1} with Avistim against CE, which were infected by only the virus at the same concentration. There was a decrease in the percentage of this feature in CE, infected with the VIB strain 4/91 in dilution 10^{-2} with Avistim 12.5%.



a *b* *c*

Fig. 2. CE infected with VIB strain 4/91: *a* – control; *c* – infected by VIB in dilution 10^{-2}; *e* – infected VIB in dilution 10^{-1} simultaneously with Avistim; *g* – infected with VIB in dilution 10^{-3} simultaneously with Avistim

The increase of this feature occurred after the injection of the virus at a concentration of 10^{-3} simultaneously with Avistim, that was 28.5%. The decrease in the percentage of CE with an increased amount of extraembryonic liquid was registered after the injection of the virus in studied concentrations, with Avistim at the same time.

Thus, the reduction of this index (by 73.2%) occurred in CE which had been infected by 10^{-3} dilution of the virus with Avistim, when compared with CE which were infected with the virus at the same concentration without Avistim.

In the control CE group and the groups that have been injected only by Avistim or Trifuzol, in contrast to the experimental CE, the amount of allantoic fluid decreased. The presence of uremic salts in CE of these groups was observed at the level of 85.7% in the control group, and 28.5% after the injection of Avistim, and 14.3% in the group receiving Trifuzol. When compared with the CE control group, the percentage of this index in CE infected by the virus in dilution 10^{-1} decreased by 56.9%, whereas in the CE group after the injection of Avistim, the difference was 57.5%. The percentage of uremic salts in allantoic fluid infected by the virus at a concentration of 10^{-3} and infected by the virus at a concentration of 10^{-3} with Avistim was 14.3%, which is believed to be lower than control by 71.4% (Fig. 3).

In the CE group, infected by the virus from dilution of 10^{-3} simultaneously with Trifuzol, the presence of uremic salts was detected at a large value, and was 42.9%.

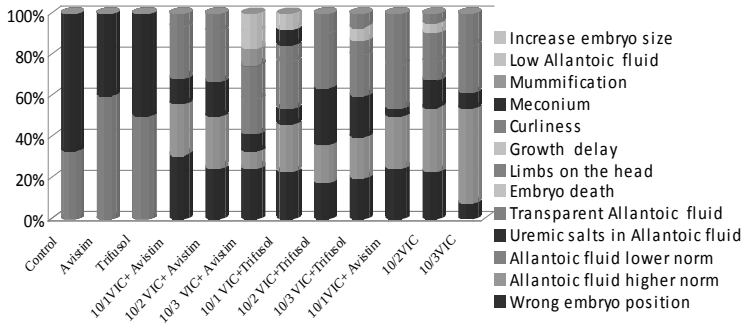


Fig. 3. Pathological changes in CE, induced by VIB with the studied compounds

Transparent allantoic fluid in CE was present in the group which had been injected by the virus in the studied concentrations, in contrast to the control group and CE groups which had been injected by Avistim and Trifuzol. The highest percentage of transparent allantoic fluid was observed in CE infected by the virus in dilution of 10^{-1} (71.4%), the lowest rate index in CE, infected by the virus in dilution 10^{-2} (25.0%). This index remained stable in CE infected by all investigated concentrations with the simultaneous injection of Trifuzol compounds and amounted to 42.9%. In the CE group infected with the virus in combination with Avistim, this feature decreased simultaneously with the decrease of virus concentration.

The highest death rate of the embryo was in the CE group infected by the virus in dilution 10^{-3} (57.1%). The death of the embryos rose to 28.5%, while in the CE infected by the virus at the same concentration simultaneously with Avistim and in the group infected by Trifuzol the death rate was 42.9%. The position of the embryos during the autopsy in many cases was accompanied by the sticking of the limbs to the head, which is one of the specific reproduction features of the bronchitis infectious virus in CE. At the same time, the highest percentage was noted in CE infected with the virus in dilution 10^{-3}, was 85.7%. The decrease of this indicator occurred both with a decrease in the concentrations of the virus, and with the simultaneous injection of the virus and the studied compounds. The lowest percentage of CE with the limbs on the head was in the group CE which were infected with different dilutions with the simultaneous Trifuzol injection, which was 14.3%.

The percentage of CE with limbs on the head in the CE group, which was infected with the virus, simultaneously with infection of Avistim was lower than in CE which had been infected with only the virus, however it was higher than in the CE group with Trifuzol.

Growth delay was marked in CE infected by the virus at the concentrations of 10^{-2}, 10^{-3} (12.5% and 14.3% respectively) and in the group infected by the virus in dilution 10^{-3} simultaneously with Trifuzol (14.3%). The Avistim and Trifuzol compounds reduce the VIB infectious activity during its cultivation in CE, reducing the titre of the virus (strain 4/91) by 3 lg EID_{50}/cm^3.

Discussion

Use of the preparations, containing the nucleus of 1,2,4-triazole into the veterinary practice is increasing very rapidly (Dal Pozzo & Thiry, 2014; Krajczyk et al., 2014; Jefferey et al., 2015; Wenda et al., 2017).

Search for new antiviral and antibacterial means is being undertaken all over the world. This is connected with the emergence of new variants of viruses and antibioticresistant bacteria (Wang & Zhou, 2011; de Oliveira et al., 2012; Asif, 2015). Testing a number of triazole derivative compounds that were obtained as the result of directed synthesis has proven their ability to suppress the reproduction of test-cultures of microorganisms (Zhuo Chen et al., 2010; Ferreira et al., 2013).

Derivatives of 1,2,4-triazole are distinguished among other drugs by the capacity for a wide range of biological activity and low toxicity. The priority of their use in antimicrobial therapy is confirmed by the prolonged effect of the formation of resistance bacteria (Siddiquia et al., 2011; Gross & Bryson, 2015). Reduction of the infectious activity of the infectious bronchitis virus (strain 4/91) in the EC biosystem, caused by Trifuzol and Avistim, makes it possible to register them as antiviral and immunomodulatory agents for use in veterinary medicine.

Investigation of the compound 2-[5-(furan-2-yl-4-phenyl-1,2,4-triazole-3-ylthio] acetate on 9-day chicken embryos against the action of the infectious encephalomyelitis virus (Calnek 1143 strain) and the infectious bronchitis virus H-120) revealed the decrease of titre of strain H-120 at 1,8 log and strain Calnek 1143 at 1.0 log (Parchenko et al., 2009; Fotina, 2015).

A comparative assessment of the sensitivity *E. coli* and *S. aureus* cultures, isolated from chickens and test-cultures, against new triazolin compounds with the results of antibacterial drug sensitivity proves that they are worth studying further. The growth inhibition zones of test-cultures, caused by compounds I and II, were indexed in the range from 12.0 to 18.8 mm. Norfloxacin, Levomicitin, Doxycycline and Eritromicin induced similar inhibition of growth zones of test-cultures, simultaneously. Sahu (2014) defined the MIC of new compounds that are derivatives of 1,2,4-triazole compare to Gentamycin. By the method of dilutions, it was determined that MIC ranges for compounds against *E. coli* and *S. aureus* were from 1.6 to 25.0 mg/ml, and for Gentamycin for these test-cultures – 1.6 mg/ml. The diameter of inhibition of growth zone of test-cultures of *E. coli* and *S. aureus*, induced by the compounds, changed from 0.6 to 5.0 mm, while for Gentamycin – 16.0 mm.

According to Malladi (2013), MIC of Ceftriaxon for *E. coli* was 1.61 mg/ml and for *S. aureus* – 3.13 mg/ml. Seelam (2016) showed the ability of triazolin compounds to suppress the growth of *E. coli* test-culture (MTCC 433) at MIC from 50 to 3.13 mg/ml. In this case, Streptomycin and Chloramphenicol had a MIC of 6.25 mg/ml.

Wahi et al. (2011) proved the antimicrobial activity of new derivatives of 1,2,4-triazole by the method of serial dilutions. Only 2 compounds out of the 8 compounds were indexed against Gram-negative and Gram-positive bacteria in low MIC (3.12 mg/ml). MIC of the compounds against *Streptococcus pyogenes*, *Micrococus luteus*, *Staphylococcus epidermidis*, *Clostridium sporogenes*, *Salmonella typhimurium* ranged from 3.12 to 50 mg/ml. The standard example had a lower MIC (1.56–6.25 mg/ml).

In research by Popiołek et al. (2013), MIC of novel 1,2,4-triazole and 1,3,4-thiadiazole derivatives against Gram-negative and Gram-positive bacteria (*S. aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876, *M. luteus* ATCC 10240) ranged from 15.6 to 500 mg/ml. The inhibitory concentration of Ceftriaxone on *S. epidermidis* ATCC 12228 was 0.24 mg/ml, and for *B. cereus* ATCC 10876 – 62.5 mg/ml.

Screening of 15 new derivatives of 1,2,4-triazole with antibacterial activity made by Bektaş et al. (2010) detected for 4 compounds: MIC for *E. coli* ATCC 25923 ≥ 500 mg/ml to 1.95 mg/ml. *S. aureus* ATCC 25923 was susceptible to these compounds in the range of 1.95 to 500 mg/ml. Ampicillin had a higher MIC compared to *E. coli* (10 mg/ml) and 35 mg/ml – to *S. aureus*. The ability of new derivatives of 1,2,4-triazole to have an effect against the bacterial and viral pathogens of poultry infections, has been carried out in the process of the research, and is the basis for their use in schemes for prevention of infectious diseases. The agents of Trifuzol and Avistim have antiviral action against the chicken infectious virus bronchitis strain 4/91 (reduction of the virus titre by 3.0 logs). In this case, defined antiviral activity was higher than the data obtained by Fotina et al. (2015), against the VIB strain N-120 and the virus of encephalomyelitis of bird strain Calnek 1143.

The biological activity of the new derivatives of 1,2,4-triazole, determined by us against the different groups of microorganisms, proves the prospect of their further use in veterinary medicine.

Conclusion

Antibacterial activity of compounds I and II against collection strains, isolated from broiler chickens is established. Against *S. aureus* , compound I in 50 μg concentration on a disk, induced the growth inhibition zone at 18.8 ± 1.65 mm and for *E. coli* – 17.8 ± 1.44 – 17.8 ± 0.25 mm respectively to serovariants k88 ad and ATCC 3912/41. Compound II in a concentration of 50 μg on a disk induced the growth inhibition zone of *S. aureus* 99, isolated from chicken broilers, at a level of 17.3 mm. Trifuzol and Avistim causde antiviral activity against the VIB strain 4/91 during cultivation in EC. The percentage of deaths of EC infected with the virus in dilution 10^{-3} in the presence of Avistim was lower by 28.6%, and with Trifuzol lower by 14.3%. The Avistim and Trifuzol compounds reduced the VIB infectious activity when it was cultivated in CE, reducing the titre of the virus (strain 4/91) by 3.0 log EID_{50}/cm^3.

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