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ANTIMICROBIAL PROPERTIES OF 4-((5-(DECYLTHIO)-4-METHYL-4H-1,2,4-TRIAZOLE-3-YL)-METHYL)MORPHOLINE

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ABSTRACT

The study of the antimicrobial properties of 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazol-3-yl)-methyl)morpholine against new strains of microorganisms is an urgent issue, which in turn can expand the list indications for the use of this compound. The drug «Vetmikoderm» is recommended for the treatment of animals with various skin diseases of fungal and microbial etiology. Thus, further studies of this compound with the aim of improving the drug are relevant and have theoretical and practical significance.

KEYWORDS: 1,2,4-triazole derivatives, antimicrobial activity, «Vetmikoderm», strains of the Enterobacteriaceae, Enterococcaceae, Staphylococcaceae, Pseudomonadaceae Bacillaceae families, in vitro.

INTRODUCTION

Despite the constant criticism of synthetic drugs, their role in providing health care cannot be overestimated. Due to the constant improvement of synthetic molecules' possibility, original medicines become more effective and they are in permanent demand. In addition, synthetic organic compounds are used not only as drug substances, they are known as effective fungicides, herbicides, plant growth regulators, anti-corrosion agents, plasticizers for plastics, additives for various types of fuels, etc.

Nowadays, an undoubted fact is the high attractiveness of the 1,2,4-triazole derivatives. These compounds are in the center of scientists' attention of various research fields due to the unique properties of this heterocycle. For a long time, they have been the «foundation» for the search of new biologically active substances, exhibiting a wide range of properties and having little toxicity. The most promising molecules among them can eventually be transformed into substances of effective drugs. In 2022, a new original domestic drug appeared on the Ukraine veterinary market in the form of «Vetmikoderm» liniment, the active substance of which belongs to the 1,2,4-triazole derivatives. Particular attention should be paid to the antimicrobial and antifungal properties of the 1,2,4-triazole derivatives due to the relevance of this problem in the context of the development of modern pharmaceutical science.

Antibiotic resistance in the scientific literature is defined as the resistance of bacteria to one or more antibiotics.[1] There are many causes and consequences of this condition.[2] In our opinion, the efforts of scientists to expand the arsenal of new biologically active compounds, a certain number of which can show antimicrobial activity and effectively counteract resistance to antimicrobial agents, remain promising.[3] It is well known, that the 1,2,4-triazole derivatives are biologically active compounds with a wide range of properties.[4,5] In some cases, derivatives of this heterocyclic system show high indicators of antimicrobial action.[6,7] Therefore, a scientifically based approach is a purposeful search for new antimicrobial molecules among the 1,2,4-triazole derivatives.[8-10]

Earlier, it was reported that the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine exhibits high antifungal activity and has an antimicrobial effect against some strains of microorganisms, and also has insignificant indicators of toxicity.[11] As a part of the liniment «Vetmikoderm», the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine has desensitizing properties and wound-healing, anti-inflammatory, anti-itching effects. The drug is recommended in the animals' treatment with various skin diseases of fungal and microbial etiology. Thus, further studies of this compound with the aim of improving the drug are relevant and have theoretical and practical significance.

The purpose of our work is to study the antimicrobial properties of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine against new strains of microorganisms, which in turn can expand a list of indications for this drug usage.

MATERIALS AND METHODS

Research was conducted on 13 microorganisms' strains at a temperature of 37°C. The release of the treated bacteria from the experimental disinfectant was carried out by washing them with a sterile physiological solution, followed by sedimentation of the bacteria by centrifugation at 3,000 rpm for 5 minutes. The supernatant liquid is drained, the sediment is resuspended in 4,5 cm^3 of sterile physiological solution with three-fold repeatability of centrifugation under the specified regime.

After the last centrifugation, the sediment of bacteria is resuspended in 4,5 cm^3 of sterile physiological solution to the initial concentration for further cultivation. To determine the results of the working concentrations' bactericidal activity of the experimental disinfectant in 0,1; 0,5; 1% mass concentrations on the test bacteria and confirmation of the bacteriostatic effect's absence in the latter, the washed bacteria resuspended sediment of the each test cultures is sown on Petri dishes with solid selective agar (TSA) in three repetitions in 0,1 cm3 volume and incubated for 24 hours at room temperature regime of 37±1,0°C. Gravimetric measurements were performed on ESJ-200-4 laboratory electronic analytical balances (the USA).

Streptomycin 1,0% solution (Streptomycin S^{300}, Himedia) served as a positive control. The results of the tests are evaluated by the suspension method after 24 hours of cultivation in a thermostat according to the presence or absence of growth of test microorganisms on solid nutrient media, comparing with the typical growth of the corresponding test cultures in growth control. The concentration of the test disinfectant is considered effective, at which the experiment repeated three times with the appropriate exposure time (30 min) ensured the growth of the test microorganisms' absence on solid nutrient media in the typical growth presence of the test cultures in growth controls (negative control).

RESULTS AND THEIR DISCUSSION

After 24 hoyrs of incubation, the diameter of the growth inhibition zone of the culture was measured using a template for the size measuring of the microorganisms'growth inhibition zones (Antibiotic Zone Scale-C, model RW297, India) and the TpsDig2 program (2016, F. James Rohlf). Data in the tables are presented as $x \pm 1.96 \bullet SD$.

The antibacterial effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on microorganisms' strains of the Enterobacteriaceae family in vitro after 24 hours of cultivation in a thermostat is summarized in the table 1.

Table 1: Antibacterial effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on microorganisms' strains of the Enterobacteriaceae and Enterococcaceae families (24 h).

Strains of microorganisms	Concentration,%			Control*
	0,1	0,5	1,0	1,0
Enterobacter cloacae	6.1±0.2	9.8±0.1	10.5±0.2	10.6±0.1
Enterobacter aerogenes	3.1±0.1	7.2±0.2	9.5±0.3	12.2±1.3
Enterococcus faecium	2.1±0.2	2.3±0.4	6.5±0.3	7.1±0.4
Enterococcus faecalis	2.5±0.1	4.3±0.2	7.1±0.5▪	4.2±0.3
Klebciella ozaenae	2.1±0.1	2.2±0.2	2.2±0.1	15.3±1.4
Klebciella rhinoscleromatis	6.1±0.3	7.2±0.4	7.2±0.6	10.1±0.5
Morganella morganii	2.2±0.1	4.3±0.1	6.5±0.2	14.0±2.1
Proteus vulgaris	7.5±0.5	7.9±0.9	11.3±0.3▪	6.1±0.9
Proteus vulgaris НХ19№222	7.5±0.7	10.1±1.2	10.2±1.4	14.6±1.8
Escherichia coli ATCC № 25923	5.2±0.5	5.3±0.4	6.5±0.2	8.2±0.3
Salmonella typhimurium UNCSM-014	3.1±0.4	5.7±0.6	6.6±0.8	9.2±0.7

* Streptomycin (300.0 µg) was used as a positive control (Valle et al., 2015). (n = 3), ▪P<0,05

We found the bactericidal effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine working concentrations in 0,5 and 1,0% mass concentrations of the drug on *Enterobacter cloacae* (9,8 and 10,5 mm), *Enterobacter aerogenes* (7,2 and 9,5 mm, Fig. 1), *Klebciella rhinoscleromatis* (7,2 mm, Fig. 2), *Proteus vulgaris* НХ19№222 (10, 1 and 10,2 mm, Fig. 3), *Salmonella typhimurium* UNCSM-014 (5,7; 6,6 mm).

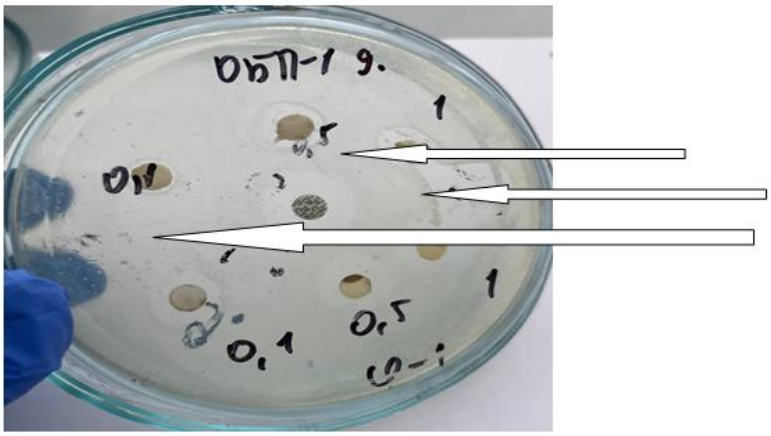

Figure 1: Antibacterial effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on the field strain of *Enterobacter aerogenes*

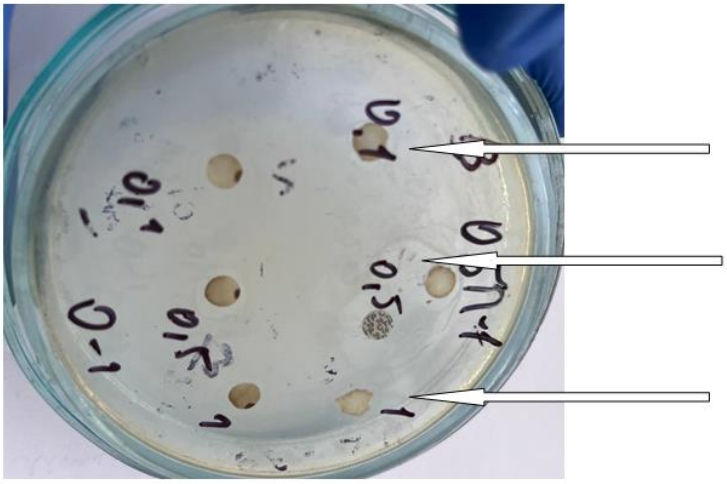

Figure 2: Bactericidal effect of working concentrations of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on the epizootic strain of *Klebciella rhinoscleromatis.*

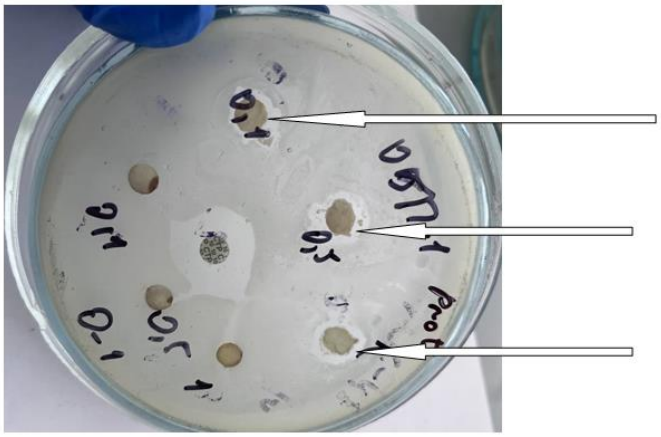

Figure 3: Effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on the epizootic strain *Proteus vulgaris* X19#222 at different concentrations.

Moderate antibacterial efficiency was determined using the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on *Escherichia coli* ATCC №25923 at concentrations of 0,1; 0,5 and 1,0% (5,2; 5,3 and 6,5 mm), respectively. The preparation of 1,0% concentration inhibits the growth of field strains of *Enterococcus faecium* and *Morganella morganii* (growth retardation zone of 6,5 mm).

A high inhibitory property of the specified compound on *Enterococcus faecalis* was determined. Thus, the 0,5% solution has already exceeded the control (4,3 vs. 4,2 mm), and the 1,0% solution has a significant difference (P<0,05). The bactericidal effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on the field epizootic strain

of *Proteus vulgaris* was also proven: all concentrations of the drug exceeded the control (7 ,5; 7,9 mm), and 1,0% even with (11,3 mm, P<0.05) probability. No bacteriostatic effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on *Klebciella ozaenae* (2,1-2,2 mm vs. 15,3 mm control) was detected.

We consider inappropriate use of streptomycin as an antibacterial drug against antibiotic-resistant epizootic strains of *Enterococcus faecalis, Proteus vulgaris* and deposited *Escherichia coli* ATCC №25923 (Fig. 4).

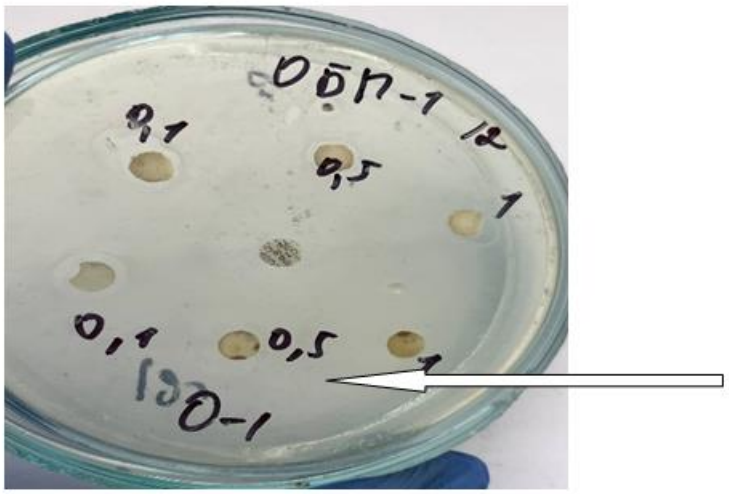

Figure 4: Resistance of the deposited strain of *Escherichia coli* ATCC № 25923 to streptomycin.

The results shown in the table. 2, have proved the high inhibitory property of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on the microorganisms' strains of the Staphylococcaceae family. All the concentrations of the test sample (0,1, 0,5 and 1.0%) have a bactericidal effect against *Staphylococcus aureus* UNCSM-017 (11,5, 14,7 and 16,5 mm) in comparison with the control (Streptomycin, 16, 2 mm). The bacteriostatic activity of a 1,0% experimental drug's solution on the field strain of *Pseudomonas aeruginosa* (6,3 mm) was determined.

Table 2: Antibacterial effect of the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine on microorganisms' strains of the Staphylococcaceae, Pseudomonadaceae and Bacillaceae families (24 hours).

Strains of microorganisms	Concentration,%			Control*
	0,1	0,5	1,0	1,0
Staphylococcus aureus UNCSM-017	11.5±1.3	14.7±1.0	16.5±0.9	16.2±1.8
Pseudomonas aeruginosa	4.1±0.8	4.2±0.6	6.3±0.5	12.2±1.3
Bacillus subtilis ATCC №6633	0.0±0.0	4.0±0.5	4.1±0.7	11.9±1.2

* Streptomycin was used as a positive control (Valle et al., 2015). (n = 3)

The slow growth retardation zone of 0,5 and 1,0% of the compound sample on *Bacillus subtilis* ATCC № 6633 (4.0; 4.1 mm) versus the control (11,9 mm), fig. 5.

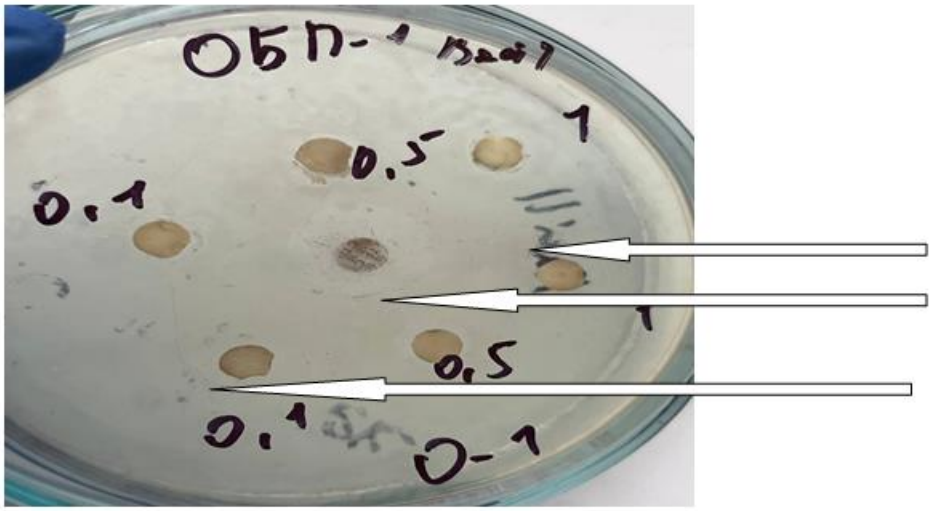

Figure 5: Resistance of the deposited *Bacillus subtilis* strain ATCC №6633 to the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine.

CONCLUSION

Therefore, after analyzing the results of our research, it was established that the 4-((5-(decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine can compete with the streptomycin, having a bactericidal effect on cryogenic strains of microorganisms cultivated in the environment at a temperature of 37 °C: *Enterobacter cloacae, Enterobacter aerogenes, Enterococcus faecalis, Proteus vulgaris, Staphylococcus aureus,* bacteriostatically against *Klebciella rhinoscleromatis, Escherichia coli* and *Pseudomonas aeruginosa*. The 4-((5-(Decylthio)-4-methyl-4H-1,2,4-triazole-3-yl)-methyl) morpholine can be recommended for further studies against multiresistant strains of these microorganisms.

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