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Influence Of 3-(3-Fluorophenyl)-6-(4-Methoxyphenyl)-7H-[1,2,4]-Triazolo-[3,4-B][1,3,4]Thiadiazine On The Cultural Properties Of Pathogenic Mycobacterium Bovis.

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ABSTRACT

Derivatives of 1,2,4-triazole are widely used in many fields of human activity. Regular creation of new and modern drugs. The outstanding scientists are actively working in this direction and this gives its results. Today, the treatment and prevention of many types of tuberculosis is a priority task in many countries around the world. The purpose of our work is to investigate the effect of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b]-[1,3,4]-thiadiazine on the cultural properties of pathogenic Mycobacterium bovis. Having analyzed the results of the conducted studies, the effect of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b]-[1,3,4]-thiadiazine on the cultural properties of pathogenic Mycobacterium bovis.

Keywords: 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b]-[1,3,4]-thiadiazine, antitubercular activity, pathogenic strains.

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INTRODUCTION

Current trends in the pharmaceutical industry in Ukraine require the introduction of new highly effective and low-toxic drugs. Today, the derivatives of 1,2,4-triazole remain leading positions among a huge variety of biologically active substances.

For many decades, this class of heterocyclic compounds attracts the attention of specialists not only in the pharmaceutical industry. A well-known fact is the high biological activity of compounds formed by combination of 1,2,4-triazole nucleus and various functional substituents, slight toxicity, high reactivity. Over the past five years, a whole series of new veterinary drugs and fertilizers have been registered in Ukraine, with the active substances being derivatives of 1,2,4-triazole (Avistem RP AB-05365-01-14, Trifuzol RP AB-05486 - 01-14, "TRIFUZOL-NEO", RP AV-07793-01-18, "Fortis Combi", RP A 06016). All this in the complex indicates that further research of new substituted 1,2,4-triazoles is promising, having both theoretical and practical interest.

It should be noted separately that some substituted 1,2,4-triazoles has the ability to suppress the growth of mycobacteria [1, 2]. The analysis of literary sources clearly shows some regularities and peculiarities of the compounds structure and indicated biological properties [3]. The results of previous studies argue that not only certain functional substituents influence on anti-TB activity, but also different concentrations, temperature regimes, pH values of the medium, etc do. [4].

The purpose of our work was to investigate and analyze the effect of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4] thiazidine on the cultures of pathogenic *Mycobacterium bovis*, make some conclusions about further trials and promising implementations.

MATERIALS AND METHODS

Method of synthesis, physicochemical characteristics of the molecule 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine is described in detail in [5]. Therefore, for the further studies of the effect on the cultures of pathogenic *Mycobacterium bovis*, the known compound 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine.

In the first stage of the study, cultivation of the pathogenic strains of *M. bovis* at a temperature of 37°C in a medium with pH 6.5 and 7.1, further comprising 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine at a mass concentration of 0.1; 0.5; 1.0%. The cultivation and accumulation of biomass of the studied *M. bovis* strains was carried out on an egg nutrient medium, which according to the composition was identical to the standard, manufactured by the State Veterinary Medicine Center (Kharkiv, Ukraine). 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine was added to the medium to obtain concentrations of 0, 1%; 0.5%; 1%. In selected and accumulated mycobacteria studied tincture properties, morphological signs, as well as the terms of the appearance of primary growth, its intensity and the nature of the subculture. The analysis and evaluation of the colonies was carried out in terms of quantity, size, shape, surface, consistency, pigmentation, transparency, shine and emulsification in a physiological solution (V.M.Manchenko, Z.R. Trotsenko, M.S. Pavlenko and others, 1994; O. A. Tkachenko, MV Bilan, V. V. Zazharsky, 2010). The tinctorial properties of mycobacteria were determined by preparing smears from colonies (cultures), painted on the Tsiel-Nielsen method, and exploring them under the imsection of the XUN series SUNNY microscope with the micromedic micrometer complex "Mikmed-2-1600" (St. Petersburg, Russia). The morphology of mycobacteria was determined by: length, thickness, shape, character of the ends of cells, grainy and location. The gravimetric measurements were performed on laboratory electronic analytical scales of the model ESJ-200-4 (USA).

RESULTS AND DISCUSSION

At the beginning of the experiment, the effect of the substance concentration and pH on the growth intensity of the culture was observed at 37 °C. To this end, *M. bovis* 100 passages which were cultured at 37°C. in a 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3, 4-b][1,3,4]thiadiazine at the indicated concentrations in a thermostat for three months in a medium with a pH 6.5 (in the number of ten samples with each concentration of the preparation) and pH 7,1 (in the number of ten samples with each concentration drug).

As control, *M. bovis* 100 passages were used without adding to the medium the active ingredient. After the end of these terms, as well as after cultivation at a temperature of 37 °C, records were made and characterized the growth of colonies of mycobacteria (V.M.Manchenko, Z.R. Trotsenko, M.S. Pavlenko, etc., 1994). As a result, data on the influence of the concentrations of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine on cultivation of *M. bovis* at a temperature of 37 °C in a medium with different pH at the intensity of growth of culture.

Table 1 summarizes the data on the crop properties of *M. bovis* 100 passage cultured on a medium with a pH of 7.1, further comprising 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine in three concentrations. Up to 7 days of the experiment, the growth of the culture of 100 passages *M. bovis* in the control group was not observed at pH 7,1 at 37 °C.

Table 1: Characteristics of the cultural properties of *M. bovis* 100 passages cultivated on a medium with a pH of 7.1 at 37 °C

7th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Raidplaque	Thereisnogrowth	Thereisnogrowth	Thereisnogrowth
14th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
A roughplaqueand a fewwhitecoloniesalongthelineofsowing	Thereisnogrowth	Thereisnogrowth	Thereisnogrowth
30th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Continuousgrowth. Smooth, smallcoloniesofwhitishcolor	Nochange	Nochange	Nochange
60th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Continuousgrowth. Smooth, smallcoloniesofwhitishcolor	Thereisnogrowth	Thereisnogrowth	Thereisnogrowth
90th, addexperiment			
Control	Concentration of the preparation 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Continuousgrowth	Thereisnogrowth	Thereisnogrowth	Thereisnogrowth

The results are shown in the table. 1, showed no growth of *M. bovis* culture in 100 passages for all (0.1, 0.5 and 1%) of substance concentrations throughout the observation period (90 days), indicating tuberculostatic effect of the drug.

In Table 2 systematizes data on the culture properties of *M. bovis* 100 passage cultured on a medium with a pH of 6.5, which additionally contained a specified substance in three concentrations. By the 7th day of the experiment, the growth of culture of 100 passages of *M. bovis* in a medium with a pH of 6.5 at 37 °C was not observed.

Table 2: Characteristics of the cultural properties of M. bovis 100 passages cultivated in a medium with a pH of 6.5 at 37 °C

7th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Separate colonies behind the sowing line	There is no growth	There is no growth	There is no growth
14th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Continuous growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth
30th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Continuous growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth
60th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Continuous growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth
90th, addexperiment			
Control	Concentration of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine		
	0,1%	0,5%	1%
Continuous growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth

The results of the experiment show that the action of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine in different concentrations in a medium with a pH 6.5 does not differ from pH 7,1. In all experimental (0.1, 0.5 and 1.0%) concentrations, we observed a lack of growth of the pathogenic strain of M. bovis 100 passages throughout the observation period (90 days). Thus, it can be concluded that 0.1, 0.5% and 1.0% concentrations of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo-[3,4-b][1,3,4]thiadiazine actively influence the culturing properties of the pathogenic strain M. bovis, cultured on a medium with pH 6.5 at 37 °C, restraining growth and development, having tuberculostatic action.

CONCLUSIONS

According to the results of an experimental study, the effect of 3-(3-fluorophenyl)-6-(4-methoxyphenyl)-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]thiadiazine on the cultural properties of pathogenic Mycobacterium bovis. It has been found that the compound in various effective concentrations has a tuberculostatic effect. Positive influence on this type of biological activity at different pH values of the medium and temperature regimes. Thus, it should be noted the need for further in-depth studies of this compound that can be used in the future to create new original drugs.

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