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Synthesis and research of the impact of new derivatives of 4-R-3-(morpholinomethyl)-4H-1, 2, 4-triazole-5-thiol on cultural attributes of pathogenic *M. Bovis.*

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ABSTRACT

Synthesis and research of the impact of new derivatives of 4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thiol on cultural attributes of pathogenic *M. bovis*. Problematics of collateral impact of xenobiotics and counteraction to their toxic influence continues to be extremely significant. Resistant tuberculosis is a serious problem of health defense of population in many developing countries. Treatment of such form of tuberculosis takes more time and demands more and more expensive medicines. The aim of this work is synthesis and research of new derivatives of 4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thiols and their ability of influencing cultural attributes of pathogenic *M. bovis.* The object of research is new derivatives of 4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thiols. Cultivation of pathogenic strains of *M. bovis* was carried out at the temperature of 37°C in the environment of pH 6,5 and 7,1, where synthesized compounds were additionally placed in mass concentrations of 0,1; 0,5; 1%. As a comparative substance a classical suberculosis-static preparation isoniazid was used. As a result of the research held is was concluded that the most pronounced tuberculosis-static influence among compounds under analysis had N'-(2-((4-amino-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotinohydrazide hydrobromide. Promising application of further research of synthesized substances as anti-tuberculosis means was demonstrated.
Keywords: 1,2,4-triazol, synthesis, *M. bovis*, anti-tuberculosis action.

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INTRODUCTION

The issues of side influence of xenobiotics and counteraction to their toxic impact remains to be very important. Up-to-date and effective approaches in the treatment of tuberculosis patient has significant scientific and social value within the global epidemics of this disease in the world [1]. Resistant tuberculosis is a serious problem of health defence of population of developing countries [2]. Treatment of such form of tuberculosis takes more time and demands more expensive medicines. Multidrug resistant tuberculosis (MDR-TB) - is a form of tuberculosis which is not influences by two most effective medical means: Rifampicinum and Isoniazid [3].

Frequency of persistence of M. bovis to such strains in the organism of cattle in insecure countries in concern of tuberculosis is relatively high (within 30 %), in order to make the significant tendency of epizootic process. The issues of biological activity of micobacteria, of bovine type in particular, still need investigation as far as there always appear new information on specific features of some of them provided by authors. Thus, in 2004 it was informed about M. bovis rapidly growing strain, the next year – on loss of reproduction because of artificial nourishing circumstances. It is also noted that this mentioned above strain of M. bovis, in such circumstances converses into non-acid-resistant bacilliform and filamentary forms with parallel change of culture growth nature: patina, haze [4].

The fact is important that derivatives of 1,2,4-triazole are studied from the point of view of their ability to have anti-tuberculosis action. Thus, quite a lot of scientists pay attention to this issue [5-7] and it gives results [8, 9]. Therefore we decided it would be relevant to synthesize and research new derivatives of 4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thiols from the point of view of their ability to influence cultural attributes of pathogenic M. bovis.

MATERIALS AND METHODS

The temperature of melting of synthesized compounds is defined by means of automatic device OptiMelt Stanford Research Systems MPA100. Elementary contents of compounds are determined with the help of analyzer Elementar Vario L cube (CHNS) (standard – sulphanilamide). ^{1}H NMR-spectres of compounds were made by means of spectrometer Varian Mercury VX-200 (1H, 200 MHz), solvent – DMSO-$_{d6}$, inner standard – tetramethylsilane (TMS) and deciphered with the help of computer software SpinWorks 3.1.8. Chromato-mass-spectrometry research was held by means of gas-liquid chromatograph Agilent 1260 Infinity HPLC with equipped mass-spectrometer Agilent 6120 (ionization in electro-spray (ESI) [10-12].

Investigation of anti-tuberculosis features was held by the Chair of epizootology and infectious diseases of animals of the Faculty of veterinary medicine of Dnipropetrovsk State Agrarian Economic University. Cultivation of pathogenic strains of M. bovis is held at the temperature of 37°C in the environment of pH 6,5 and 7,1, where synthesized derivatives of 4-R-3-(morpholinomethyl)-4H-1,2,4-triazole-5-thiols in mass concentrations 0,1; 0,5; 1% were added.

The researching strains of M. bovis, biomass' cultivation and accumulation was carried out on an egg nutrient environment, which according to the composition was identical to the standard, manufactured by the State Veterinary Medicine Center (Kharkiv, Ukraine). The solutions of compounds were prepared according to the methods describing in GOST 4212-76 and GOST 4517-87. The gravimetric measurements were performed on laboratory electronic analytical scales (ESJ-200-4 (USA)).

As a comparison drug, the classic tuberculosis-static preparation of isoniazid was used at various concentrations, pH, and temperature of 37 ° C. At the beginning of the experiment, the influence of the concentration of compounds **2.1-2.3** and the pH environment on the intensity culture growth was observed at 37 °C. For this, M. bovis of 100 passages were harvested at a given temperature in the media with compounds 2.1-2.3 at the indicated concentrations in the thermostat for three months in the environment with pH 6.5 and 7.1 (in the number of ten samples with each concentration of compounds for each pH value). As the control, M.bovis of 100 passages were used without adding substances to the environment of **2.1-2.3**.

In selected and accumulated mycobacteria have researched the terms of the appearance of primary growth, its intensity and the nature of the subculture.

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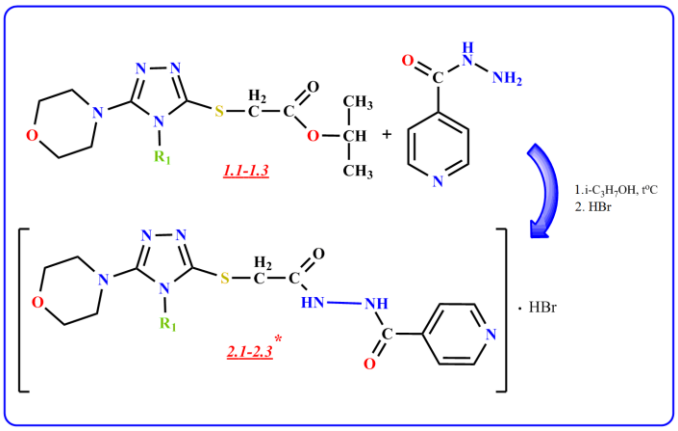
The analysis and evaluation of the colonies were carried out in terms of quantity, size, shape, surface, consistency, pigment formation, transparency, luster and emulsification in the physiological solution [13].

RESULTS AND DISCUSSION

Chemical experimental part

Synthesis of the target compounds of N'-(2-((4-methyl-3-morpholinomethyl-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotinohydrazide **(2.1)**, N'-(2-(4-phenyl-3-morpholinomethyl-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotinohydrazide hydrobromide **(2.2)** and N'-(2-((4-amino-3- (morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotinhydrazide hydrobromide was carried out according to Scheme 1. So, as the starting compounds, the esters of 2-((4-R_1-3-morpholinomethyl-1,2,4-triazole-5-yl)thio)acetic acids (**1.1-1.3**) that were synthesized and described by us earlier [14].

To reach the target, the synthesis was carried out in the environment of i-propanol by boiling on a water stove for 8 hours with the addition of the isonicotinic acid hydrazide equivalent (CAS # 54-85-3) followed by evaporation. Compounds **2.2** and **2.3** were converted into hydrobromide forms for ease of further research.



where, **2.1** R_1 = -CH_3; **2.2** R_1 = -C_6H_5; **2.3** R_1 = -NH_2,

Note:*Compounds **2.2** and **2.3** are obtained in the form of hydrobromides. Substance **2.1** is isolated in the form of a base without conversion into the halogenated water.

Scheme1. The Synthesis of N'-(2-((4-R_1-3-morpholinomethyl-4H-1, 2, 4-triazole-5-yl) thio) acetyl) is onicotin hydrazides (**2.1-2.3**)

N'-(2-((4-methyl-3-(morpholinomethyl)-4H-1,2,4-triazole-5-yl)thio)acetyl)isonicotinic hydrazide (2.1)

0.01 mole of isonicotinic acid hydrazide is added to a solution of isopropyl 2-((4-methyl-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetate (3.14 g) in 50 ml of i-propanol. The solution is boiled on a water stove for 8 hours and evaporated to dryness. For analysis, the compound is recrystallized from i-propanol. The crystalline substance is yellow color with melting point 111-113°C.

Yield 84%, ^{1}H NMR (200 Mz, DMSO-d_6) δppm: 8.87 (d, 2H, pyridin-4-yl), 7.95 (s, 2H, -NH-), 7.41 (d, 2H, pyridin-4-yl), 4.55(s, 2H, -CH_2), 4.01(s, 2H, -CH_2), 3.65 (t, 4H, morpholine), 3.23 (s, 3H, -CH_3), 2.55 (t, 4H,morpholine). m/z 391.

Elemental analysis: $C_{16}H_{21}N_7O_3S$; Calculated (%); C 49.09, N 5.41, N 25.05, S, 8.19. Found (%): C 49.19, H 5.42, N 25.09, S 8.21.

N'-(2-((3-(morpholinomethyl)-4-phenyl-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide hydrobromide (2.2)

0.01 mole of isonicotinic acid hydrazide is added to a solution of isopropyl 2-((4-phenyl-3-(morpholinomethyl)-4H-1,2,4-triazole-5-yl)thio)acetate (3.48 g) in 30 ml of i-propanol. The solution was boiled on a water stove for 8 hours, evaporated to dryness and isolated as hydrobromide. For analysis, the compound is recrystallized from methanol.

The crystalline substance is yellow color with melting point 195-197 °C. Yield 81%, ^{1}H NMR (200 Mz, DMSO-d_6) δppm: 8.89 (d, 2H, pyridin-4-yl), 8.01 (m, 5H, Ar), 7.71 (s, 2H, -NH-), 7.44 (d, 2H, pyridin-4-yl), 4.59 (s, 2H, -CH 2), 4.06 (s, 2H, -CH 2), 3.69 (t, 4H, morpholine), 2.50 (t, 4H, morpholine). m / z 535.

The elemental analysis: $C_{21}H_{24}BrN_7O_3S$, Calculated (%): 47.20, H 4.53, N 18.35, S 6.00. Found (%): C, 47.29, H, 4.55, N, 18.39, S, 6.03.

N'-(2-((4-amino-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide hydrobromide (2.3)

0.01 mole of isonicotinic acid hydrazide is added to a solution of isopropyl 2-((4-amino-3-(morpholinomethyl)-4H-1,2,4-triazole-5-yl)thio)acetate (3.15 g) in 30 ml of i-propanol. The solution was boiled on a water stove for 8 hours, evaporated to dryness and isolated as hydrobromide. For analysis, the compound is recrystallized from i-propanol. Crystal substance is white color with melting point 157-159 °C.

Yield 81%, ^{1}H NMR (200 Mz, DMSO-d_6) δppm: 8.82 (d, 2H, pyridin-4-yl), 8.01 (s, 2H, -NH-), 7.55 (d, 2H, pyridin-4- (t, 4H, morpholine), 5.77 (s, 2H, -NH2) . m / z 473.

The elemental analysis: $C_{15}H_{21}BrN_8O_3S$, Calculated (%): C 38.05, H 4.47, N 23.67, S 6.77; Found (%): C 38.10, N 4.48, N 23.77, S 6.79.

Biological experimental part

On the Table 1 - the data about the cultural M. bovis properties of 100 passages, which were cultivated in a medium with a pH of 7.1, which additionally contains compounds **2.1** to **2.3** at 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. Beginning from the 7th day, along with the line of sowing, a rough plaque was discovered, from the 14th day, there were characteristic single colonies, which up to the 90th day of the experiment formed a significant number of macroscopically formed colonies of mycobacteria in the form of continuous growth.

The results are on the table 1, prove that 0.1% concentration of compound **2.1** is characterized by the gradual growth of colonies, from the 30th to the 60th day of the experiment - along with the line of sowing a rough plaque, at the 90th day there are some smooth (S-forms), small colonies of white color. We have established a lack of growth of M. bovis culture of 100 passages at 0.5 and 1% of compound concentrations 2.1 throughout the whole period (90 days), indicating a moderate tuberculosis-static effect of the drug.

Later, analyzing the results of table 1, the absence of growth of M. bovis 100 culture passages for all (0.1, 0.5 and 1%) of the concentrations of compounds **2.2** and **2.3** throughout the observation period (90 days) was established, which indicates their tuberculosis-static effect.

Analyzing the data in Table 1 and 2, it was found the effects of compounds **2.1** and **2.3** at various concentrations in a medium with a pH of 6.5 are the same from pH 7.1. In all of the experimental concentrations (0.1, 0.5 and 1.0%) of these compounds, we observed a lack of growth of the pathogenic strain of M.bovis 100 passages throughout the observation period (90 days). However, interpreting the results of compound **2.2**, it is noted that gradually increasing colonies are observed at 0.1% concentration of the substance, starting from the 30th to the 60th day of the experiment - along the line of sowing, a rough plaque, at the 90th day there are some smooth (S-forms), small colonies of white color. Although, concentrations of 0.5 and 1.0% of compound **2.2** constrain the growth of M. bovis 100 culture.

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

N'-(2-((4-methyl-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide **(2.1)**																
7th day of experiment				**14th day of experiment**				**30th day of experiment**				**60th day of experiment**				**90th day of experiment**			
Testing	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**		
	0,1%	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**
A rough plaque	**There is no growth**	**There is no growth**	**There is no growth**	**A rough plaque and a few white colonies along the line of sowing**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Smooth, small colonies of white color**	**A rough plaque is along the line sowing**	**Unchanged**	**Unchanged**	**Continuous growth. Smooth, small colonies of whitish color**	**A rough plaque is along the line sowing**	**There is no growth**	**There is no growth**	**Continuous growth.**	**A few white colonies**	**There is no growth**	**There is no growth**
N'-(2-((3-(morpholinomethyl)-4-phenyl-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide hydrobromide **(2.2)**																
7th day of experiment				**14th day of experiment**				**30th day of experiment**				**60th day of experiment**				**90th day of experiment**			
Testing	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**		
	0,1%	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**
A rough plaque	**There is no growth**	**There is no growth**	**There is no growth**	**A rough plaque and a few white colonies along the line of sowing**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Smooth, small colonies of white color**	**Unchanged**	**Unchanged**	**Unchanged**	**Continuous growth. Smooth, small colonies of white color**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth.**	**There is no growth**	**There is no growth**	**There is no growth**
N'-(2-((4-amino-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide hydrobromide **(2.3)**																
7th day of experiment				**14th day of experiment**				**30th day of experiment**				**60th day of experiment**				**90th day of experiment**			

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Testing	Concentration			Testing	Concentration			Testing	Concentration			Testing	Concentration			Testing	Концентрація		
	0,1%	0,5%	1%		0,1%	0,5%	1%		0,1%	0,5%	1%		0,1%	0,5%	1%		0,1%	0,5%	1%
A rough plaque	There is no growth	There is no growth	There is no growth	A rough plaque and a few white colonies along the line of sowing	There is no growth	There is no growth	There is no growth	Continuous growth. Smooth, small colonies of white color	Unchanged	Unchanged	Unchanged	Continuous growth. Smooth, small colonies of white color	There is no growth	There is no growth	There is no growth	Continuous growth.	There is no growth	There is no growth	There is no growth
Isonicotinic acid hydrazide																			
7th day of experiment				14th day of experiment				30 th day of experiment				60 th day of experiment				90 th day of experiment			
Testing	Concentration			Testing	Concentration			Testing	Concentration			Testing	Concentration			Testing	Concentration		
	0,1%	0,5%	1%		0,1%	0,5%	1%		0,1%	0,5%	1%		0,1%	0,5%	1%		0,1%	0,5%	1%
A mucus plaque	A mucus plaque of yellow color	A mucus plaque	There is no growth	Unchanged	Unchanged	Single and small colonies	There is no growth	Numerous colonies of white color	Single colonies of white color	Small colonies of white color	There is no growth	Continuous growth. Smooth, small colonies of white color	The quantity of single and small colonies has some increased	Small colonies of white color	There is no growth	Continuous growth.	Continuous growth	The quantity of single and small colonies has some increased	There is no growth

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Table 2: Characteristics of the cultural properties M. bovis of 100 passages, cultivated on the environment with a pH of 6.5 at a temperature of 37 ° C

N'-(2-((4-methyl-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide **(2.1)**																	
7 th day of experiment				**14 th day of experiment**				**30 th day of experiment**				**60 th day of experiment**				**90 th day of experiment**			
Testing	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**		
	0,1%	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**
Single colonies along with the sowing line	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**
N'-(2-((3-(morpholinomethyl)-4-phenyl-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide hydrobromide **(2.2)**																	
7 th day of experiment				**14 th day of experiment**				**30 th day of experiment**				**60 th day of experiment**				**90 th day of experiment**			
Testing	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**		
	0,1%	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**

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Single colonies along with the sowing line	There is no growth	There is no growth	There is no growth	Continuous growth. Colonies are small, white and smooth	There is no growth	There is no growth	There is no growth	Continuous growth. Colonies are small, white and smooth	A rough plaque is along the line sowing	There is no growth	There is no growth	Continuous growth. Colonies are small, white and smooth	A rough plaque is along the line sowing	There is no growth	There is no growth	Continuous growth. Colonies are small, white and smooth	Small colonies of white color	There is no growth	There is no growth
N'-(2-((4-amino-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl)isonicotino hydrazide hydrobromide **(2.3)**																			
7 th day of experiment				**14 th day of experiment**				**30 th day of experiment**				**60 th day of experiment**				**90 th day of experiment**			
Testing	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**		
	0,1%	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**
Single colonies along with the sowing line	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**	**Continuous growth. Colonies are small, white and smooth**	**There is no growth**	**There is no growth**	**There is no growth**
Isonicotinic acid hydrazide																			
7 th day of experiment				**14 th day of experiment**				**30 th day of experiment**				**60 th day of experiment**				**90 th day of experiment**			
Testing	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**			**Testing**	**Concentration**		
	0,1%	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**		**0,1%**	**0,5%**	**1%**

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A mucus plaque	Single colonies of white color	A rough plaque	There is no growth	Single colonies of white color	Unchanged	Single and smooth colonies	There is no growth	Numerous colonies of white color	Single colonies of white colors	Small colonies of white color	Single and small colonies along with the sowing line	Continuous growth. Colonies are small, white and smooth	Growth of white, single and smooth colonies	Growth of white, single and smooth colonies	Unchanged	Continuous growth. Colonies are small, white and smooth	The quantity of single and small colonies has increased	The quantity of single and small colonies has increased	The quantity of single and small colonies has increased

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CONCLUSION

- The synthesis and studing of tuberculosis-static properties of new derivatives 4-R-3- (morpholinomethyl)-4H-1,2,4-triazole-5-thiols on the crop properties of pathogenic M. bovis were carried out.
- It was found that among the compounds obtained the most pronounced tuberculosis-static effect is N'-(2-((4-amino-3-(morpholinomethyl)-4H-1,2,4-triazol-5-yl)thio)acetyl) isonicotinohydrazide hydrobromide **(2.3)**
- As a result of the experiment, the availability of further studies of synthesized substances as anti-TB agents is demonstrated.

REFERENCES

[1] Hoppe, L., Kettle, R., Eisenhut, M., & Abubakar, I. (2016). Tuberculosis -diagnosis, management, prevention, and control: summary of updated NICE guidance. BMJ, h6747. http://dx.doi.org/10.1136/bmj.h6747

[2] World Health Organization. (2015). Guide to Monitoring and Evaluation for Collaborative TB/HIV Activities-2015 Update. World Health Organization.

[3] Caminero, J. A., Van Deun, A., & Fujiwara, P. I. (2013). Guidelines for clinical and operational management of drug-resistant tuberculosis. Paris, France: International Union Against Tuberculosis and Lung Disease, 18-19.

[4] Tkachenko, O. A., Bilan, M. V., Zazharskyi, V. V., & Davydenko, P. O. (2015). Vplyv preparatu 5-tiienil-1,2,4-tryazol-3-tionu na morfolohiiu, tynktorialni ta kulturalni vlastyvosti patohennykh ta dysotsiatyvnykh form Mycobacterium bovis. Naukovo-tekhnichnyi biuleten Naukovo-doslidnoho tsentru biobezpeky ta ekolohichnoho kontroliu resursiv APK, (3,№ 1), 91-100

[5] Rode, N. D., Sonawane, A. D., Nawale, L., Khedkar, V. M., Joshi, R. A., Likhite, A. P., ... & Joshi, R. R. (2017). Synthesis, Biological Evaluation and Molecular Docking studies of Novel 3-aryl-5-(alkyl-thio)-1H-1,2,4-triazoles Derivatives targeting Mycobacterium tuberculosis. Chemical Biology & Drug Design. 90(6):1206-1214. doi: 10.1111/cbdd.13040.

[6] Thakkar, S. S., Thakor, P., Doshi, H., & Ray, A. (2017). 1,2,4-triazole and 1,3,4-oxadiazole analogues: synthesis, MO studies, in silico molecular docking studies, antimalarial as DHFR inhibitor and antimicrobial activities. Bioorganic & Medicinal Chemistry. 25(15):4064-4075. doi: 10.1016/j.bmc

[7] Pattan, S., Gadhave, P., Tambe, V., Dengale, S., Thakur, D., Hiremath, S. V., & Deotarse, P. (2012). Synthesis and evaluation of some novel 1, 2, 4-triazole derivatives for antmicrobial, antitubercular and anti-inflammatory activities. Indian Journal of Chemistry Section B 51(1):297-301

[8] Kumar, G. S., Prasad, Y. R., & Chandrashekar, S. M. (2013). Synthesis and pharmacological evaluation of novel 4-isopropylthiazole-4-phenyl-1, 2, 4-triazole derivatives as potential antimicrobial and antitubercular agents. Medicinal Chemistry Research, 22(2), 938-948.

[9] Patel, N. B., Khan, I. H., Pannecouque, C., & De Clercq, E. (2013). Anti-HIV, antimycobacterial and antimicrobial studies of newly synthesized 1, 2, 4-triazole clubbed benzothiazoles. Medicinal Chemistry Research, 22(3), 1320-1329.

[10] Saidov, G., Sverdlova, O. and Bakhshiev, N. (1995). Prakticheskoe rukovodstvo po molekuliârnoĭ spektroskopii. 1st ed. Sankt-Peterburg: Izd-vo Sankt-Peterburgskogo universiteta.

[11] Kazitsena, L. and Kupletskaya, N. (1979). Primenenie UF-, IK-, YaMR - i MASS - spectrometrii v orhanicheskoy khimii. 2nd ed. Moskow: University of Moscow, p.240.

[12] Varynskyi, B., Knysh, Y., Parchenko, V., Panasenko, O., & Kaplaushenko, A. (2015). The study of retention regularities for the potential drug substances of 1,2,4-triazol-3-ylthioacetic acids and their salts series by the method of HPLC/DAD-MS. Journal Of Organic And Pharmaceutical Chemistry, 13(4), 68-75.

[13] Tkachenko, A., Zazharsky, V., Bilan, M., & Kovaleva, L. (2010). Some peculiarities of bovis tuberculosis display in long not favourably farm. News of Dniproptrovsk State Agrarian and Economic University, (1), 100-103

[14] Shcherbyna, R., Pruglo, E., Panasenko, O., & Knysh, Y. (2015). Synthesis, physical and chemical properties of esters of 2-((4-R-3-R_1-1,2,4-triazole-5-yl)thio)acetic acids. Journal Of Chemical And Pharmaceutical Research, 7(4), 1418-1422.