Research Article

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N'-(2-(5-((Theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl) isonicotinohydrazide As Antitubercular Agents

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ABSTRACT

The paper presents the results of histological studies of tuberculosis in rabbits in an experimental model with comparative treatment with isoniazid and N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide. The calculated dose of N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide for subcutaneous administration indicates its prospects for veterinary practice as an effective and safe tuberculocidal drug.

Keywords: tuberculosis, experimental model, rabbits, 1,2,4-triazole

1. Introduction

One-third of the world's population is infected with the tuberculosis bacterium [1]. Such people are not sick or contagious. However, they are at increased risk of developing tuberculosis, especially if they have a weakened immune system. Providing such people with preventive therapy not only protects them from the disease, but also reduces the risk of transmission among the population.

Improper use of antimicrobials or ineffective dosage forms, as well as premature discontinuation of treatment can lead to the development of drug resistance [3-8].

Bacteria that cause tuberculosis (TB) may develop resistance to antimicrobial drugs used to treat the disease. Multidrug-resistant tuberculosis (MDR-TB) is TB that does not respond to at least isoniazid and rifampicin, the two most potent anti-TB drugs [9].

In some countries, the treatment of MDR-TB is becoming increasingly problematic. In some cases, tuberculosis may develop with even more severe drug resistance. Extensively drug-resistant tuberculosis (DR-TB) is a form of multidrug-resistant tuberculosis that responds to even fewer drugs. It is registered in 117 countries.

Tuberculosis remains the leading cause of death from infectious diseases in the world [10].

The creation of medicines based on heterocyclic systems makes it possible to make significant progress in the treatment of various diseases. Among the compounds of this class, derivatives of 1,2,4-triazole are particularly distinguished [12, 13].

Study of the tuberculocidal effect of 1,2,4-triazole derivatives *in vivo* infected with *M. bovis* is poorly understood and requires further research.

The aim of the work was to study the structural changes of rabbit organs in modeling tuberculosis under conditions of correction with isoniazid and *N*'-(2-(5-((theophylline-7-yl) methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide. The synthesis of this substance is given in the previous work [17]. The prospects of this area of work were determined by previous stages of research using *in vitro* and *in vivo* methods. In our first experiment, it was found that subcutaneous administration of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide at a dose of 10 mg/kg of animal weight leads to the absence of specific and non-specific manifestations of inflammation in the lungs, liver, kidneys, and spleen of guinea pig [17, 18].

To achieve this goal, the following tasks were solved:

- to perform a comparative analysis of treatment with isoniazid and *N'*-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide using an experimental model of rabbit tuberculosis caused by *M. bovis*;
- to establish the presence of signs of tuberculosis inflammation and non-specific changes in the organs of laboratory animals on the basis of histological studies.

2. Materials and Methods

The tuberculocidal action of *N'*-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl) isonicotinohydrazide was determined in 12 rabbits with an average weight of 2,2 kg [19, 20]. 4 Groups of 3 animals in each were formed:

1 group: subcutaneous administration of isoniazid at a dose of 10 mg/kg body weight;

2 group: subcutaneous administration of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide at a dose 10 mg/kg animal weight;

3 group: control group of animals artificially infected with pathogenic strain of *M. bovis* without treatment;

4 group: control group: clinically healthy animals - subcutaneous injection of isotonic sodium chloride solution (6 ml/kg).

The animals were kept under standard conditions. Experiments performed on live vertebrates were in line with the principles of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986).

For histological examination, each animal was taken with regional to the injection site, pieces of liver, lungs, heart and kidney and placed in 10% formalin solution.

Pathological autopsy was performed by the method of complete evisceration according to Shore. For

histopathological examination, the material was selected immediately after autopsy. Hematoxylin and eosin staining performed histological examinations. The obtained histopreparations were examined using a Leica DM1000 microscope. Photo-fixation of histopreparations was performed with a Leica DFC 295 digital camera.

3. Results and Discussion

A comparative analysis of histological changes in the organs of rabbits experimentally infected with the pathogenic strain of *M. bovis* with long-term administration of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide and isoniazid showed positive results.

These results were obtained after application of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide to animals of the 2 experimental group. Long-term use of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)-isonicotinohydrazide showed the absence of degenerative changes in internal organs and tissues of rabbits. The lungs were dominated by changes in the type of focal interstitial pneumonia in the form of uneven thickening of the interalveolar septa, there were single inflammatory foci, represented by macrophages, epithelial plates and with moderate infiltration of lymphocytes (Figure 1, 2).

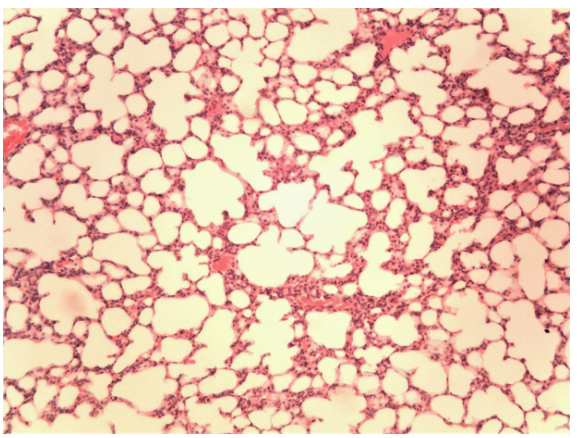

Figure 1. Lungs (*N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)-isonicotinohydrazide). Stagnant plethora, alveoli with uneven ease due to the presence of areas of thickening of the interalveolar septa with moderate infiltration of lymphocytes and macrophages. Hematoxylin-eosin, × 100

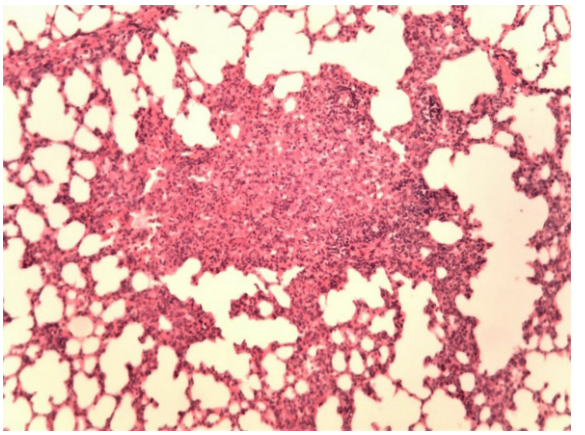

Figure 2. Lungs (*N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)-isonicotinohydrazide). Drain center of pneumonia with infiltration by macrophages, epithelioid cells, lymphocytes, on the periphery of which lung tissue with uneven ease, thickening of interalveolar sections with foci of lymphocytic infiltration. Hematoxylin-eosin, × 100.

There was a preservation of the beam structure, there were areas with focal hydropic dystrophy of hepatocytes, single dinuclear forms in the liver (Figure 3).

Minor regenerative changes of the tubular epithelium in the kidneys in the form of swelling, stagnant plethora and granular dystrophy were detected (Figure 4). In the heart - intermediate edema and granular dystrophy of myocardial cells (Figure 5).

Analyzing the effectiveness of isoniazid in animals of the first experimental group, we determined the presence of degenerative changes in the organs of rabbits. In the lungs, the formation of large drain foci of intermediate pneumonia with infiltration of macrophages, epithelial cells and lymphocytes, as well as the formation of granuloma-like structures were observed. Among the cells of the inflammatory infiltrate the appearance of foci of fibrinoid necrosis with a giant cell reaction around was revealed (Figure 6, 7).

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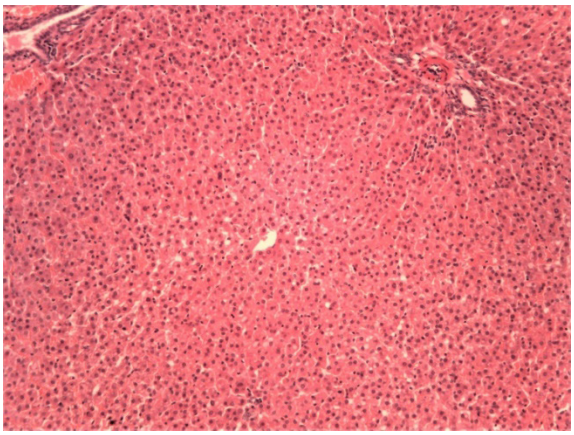

Figure 3. Liver (*N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)-isonicotinohydrazide). Moderate plethora, beam structure preserved, granular, hepatocyte dystrophy, focal hydropic dystrophy, single dinuclear forms. Hematoxylin-eosin, × 100.

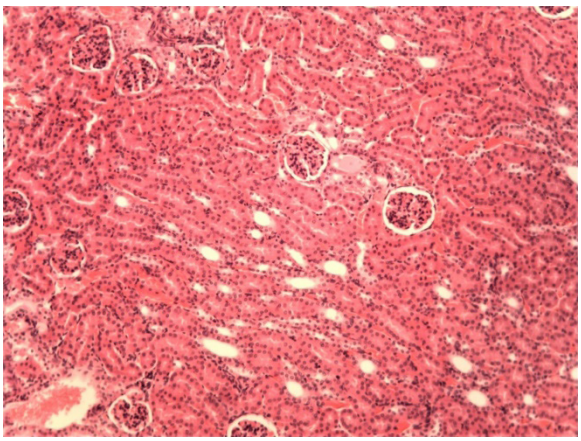

Figure 4. Kidney (*N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)-isonicotinohydrazide). Stagnant plethora, granular dystrophy, swelling of the epithelium of the renal tubules. Hematoxylin-eosin, × 100.

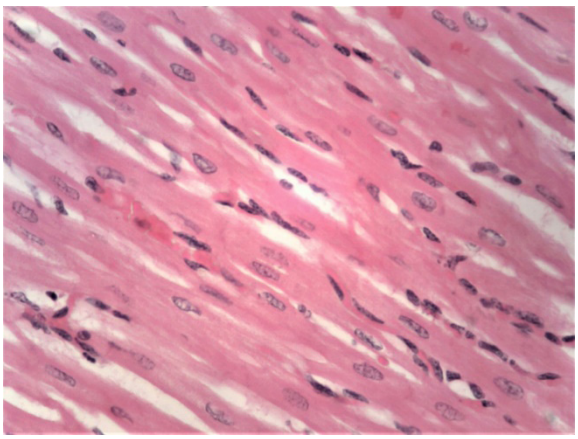

Figure 5. Myocardium (*N'*-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)-acetyl) isonicotinohydrazide). Minor intermediate edema, granular dystrophy of myocardial cells. Hematoxylin-eosin, × 400.

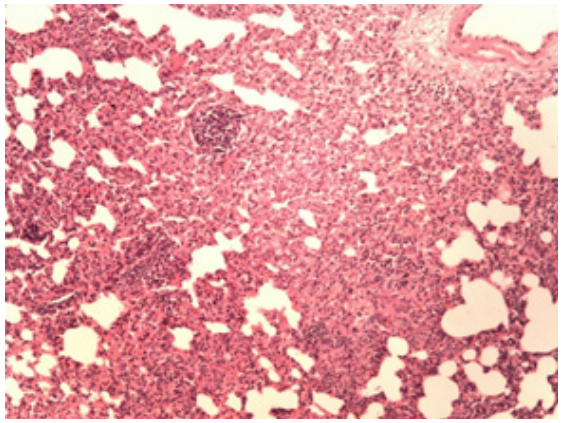

Figure 6. Lungs (isoniazid). Draining inflammation, infiltration of macrophages, epithelial cells and lymphocytes with a tendency to form granulomonophobic foci. Hematoxylin-eosin, × 100.

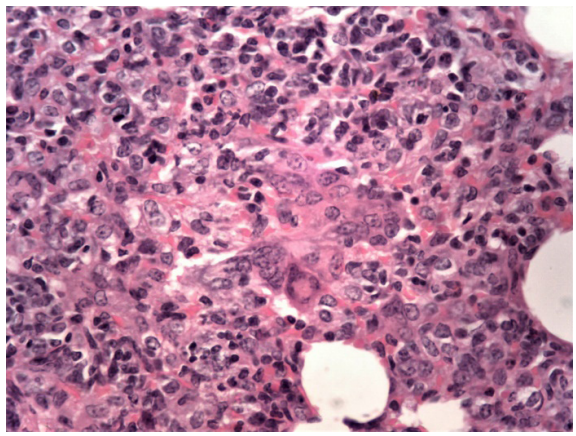

Figure 7. Lungs (isoniazid). Detail of the infiltrate. Among the cells of the inflammatory infiltrate are visible foci of fibrinoid necrosis, and the presence of a number of clusters of epithelioid cells and giant multinucleated cells. Formation of specific granulomas. Hematoxylin-eosin, × 400.

There was a violation of the beam structure of the liver particles due to pronounced diffuse dystrophic changes in the form of hydropic, and in places fatty dystrophy in the liver. Edema of the endothelium of sinusoidal capillaries was revealed (Figure 8, 9).

Dystrophic changes were pronounced in the epithelium of the renal tubules with the phenomena of hydropic dystrophy, stagnant plethora, which even led to necrosis and desquamation of the epithelium with a significant accumulation of protein masses in the lumen of the tubules in the kidney tissue (Figure 10).

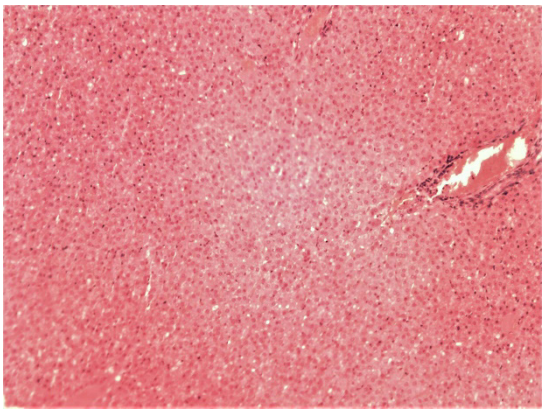

Figure 8. Liver (isoniazid). Granular, hydropic and fatty dystrophy of individual hepatocytes. The beam structure of the liver particles is erased. Hematoxylin-eosin, × 100

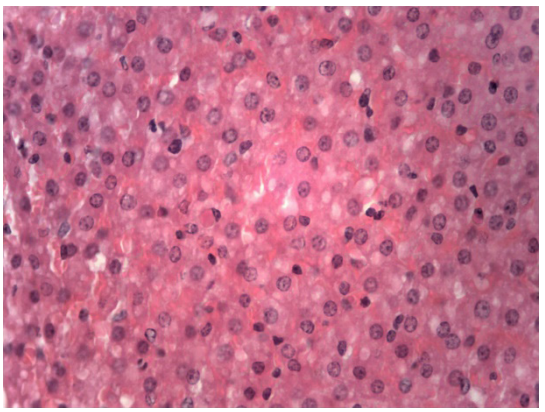

Figure 9. Liver (isoniazid). Edema of the endothelium of sinusoidal capillaries. Hematoxylin-eosin, × 400.

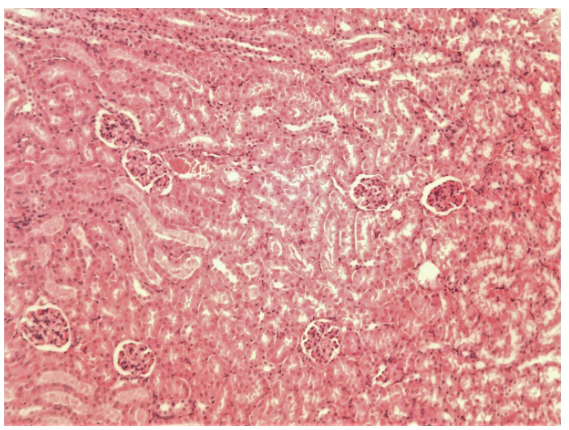

Figure 10. Kidney (isoniazid). Stagnant plethora, granular and hydropic tubular epithelial dystrophy, beam masses in the lumen of the tubules, signs of severe intoxication. Hematoxylin-eosin, × 100.

There was intermediate edema and pronounced dystrophic changes in cardiomyocytes with the disappearance of striated muscle cells, with uneven cytoplasmic staining, which indicates an uneven loss of myoglobulin by myocardiocytes in the myocardium (Figure 11).

We found that the use of *N'*-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl) isonicotinohydrazide is less promising for the treatment of animals with tuberculosis, the degree of damage to the internal organs of rabbits infected with the pathogenic strain of *M. bovis* was significantly less than with isoniazid. Dystrophic changes of internal organs in rabbits of the 2 experimental group have a regenerative character and were characterized by a lower degree of severity (lungs, liver, kidneys and heart). In turn, the use of isoniazid for the treatment of tuberculosis in rabbits leads to diffuse degenerative changes of a degenerative nature with remnants of the inflammatory process at the end of the experiment.

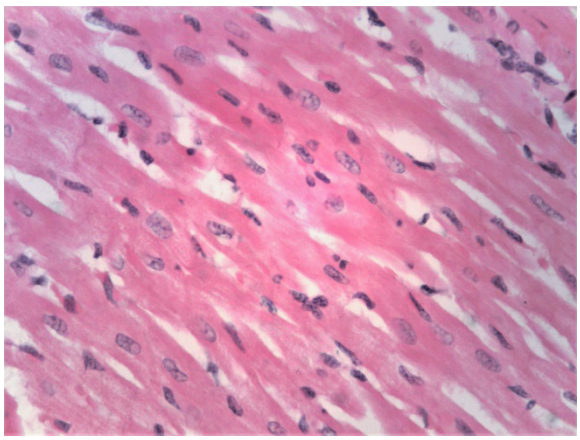

Figure 11. Myocardium (isoniazid). Interstitial edema, granular dystrophy with disappearance of transverse striation of cardiomyocytes. Uneven staining of the cytoplasm of cardiomyocytes. Hematoxylin-eosin, × 400.

N'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotino-hydrazide when administered subcutaneously has a greater tuberculocidal effect compared to isoniazid, as evidenced by the absence of degenerative pathological changes in the lungs, liver, kidneys and heart.

These examples present the results of a comparative analysis of isoniazid and *N'*-(2-(5-((theophylline-

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7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl) isonicotinohydrazide treatment based on histological studies of signs of tuberculous inflammation and nonspecific changes in the organs of rabbits with an experimental model of tuberculosis. It was found that subcutaneous administration of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide at a dose of 10 mg/kg weight of the animal leads to the absence of specific manifestations of inflammation in the lungs, liver, kidneys and spleen. The calculated dose of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide indicates the effectiveness and viability of it's use for veterinary practices in the fight against tuberculosis caused by *M. bovis*.

4. Conclusions

The positive antibacterial effect of *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide was studied in an experiment on rabbits, artificially infected with a pathogenic strain of *M. bovis.*

A comparative analysis of histological changes in the treatment of tuberculosis with *N*'-(2-(5-((theophylline-7-yl)methyl)-4-ethyl-1,2,4-triazole-3-ylthio)acetyl)isonicotinohydrazide and isoniazid allowed to establish therapeutic efficacy of the new compound.

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Conflicts of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

Statement of contribution of researchers

Concept: *A. G., V. Z., V. P.;* Design: *A. G., V. Z.;* Supervision: *V. Z., V. P.;* Resources: *V. Z., P. D., O. K.;* Materials: *A. G., V. Z.;* Data collection and/or processing: *V. Z., P. D., O. K.;* Analysis and/or interpretation: *A. G.; V. Z., T. B.;* Literature search: *V. Z., P. D., O. K.;* Writing manuscript: *V. Z., A. G.;* Critical review: *A. G., Y. Z.*

References

1. Kirimuhuzya C. Multi-Drug/Extensively Drug Resistant Tuberculosis (Mdr/Xdr-Tb): Renewed Global Battle Against Tuberculosis? Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance. 2012. http://dx.doi.org/10.5772/29667.
2. Toktogonova AA. Causes of treatment defaults in those suffering from multiple drug resistance tuberculosis in Kyrgyzstan republic. Tuberculosis and Lung Diseases. 2016;94(12):38–42. doi:10.21292/2075-1230-2016-94-12-38-42
3. Gavali D, Aring B, Gadhavi H, Nathamehta A, Dave A, Nakhva A. Prevalence of Pre-Extensively Drug Resistance Tuberculosis (Pre XDR-TB) & Extensively Drug Resistance Tuberculosis (XDR-TB) among Pulmonary Multidrug Resistance Tuberculosis at a Tertiary Care Hospital, Jamnagar. Journal of Advances in Microbiology. 2019;19(2):1-9. doi:10.9734/jamb/2019/v19i230186
4. Faridgohar M. Finding New Ways to Combat Multidrug-Resistant Tuberculosis. Microbial Drug Resistance. 2020;26(1):71–80. doi:10.1089/mdr.2018.0353
5. Maharjan S, Singh A, Khadka DK, Aryal M. Drug Resistance Pattern in Pulmonary Tuberculosis Patients and Risk Factors Associated with Multi-Drug Resistant Tuberculosis. Journal of Tuberculosis Research. 2017;05(02):106–117. doi:10.4236/jtr.2017.52012
6. Kurz SG, Furin JJ, Bark CM. Drug Resistant Tuberculosis: Challenges and Progress. Infectious Disease Clinics of North America. 2016;30(2):509–522. doi:10.1007/978-3-319-47266-9_33.
7. Kuzmina NV, Nelidova NV. Clinical characteristics of patients with disseminated tuberculosis and multiple drug resistance, residing in the northern territories. Tuberculosis and Lung Diseases. 2018;96(5):56–57. doi:10.21292/2075-1230-2018-96-5-56-57.
8. Patil JS. Epidemiology of Tuberculosis and Drug Resistance in Tuberculosis Treatment in Indian Patients. Advances in Pharmacoepidemiology & Drug Safety. 2015;04(04):e132. doi:10.4172/2167-1052.1000e132.
9. Singh PK, Singh U, Jain A. Emergence of Specific gyrA Mutations Associated High-Level Fluoroquinolone-Resistant *Mycobacterium tuberculosis* among Multidrug-Resistant Tuberculosis Cases in North India. Microbial Drug Resistance. 2021;27(5):647–665. doi:10.1089/mdr.2020.0240.
10. Sheyfer YA, Gelberg IS. Analysis of treatment outcomes for destructive pulmonary tuberculosis in patients with multiple

drug resistance of *M. tuberculosis*. Tuberculosis and Lung Diseases. 2020;98(10):23–27. doi:10.21292/2075-1230-2020-98-10-23-27.

11. Bihdan O, Parchenko V, Zazharskyi V, Fotina T, Davydenko P. Studying of physico-chemical properties of 5-(2-,3-fluorophenyl)-4-((aryl-, geteryl)yliden)amino-1,2,4-triazole-3-thiols and any of their retrieval products. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2019;10(1):464–474.
12. Zazharskyi V, Parchenko M, Parchenko V, Davydenko P, Kulishenko O, Zazharskaya N. Physicochemical properties of new *S*-derivatives of 5-(5-bromofuran-2-yl)-4-methyl-1,2,4-triazole-3-thiols. Voprosy Khimii i Khimicheskoi Tekhnologii. 2020;(6):50–58. doi:10.32434/0321-4095-2020-133-6-50-58
13. Gotsulya AS. Synthesis and antiradical activity of alkyl derivatives of 5-(5-methyl-1*H*-pyrazol-3-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol. Journal of Faculty of Pharmacy of Ankara University. 2020;44(2):211–219.
14. Frolova Y, Kaplaushenko A, Nagornaya N. Design, synthesis, antimicrobial and antifungal activities of new 1,2,4-triazole derivatives containing 1*H*-tetrazole moiety. Journal of Faculty of Pharmacy of Ankara University. 2020;44(1):70–88. doi: 10.33483/jfpau.574001
15. Samelyuk YG, Kaplaushenko AG. Synthesis Of 3-Alkylthio(sulfo)-1,2,4-triazoles, Containing Methoxyphenyl Substituents At C^5atoms, Their Antipyretic Activity, Propensity To Adsorption And Acute Toxicity. Journal of Chemical and Pharmaceutical Research. 2014;6(5):1117–1121.
16. Karpun Y, Polishchuk N. Synthesis and antimicrobial activity of s-substituted derivatives of 1,2,4-triazole-3-thiol. Science-Rise: Pharmaceutical Science. 2021;3(31):64-69.
17. Gotsulia AS, Zazharskyi VV, Davydenko PO. Synthesis and antituberculosis activity of *N*'-(2-(5-((theophylline-7'-yl)methyl)-4-R-4*H*-1,2,4-triazole-3-ylthio)acetyl)-isonicotinohydrazides. Zaporozhye medical journal. 2018;20(4):578–583. doi: 10.14739/2310-1210.2018.4.135677
18. Gotsulia AS, Zazharskyi VV, Davydenko PO. Features of experimental modeling of tuberculosis in guinea pig with the participation of *N*'-(2-(5-((thephylline-7'-yl)methyl)-4-R-1,2,4-triazole-3-ylthio)acethyl)isonicotinohydrazide. Ukrainian Journal of Ecology. 2020;10(4):191–194.
19. Наказ МОЗ України «Порядок проведення доклінічного вивчення лікарських засобів та експертизи матеріалів доклінічного вивчення лікарських засобів» від 14.12.2009 р. №944.
20. Stephanov OV. Doklinichni doslidzhennia likarskikh zasobiv [Preclinical studies of medicines]. Kyiv: Avicenna. [in Ukrainian]. Kyiv: Avicena. 2001. 528 p.

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