Anti-tuberculosis activity research of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Introduction. 1,2,4-Triazole derivatives have already proven themselves to be compounds with low toxicity and high antimicrobial, antifungal, antiviral, hepatoprotective activity.

The aim of work is to research the anti-tuberculosis activity of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazoles-3-thiol on the bacteria strain *M. bovis*.

Materials and methods. At the beginning of the experiment, the effect of the drug concentration and the pH of the medium on the growth rate of the culture at 37 °C was detected. *M. bovis* 100 passage was selected for this purpose, which was cultured at 37 °C with 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol at the indicated concentrations in a thermostat for 3 months on medium with pH 6.5 (ten test tubes with each medicine concentration) and pH 7.1 (ten test tubes with each medicine concentration). *M. bovis* 100 passage was used as a control without the addition of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol to the medium.

Results. The results of the experiment show that the effect of the drug at different concentrations on the medium with a pH 6.5 same with pH 7.1.

Growth of pathogenic strain *M. bovis* 100 passages throughout the observation period (90 days) have been absence for all of the test (0.1 %, 0.5 % and 1.0 %) concentrations of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazoles-3-thiol.

Conclusion. It can be concluded that 0.1 %, 0.5 % and 1.0 % concentration of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol influence on the culture properties pathogenic strain *M. bovis*, which cultured on the medium with pH 6.5 and pH 7.1 at 37 °C, while holding back growth, having a tuberculostatic effect.

Дослідження протитуберкульозної активності 5-(тіофен-2-ілметил)-4*H*-1,2,4-тріазол-3-тіолу

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Одна з основних проблем у сучасній оперативній і терапевтичній медицині та фармації – захворювання, що спричинені мікробними інфекціями.

Похідні 1,2,4-тріазолів зарекомендували себе як сполуки з низькою токсичністю та високою протимікробною, протигрибковою, противірусною, гепатопротекторною активністю.

Мета роботи – дослідження протитуберкульозної активності 5-(тіофен-2-ілметил)-4*H*-1,2,4-тріазол-3-тіолу щодо бактерії штаму *M. bovis*.

Матеріали та методи. На початку досліду виявили вплив концентрації препарату та pH середовища на інтенсивність росту культури за температури 37 °C. Для цього відібрали *M. bovis* 100 пасажу, який культивували за температури 37 °C із 5-(тіофен-2-ілметил)-4H-1,2,4-тріазол-3-тіолом у зазначених концентраціях у термостаті протягом 3 місяців на середовищі з pH 6,5 (10 пробірок із кожною концентрацією препарату) та pH 7,1 (10 пробірок із кожною концентрацією препарату). Як контроль використали *M. bovis* 100 пасажу без додавання до середовища 5-(тіофен-2-ілметил)-4*H*-1,2,4-тріазол-3-тіолу.

Результати. 0,1 %, 0,5 % та 1,0 % концентрації 5-(тіофен-2-ілметил)-4*H*-1,2,4-тріазол-3-тіолу активно впливають на культуральні властивості патогенного штаму *M. bovis*, що культивований на середовищі з pH 6,5 та pH 7,1 при температурі 37 °C, стримуючи ріст і розвиток, володіючи туберкулостатичною дією.

Ключові слова: 1,2,4-тріазол, протитуберкульозна активність, гетероциклічні сполуки.

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Key words: 1,2,4-triazole, anti-tuberculosis activity, heterocyclic compounds.

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Исследование противотуберкулезной активности 5-(тиофен-2-илметил)-4*H*-1,2,4-триазол-3-тиола

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Одной из основных проблем в современной оперативной и терапевтической медицине и фармации являются заболевания, вызванные микробными инфекциями. Производные 1,2,4-триазола зарекомендовали себя как соединения с низкой токсичностью и высоким противомикробным, противогрибковым, противовирусным, гепатопротекторным действием.

Цель работы – исследование противотуберкулезной активности 5-(тиофен-2-илметил)-4*H*-1,2,4-триазол-3-тиола на бактерии штамма *M. bovis*.

Материалы и методы. В начале опыта исследовали влияние концентрации препарата и pH среды на интенсивность роста культуры при температуре 37 °С. Для этого отбирали *M. bovis* 100 пассажа, который культивировали при температуре 37 °С с 5-(тиофен-2-илметил)-4*H*-1,2,4-триазол-3-тиолом в указанных концентрациях в термостате в течение 3 месяцев в среде с pH 6,5 (10 пробирок с каждой концентрацией препарата) и pH 7,1 (10 пробирок с каждой концентрацией препарата). В качестве контроля использовали *M. bovis* 100 пассажа без добавления к среде 5-(тиофен-2-илметил)-4*H*-1,2,4-триазол-3-тиола.

Результаты. Таким образом, 0,1 %, 0,5 % и 1,0 % концентрации 5-(тиофен-2-илметил)-4*H*-1,2,4-триазол-3-тиола активно влияют на культуральные свойства патогенного штамма *M. bovis*, культивируемого на среде с pH 6,5 и pH 7,1 при температуре 37 °С, сдерживая рост и развитие, обладая туберкулостатических действием.

Ключевые слова: 1,2,4-триазол, противотуберкулезная активность, гетероциклические соединения.

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One of the main problems in modern surgical, therapeutic medicine and pharmacy is diseases caused by microbial infections. The diversity of modern antibiotics is astounding. However, along with high antimicrobial activity, they have a number of side effects. One of the main problems is the addictive microorganisms to existing substances.

Thus, there is the question of the creation of new antimicrobial medicines. 1,2,4-Triazole derivatives have already proven themselves [1–8] as compounds with low toxicity and high antimicrobial, antifungal, antiviral, hepatoprotective activity [9–14].

The aim

The aim of work is to research the anti-tuberculosis activity of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thioles on the bacteria strain *M. bovis.*

Materials and methods

Cultivation and biomass accumulation for strains of *M. bovis* was carried out on an egg nutrient medium, which was identical in composition to the standard one manufactured by SE "Veterinary Medicine" (Kharkiv, Ukraine). 5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole-3-thiol was added to the medium to obtain concentrations 0.1 %, 0.5 %, 1.0 %. Measurement of the required amount of the medicine was performed according to GOST 27025-86. The solutions were prepared according to the methods described in GOST 4212-76 and GOST 4517-87.

Tinctorial properties, morphological features and the timing of primary growth, its intensity and the nature of the subculture of the isolated and accumulated mycobacteria were studied. Number, size, shape, surface, consistency, pigmentation, transparency, gloss and emulsification in saline solution performed the analysis and evaluation of the colonies.

The tinctorial properties of mycobacteria were determined by preparing smears from colonies (cultures) stained with the Zill–Nielsen method and examining them under immersion of a SUNNY XS series microscope with a digital microscopic complex "Mykmed-2-1600". The morphology of mycobacteria was determined by length, thickness, shape, nature of cell ends, granularity and location.

Gravimetric measurements were performed on laboratory electronic analytical scales model ESJ-200-4(US).

Results and discussion

At the beginning of the experiment, the effect of the medicine concentration and the pH of the medium on the growth rate of the culture at 37 °C was detected. *M. bovis* 100 passage, which was cultured at 37 °C with 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol at the indicated concentrations in a thermostat for three months on medium with pH 6.5 (ten test tubes with each drug concentration) and pH 7.1 (ten test tubes with each drug concentration) was selected for this purpose. *M. bovis* 100 passage without the addition of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol to the medium have been used as a control.

After these and after cultivation at 37°C, the growth of mycobacterium colonies was recorded and characterized.

As a result, data on the effect of concentrations of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol on the cultivation of *M. bovis* at 37°C on medium with different pH on the culture growth rate were obtained.

The data on the cultural properties of *M. bovis* 100 passage, which was cultured on medium with a pH 7.1, which contains 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol in three concentrations was summarized in *Table 1*.

Until the 7th day of the experiment, the growth of the culture of *M. bovis* 100 passage in the control group on medium with a pH 7.1 at 37 °C was not observed.

The results are given in Table 1, showed no growth of *M. bovis* culture 100 passage in all (0.1 %, 0.5 % and 1.0 %) concentrations of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol throughout the observation period (90 days), indicating the tuberculostatic effect of the drug.

Table 1. Characterization of the cultural properties of *M. bovis* 100 passage, which cultured on medium with a pH 7.1 at 37 °C

Day of experience	Control	The concentration of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol		
		0.1 %	0.5 %	1.0 %
7th day of experience	A rough raid	There is no growth	There is no growth	There is no growth
14th day of experience	Rough raid and single white colonies along the sowing line	There is no growth	There is no growth	There is no growth
30th day of experience	Solid growth. Smooth, small colonies of whitish color	Unchanged	Unchanged	Unchanged
60th day of experience	Solid growth. Smooth, small colonies of whitish color	There is no growth	There is no growth	There is no growth
90th day of experience	Solid growth	There is no growth	There is no growth	There is no growth

Table 2. Characterization of the cultural properties of 100 passages of *M. bovis*, which cultured on medium with a pH of 7.1 at 37 °C

Day of experience	Control	The concentration of the drug isoniazid		
		0.1 %	0.5 %	1.0 %
7th day of experience	Mucoid plaque	Mucoids plaque is yellow	Mucoids plaque	There is no growth
14th day of experience	Unchanged	Unchanged	Single smooth colonies	There is no growth
30th day of experience	Numerous colonies are white	Single colonies are whitish	Small colonies are white	There is no growth
60th day of experience	Solid growth. Smooth, small colonies of whitish color	The number of single small colonies increased slightly	Small colonies are white	There is no growth
90th day of experience	Solid growth	Solid growth	The number of single small colonies increased slightly	There is no growth

Table 3. Characterization of the cultural properties of *M. bovis* 100 passage, cultured on medium with pH 6.5 at 37 °C

Day of experience	Control	The concentration of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol		
		0.1 %	0.5 %	1.0 %
7th day of experience	Single colonies along the sowing line	There is no growth	There is no growth	There is no growth
14th day of experience	Solid growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth
30th day of experience	Solid growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth
60th day of experience	Solid growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth
90th day of experience	Solid growth. Colonies are small, white, smooth	There is no growth	There is no growth	There is no growth

Table 4. Characterization of the cultural properties of 100 passages of *M. bovis* cultured on medium with pH of 6.5 with isoniazid at 37 °C

Day of experience	Control	The concentration of the medicine isoniazid		
		0.1 %	0.5 %	1.0 %
7th day of experience	Mucoid plaque	Single colonies are whitish	A rough raid	There is no growth
14th day of experience	Single colonies whitish color	Unchanged	Single smooth colonies	There is no growth
30th day of experience	Numerous colonies are white	Single colonies are whitish	Small colonies are white	Single small colonies along the sowing line
60th day of experience	Solid growth. Colonies are small, white, smooth	Growth of whitish, single smooth colonies	Growth of whitish, single smooth colonies	Unchanged
90th day of experience	Solid growth. Colonies are small, white, smooth	The number of single small colonies increased	The number of single small colonies increased	The number of single small colonies increased

Table 3 summarizes the data on the cultural properties of *M. bovis* 100 passage, which was cultured in a medium with pH 6.5, which contained 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol in three concentrations.

Until the 7th day of the experiment, the growth of the culture of 100 passages of *M. bovis* on a medium with pH 6.5 at 37 °C was not observed.

The results of the experiment show that the effect of the substance at different concentrations on the medium with pH 6.5 and pH 7.1 are the same. It was marked lack of growth of pathogenic strain *M. bovis* 100 passages throughout the observation period (90 days) for the all test (0.1 %, 0.5 % and 1.0 %) concentrations of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol. Thus, it can be concluded that 0.1 %, 0.5 % and 1.0 % concentration of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol actively influence the culture properties pathogenic strain *M. bovis* cultured on medium with pH 6.5 at 37^{0}C, which holding back growth and having a tuberculostatic effect.

The low concentration (0.1 % and 0.5 %) of the medicine isoniazid on the medium with pH 6.5 and 7.1 at 37°C was not inhibited the growth of pathogenic *M. bovis* 100th passage unlike 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol.

Thus, tables 1-4 had shown that 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol had higher anti-tuberculosis activity than the medicine isoniazid.

Conclusions

Thus, it can be concluded that 0.1 %, 0.5 % and 1.0 % concentration of 5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazole-3-thiol actively influence the culture properties pathogenic strain *M. bovis* cultured on medium with pH 6.5 at 37 °C, which holding back growth and having a tuberculostatic effect.

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