5. Katarzyna Swiader, Regina Kasperek, Dorota Dwornicka. The preparation of suppositories by various methods and evaluation of physicochemical properties. Medical University in Lublin [Електроний ресурс]/ 2011. Режим доступу: www.curipms.umlub.pl/download/gfx/.../en/.../24220.pdf.

Ivanchenko H. D.
PhD, Associate Professor

Romanenko M. I.
Doctor of Pharmacy, Professor

Tikhonovska M. A.
PhD, Associate Professor

Zaporizhzhia State Medical University
Zaporizhia, Ukraine

SYNTHESIS, PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES OF 7-SUBSTITUTED 8-(3,4-DIMETHYL-6-OXOPYRANO[2,3-C]PYRAZOL-1-YL)THEOPHYLLINE

Antimicrobial resistance is a serious concern in human and veterinary medicine. Infections with antimicrobial resistance bacterial pathogens have increased globally and led to the rise of medical expenses, treatment failure and increased morbidity and mortality.

Escherichia coli are ubiquitous in the intestinal tract of humans and animals. Even though commensal *E. coli* is usually non-pathogenic, it is one of the most frequent bacteria of the Enterobacteriaceae family associated with extraintestinal infections [1, p. 916-932]. Concern has been raised that commensal *E. coli* in animals may serve as a reservoir of resistance determinants that could be transferred to pathogenic bacteria in either humans or animals. Plasmid carrying several resistance genes including genes encoding ESBL (extended-spectrum β-lactamase) has increasingly emerged in *E. coli* isolates of animal origin [2, p. 1-13]. Of particular concern is that these resistant *E. coli* may contaminate carcasses during the lairage, slaughter, and marketing processes, contributing to the spread of antimicrobial resistance.

Increasing prevalence of MDR (multidrug resistance)- and ESBL-producing *E. coli* has been recognized worldwide. However, only a few reports have attempted

to elucidate this public health crisis in food animals in developing countries, including those in South-East Asia [3, p. 575-585].

Thus, creation of new modern drugs, that have antimicrobial and antifungal properties, is important and promising trend.

The aim of our work was the synthesis of 7-substituted 8-(3,4-dimethyl-6-oxopyrano[2,3-c]pyrazol-1-yl)theophylline previously undescribed and the study of their biological action.

Materials and Methods. The melting point was determined by the open capillary method on a PTP-M device (a device for determining the melting temperature of solid substances, Russia). Elemental analysis was performed on an Elementar Vario L cube device (Germany), The NMR spectra were taken on a Bruker SF-400 spectrometer (Germany) (the working frequency – 400 MHz, the solvent – dimethylsulfoxide (DMSO), the internal standard – tetramethylsilane). The data of elemental analysis corresponded to the calculated ones.

Molecular descriptors have been calculated using the computer programs ALOGPS and DRAGON, whereas biological properties of the synthesized compounds have been calculated with the help of GUSAR and ACD / Percepta Platform.

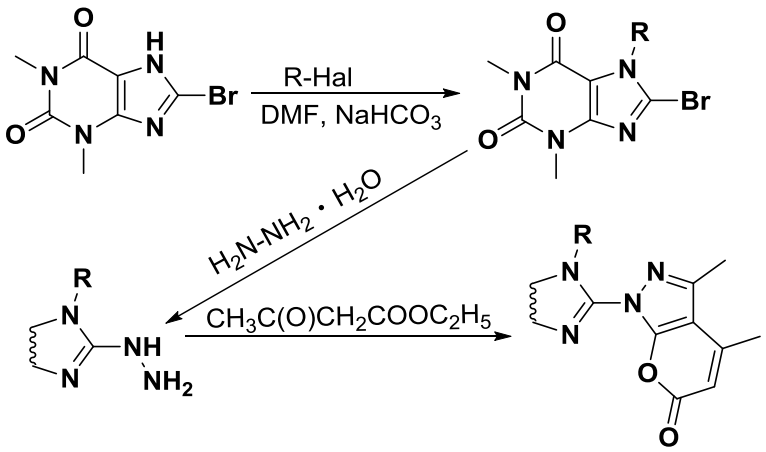
The assessment of the antimicrobial and antifungal activity was carried out using the standard test strains of microorganisms obtained in the bacteriological laboratory of the SI “Zaporizhzhia Regional Laboratory Centre of the State Sanitary and Epidemiological Service of Ukraine”. Such strains as *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 885-653) were used in our studies. The sensitivity of microorganisms to the new promising antimicrobial compounds synthesized was determined according to the methodical recommendations [9, p. 1-38]. To incubate bacteria the Mueller-Hinton broth and agar (pH 7.2-7.4) were used, and for fungi the Sabouraud’s medium (pH 6.0-6.8) was applied.

The minimal inhibitory concentration (MIC) was determined. Dimethylsulfoxide (DMSO) was used as a solvent for the compounds in our studies, the initial solutions were adjusted to the concentration of 1 mg/ml. Additionally monitoring of nutrient media and the solvent was performed using conventional methods. As the reference drugs ampicillin (PJSC "Kyivmedpreparat", Ukraine) and nistatine (PJSC SIC “Borshchahivskiy CPP”, Ukraine) were used.

Results and Discussion. As it is shown in Scheme 1, appropriate 7- substituted of 8-bromotheophylline were synthesized by the reaction of 8-bromotheophylline [4, p. 133-136] with alkyl-, benzylhalogenides in dimethylformamide in the presence of equimolar amounts of $NaHCO_3$. Through the interaction of bromotheophyllines with the excess of hydrazine hydrate in the aqueous dioxane

were obtained 7-substituted of 8-hydrazinotheophylline, which under heating up with ethyl acetoacetate in acetic acid form respective 7-substituted 8-(3,4-dimethyl-6-oxopyrano[2,3-c]pyrazol-1-yl)theophylline.

Scheme 1



The structure of the synthesized substances has been proven by elemental analysis, NMR spectroscopy data.

Further properties of the synthesized compounds were calculated. It has been found that all compounds satisfy to the Rule of five [5, p. 3-26], which means that the Lipinski index for all substances is 0. Further the Ghose filter has been used. It should be noted that compounds 6 and 7 in terms of polar surfaces do not satisfy to all criteria the Ghose filter [6, p. 55-68].

7-Substituted 8-(3,4-dimethyl-6-oxopyrano[2,3-c]pyrazol-1-yl)theophylline have been shown the antimicrobial and antifungal activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, and it is higher than that of the reference drugs – ampicillin and nistatine. Some regularities in the "chemical structure – biological activity" relationship have been determined.

The above facts clearly demonstrate reasonability and prospects for further search of antimicrobial and antifungal agents in the series of xanthines, especially among their 7-substituted 8-(3,4-dimethyl-6-oxopyrano[2,3-c]pyrazol-1-yl)theophylline. For final conclusions it is necessary to significantly expand both the spectrum of pathogenic microorganisms, and the number of the compounds synthesized.

REFERENCES:

1. Mellata M. Human and avian extraintestinal pathogenic *Escherichia coli*: Infections, zoonotic risks, and antibiotic resistance trends / M. Mellata // Foodborne Pathog Dis. – 2013. – vol. 10, № 11. – P. 916-932.

2. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health / D. J. O'Sullivan, L. Giblin, P. L. H. McSweeney, J. J. Sheehan, P. D. Cotter // Front Microbiol. – 2013. – vol. 4. – P. 1–13.

3. Trongjit S. Occurrence and molecular characteristics of antimicrobial resistance of Escherichia coli from broilers, pigs and meat products in Thailand and Cambodia provinces / S. Trongjit, S. Angkittitrakul, R. Chuanchuen // Microbiology and Immunology. – 2016. – vol. 60, № 9. – P. 575-585.

4. Uber die oxidative bromierung von methylxantinen / M. Eckstein, M. Gorczyca, A. Zlyc // Acta Pharm. Jugoslav. – 1972. – № 4. – P. 133-136.

4. Вивчення специфічної активності протимікробних лікарських засобів : Метод. реком. / Ю. Л. Волянський, І. С. Гриценко, В. П. Широбоков та ін. – К. : ДФЦ МОЗ України, 2004. – 38 с.

5. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings / Ch. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney // Adv. Drug Del. Rev. – 2001. – № 46. – P. 3-26.

6. Ghose A. K. A Knowledge-Based Approach in Designing Combinatorial or Medicinal Chemistry Libraries for Drug Discovery. 1. A Qualitative and Quantitative Characterization of Known Drug Databases / A. K. Ghose, V. N. Viswanadhan, J. J. Wendoloski // J. Comb. Chem. – 1999. – № 1. – P. 55-68.