UDC 54.057:547.812:547.594.1:615.281.9 https://doi.org/10.24959/ophcj.18.931

G. V. Grygoriv, D. A. Lega, V. P. Chernykh, T. P. Osolodchenko*, L. A. Shemchuk
National University of Pharmacy
53, Pushkinska str., Kharkiv, 61002, Ukraine. E-mail: galkagrigoriv@gmail.com
* State Institution "Institute of Microbiology and Immunology named after I. I. Mechnikov of the National Academy of Medical Sciences of Ukraine"

Domino-reactions of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide, hetarenecarbaldehydes and active methylene nitriles in the construction of new 2-amino-4*H*-pyrans and the study of their antimicrobial properties

Multicomponent domino reactions are an effective modern approach in the synthesis of different types of organic compounds, including biologically active pyrans.

Aim. To study the three-component interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with different hetarenecarbaldehydes and active methylene nitriles in order to synthesize new 2-amino-4*H*-pyran derivatives, as well as the antimicrobial activity of the compounds obtained.

Results and discussion. 2-Amino-4-heteryl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides were obtained by stepwise and multicomponent reactions of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with hetarenecarbaldehydes and malononitrile. For the same interaction with ethyl cyanoacetate the reaction selectivity decreased and not only target ethyl 2-amino-4*H*-pyran-3-carboxylates were obtained, but also triethylammonium salts of bis(1,2-benzoxathiin-2,2-dioxo-4-ol-3-yl)(heteryl)methane. The latter were also purposefully synthesized by the two-component reaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with hetarenecarbaldehydes in the presence of triethylamine. The compounds obtained revealed a higher antimicrobial activity against gram-positive bacteria and fungi compared to the reference drugs.

Experimental part. 3-Amino-4-heteryl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides and triethylammonium 3-[1-(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)heteryl]-1,2-benzoxathiin-4-olate 2,2-dioxides were synthesized. The antimicrobial activity of the compounds synthesized was studied by the agar diffusion method.

Conclusions. It has been proven that the multicomponent format for the three-component interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with hetarenecarbaldehydes and active methylene nitriles is more favorable and convenient than the stepwise approach to obtain new derivatives of 2-amino-4*H*-pyrans. Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)heteryl]-2,1-benzoxathiin-5-olate 2,2-dioxides have been also synthesized. The antimicrobial properties of the compounds obtained are higher than in the reference drugs, especially against gram-positive bacteria and fungi.

Key words: 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide; domino reactions; 2-amino-4*H*-pyran; triethylammonium salt; antimicrobial activity

Г. В. Григорів, Д. О. Лега, В. П. Черних, Т. П. Осолодченко, Л. А. Шемчук

Доміно-реакції 1,2-бензоксатин-4(3*H*)-он 2,2-діоксиду, гетаренкарбальдегідів та активних метиленових нітрилів у побудові нових 2-аміно-4*H*-піранів і вивчення їх антимікробних властивостей

Одним із ефективних сучасних підходів до синтезу органічних сполук, в тому числі біологічно активних піранів, є багатокомпонентні доміно-реакції.

Метою даної роботи було дослідити трикомпонентну взаємодію 1,2-бензоксатіїн-4(3*H*)-он 2,2-діоксиду з гетероциклічними альдегідами та метиленактивними нітрилами для синтезу похідних 2-аміно-4*H*-пірану та визначити антимікробну активність одержаних сполук.

Результати та їх обговорення. 2-Аміно-4-гетерил-4,6-дигідропірано[3,2-*c*][2,1]бензоксатіїн-3-карбонітрил 5,5-діоксиди були одержані шляхом ступінчатих та багатокомпонентних реакцій 1,2-бензоксатіїн-4(3*H*)-он 2,2-діоксиду з гетероциклічними альдегідами і малонодинітрилом. При використанні як метиленактивного нітрилу етилціаноацетату були одержані не лише цільові етил 2-аміно-4*H*-піран-3-карбоксилати, але також триетиламонієві солі біс(1,2-бензоксатіїн-2,2-діоксо-4-ол-3-іл)(гетерил)метану. Останні також були цілеспрямовано синтезовані шляхом двокомпонентної реакції 1,2-бензоксатіїн-4(3*H*)-он-2,2-діоксиду з гетероциклічними альдегідами в присутності триетиламіну. Одержані сполуки показали більш високу антимікробну активність щодо грампозитивних бактерій і грибів, ніж препарати порівняння.

Експериментальна частина. Були одержані 3-аміно-4-гетерил-4,6-дигідропірано[3,2-*c*][2,1]бензоксатіїн-3-карбонітрил-5,5-діоксиди та триетиламоній 3-[1-(4-гідрокси-2,2-діоксидо-1,2-бензоксатіїн-3-іл)гетерил]-1,2-бензоксатіїн-4-олат 2,2-діоксиди. Антимікробну активність синтезованих сполук вивчали методом дифузії в агар.

Висновки. В ході дослідження була показана перевага багатокомпонентного підходу для синтезу нових похідних 2-аміно-4*H*-пірану шляхом взаємодії 1,2-бензоксатіїн-4(3*H*)-он 2,2-діоксиду з гетероциклічними альдегідами та метиленактивними нітрилами. Триетиламоній 3-[(4-гідрокси-2,2-діоксидо-2,1-бензоксатіїн-3-іл)гетерил]-2,1-бензоксатіїн-5-олат 2,2-діоксиди були одержані двокомпонентною взаємодією 1,2-бензоксатіїн-4(3*H*)-он 2,2-діоксиду з гетероциклічними альдегідами. Антимікробна активність отриманих сполук вище, ніж у препаратів порівняння, особливо щодо грампозитивних бактерій і грибів.

Ключові слова: 1,2-бензоксатіїн-4(3*H*)-он 2,2-діоксид; доміно-реакції; 2-аміно-4*H*-піран; триетиламонієва сіль; антимікробна активність

Г. В. Григорив, Д. А. Лега, В. П. Черных, Т. П. Осолодченко, Л. А. Шемчук

Домино-реакции 1,2-бензоксатин-4(3*H*)-он 2,2-диоксида, гетаренкарбальдегидов и активных метиленовых нитрилов в построении новых 2-амино-4*H*-пиранов и изучение их антимикробных свойств

Многокомпонентные домино-реакции представляют собой эффективный современный подход в синтезе различных органических соединений, включая биологически активные пираны.

Целью данной работы было изучить трехкомпонентное взаимодействие 1,2-бензоксатиин-4(3*H*)-он 2,2-диоксида с различными гетероциклическими альдегидами и метиленактивными нитрилами для синтеза производных 2-амино-4*H*-пирана, а также исследовать антимикробную активность полученных соединений.

Результаты и их обсуждение. 2-Амино-4-гетерил-4,6-дигидропирано[3,2-*c*][2,1]бензоксатиин-3-карбонитрил 5,5-диоксиды получали путем ступенчатых и многокомпонентных реакций 1,2-бензоксатиин-4(3*H*)-он 2,2-диоксида с гетероциклическими альдегидами и малонодинитрилом. При использовании в качестве метиленактивного нитрила этилцианоацетата избирательность реакции снижалась, и были получены не только целевые этил 2-амино-4*H*-пиран-3-карбоксилаты, но также триэтиламмониевые соли бис(1,2-бензоксатиин-2,2-диоксо-4-ол-3-ил)(гетерил)метана. Последние также были целенаправленно синтезированы путем двухкомпонентной реакции 1,2-бензоксатин-4(3*H*)-он-2,2-диоксида с гетероциклическими альдегидами в присутствии триэтиламина. Полученные соединения показали более высокую противомикробную активность в отношении грамположительных бактерий и грибов, чем препараты сравнения.

Экспериментальная часть. Были получены 3-амино-4-гетерил-4,6-дигидропирано[3,2-*c*][2,1]бензоксатиин-3-карбонитрил-5,5-диоксиды и триэтиламмоний 3-[1-(4-гидрокси-2,2-диоксидо-1,2-бензоксатиин-3-ил)гетерил]-1,2-бензоксатиин-4-олат 2,2-диоксиды. Антимикробную активность синтезированных соединений изучали методом диффузии в агар.

Выводы. В ходе исследования была показана предпочтительность многокомпонентного формата для получения новых производных 2-амино-4*H*-пирана путем трехкомпонентного взаимодействия 1,2-бензоксатиин-4(3*H*)-он 2,2-диоксида с гетероциклическими альдегидами и метиленактивными нитрилами. Триэтиламмоний 3-[(4-гидрокси-2,2-диоксидо-2,1-бензоксатиин-3-ил)гетерил]-2,1-бензоксатиин-5-олат 2,2-диоксиды были синтезированы двухкомпонентной реакцией 1,2-бензоксатиин-4(3*H*)-он 2,2-диоксида и гетероциклических альдегидов. Антимикробная активность полученных соединений выше, чем у препаратов сравнения, особенно в отношении грамположительных бактерий и грибов.

Ключевые слова: 1,2-бензоксатиин-4(3*H*)-он 2,2-диоксид; домино-реакции; 2-амино-4*H*-пиран; триэтиламмониевая соль; антимикробная активность

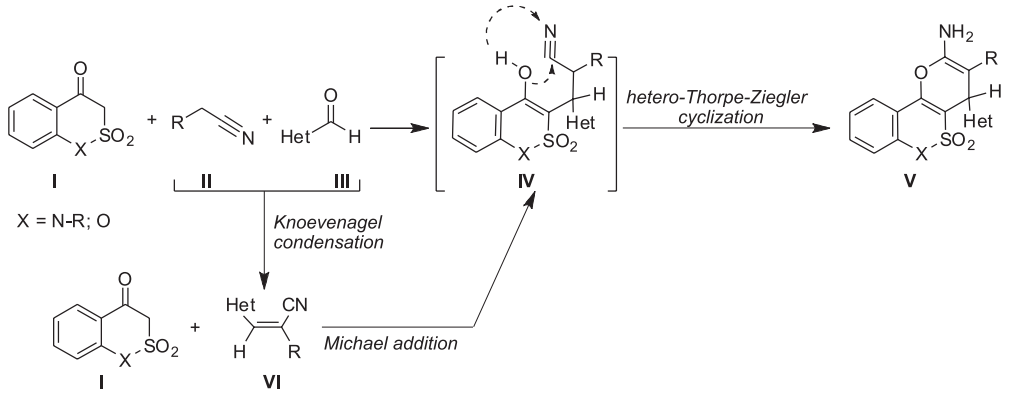
When planning the synthesis of any organic compound it is important to determine all possible synthetic paths. After choosing a clearly defined pathway among this set the paramount attention is paid to the number of stages and the final yield of the product. Considering that organic reactions always occur with less than 100 % yield the synthesis becomes more valuable if it contains fewer stages with a high yield at each of them. Even such acceptable organic yield as 75 % at the 5-th stage gives the total yield of approximately 24 %, for 50 % yield it turns out to be about 3 % [1].

The first way to solve this synthetic problem is to increase the yield of each stage. However, this path is not always successful [2].

The second route relies on utilization of cascade transformations that are known as domino-reactions representing a one-pot process with construction of two or more bonds in one step in order to obtain a complex compound without isolation of intermediates. Domino reactions can take place as the one-component, two-component or multicomponent processes by the number of starting compounds. This approach turned out to be very effective, so it is not surprising that domino interactions gained popularity among synthetic chemists. Moreover, sometimes domino reactions lead to formation of the unexpected reaction products, so they are considered as an instrument for new types of the synthesis of organic compounds [3].

To date a huge variety of domino-reactions has been studied, and it gives the possibility to construct different types of organic compounds, including heterocyclic frames. One of such heterocyclic systems are pyrans, a well-known group with many biologically active representatives, including antimicrobial [4] and antitumor [5] agents. Therefore, one-pot multicomponent reactions in the case of the pyran synthesis can be considered as a useful tool for creating new bioactive substances.

In our previous work 1*H*-2,1-benzothiazin-4-one 2,2-dioxide **I** (X=N-R), active methylene nitriles **II** and hetarenecarbaldehydes **III** were used to synthesize new pyran-annulated derivatives **V** [6]. As a continuation of this research we decided to replace the benzothiazinone core with its oxygen analog – 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **I** (X=O). Such three-component interactions represent a domino Knoevenagel/Michael/*hetero*-Thorpe-Ziegler cyclization sequence, which leads to formation of 2-amino-4*H*-pyrans **V** (Scheme 1). It is possible to perform the corresponding two-step synthesis utilizing the Knoevenagel condensation products – α,β-unsaturated nitriles **VI**. However, such approach seems to be inconvenient because many of these intermediates have toxic and lachrymatory properties [7, 8].



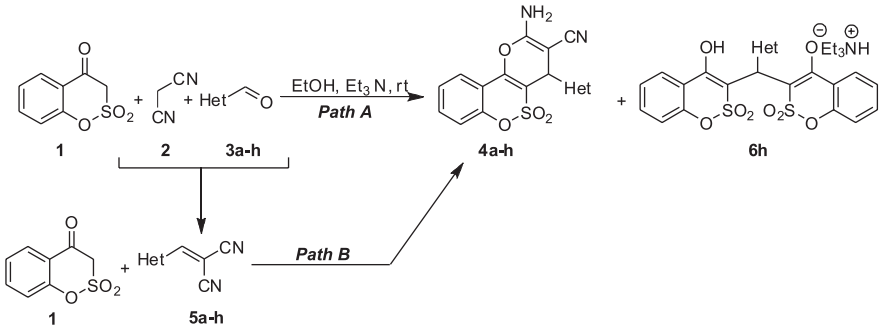
Scheme 1. Two possible ways for the synthesis of 2-amino-4*H*-pyrans

Therefore, our investigation was started with the three-component interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1**, malononitrile **2** as active methylene nitrile and representatives of aldehydes with different hetarene core type **3a-h** (Scheme 2). The reaction proceeded smoothly in the presence of the catalytic amount of triethylamine in ethanol without heating. The target 2-amino-4*H*-pyran-3-carbonitriles **4** were obtained in all cases, except aldehydes **3e** and **3h**. N-Methylpyrrol-2-carbaldehyde **3e** gave hetarylidene **5e**. Considering the reduced reactivity of the double bond in Knoevenagel product **5e** towards Michael addition due to the impact of the pyrrole electron-rich heterocyclic system triethylamine was replaced with much more basic 1,8-diazabicyclo(5.4.0)undec-7-ene (DBU) in order to increase nucleophilic properties of 3-C in 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1**, and the reaction was additionally carried out under reflux for 7-10 h. But all attempts were unsuccessful, and the only product recovered after the reaction was initial α,β-unsaturated nitrile **5e**. As for indol-3-carbaldehyde **3h** the reaction product was triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(3-indolyl)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide **6h**.

The two-step approach towards 2-amino-4*H*-pyrans with the preliminary obtaining of α,β-unsaturated nitriles **5a-h** was also performed (Scheme 2, *Path B*). It was found that this reaction pathway resulted in the lower yields of target compounds compared to those obtained by three-component reactions. Thereby, the multicomponent format for the 2-amino-4*H*-pyrans synthesis is more favorable. It is also worth noting that the two-step format in the case of **3e** and **3h** also gave no target products **4e** and **4h** and resulted in the starting hetarylidenes. Considering such result the triethylammonium salt for indol-3-carbaldehyde **3h** in the three-component reaction was probably formed due to the direct interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** and aldehyde **3h**.

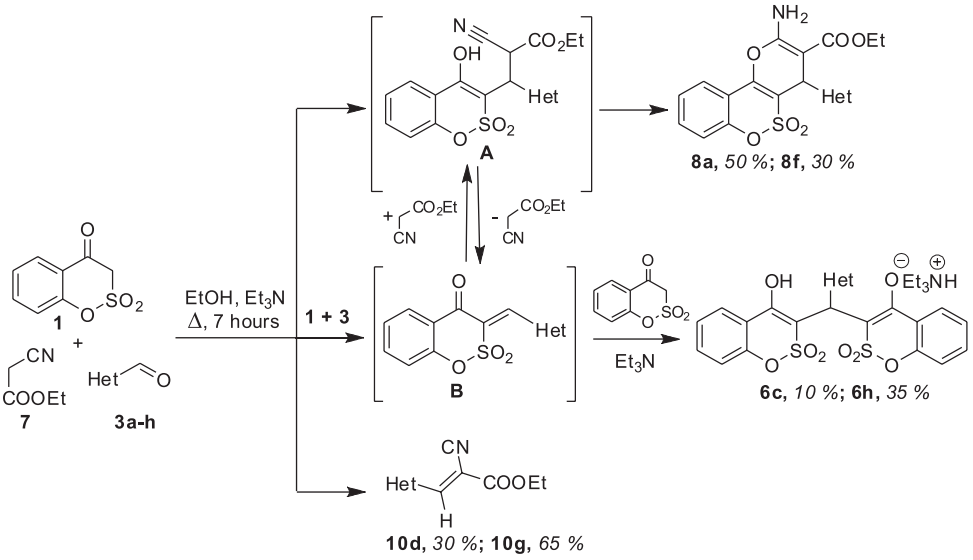
2-Amino-4-heteryl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides **4a-d, 4f,g** were obtained with moderate to high yields (Scheme 2). Different thermal conditions were also applied for these reactions. Heating for 1 to 3 h under reflux gave no appreciable growth to the yield.

At the next stage of our research ethyl cyanoacetate 7 was used instead malononitrile in the three-component interaction studied (Scheme 3) in order to obtain the corresponding ethyl 2-amino-4*H*-pyran-



Compound		5a	5b	5c	5d	5e	5f	5g	5h
Het									
Yield, %	*Path A*	93	65	60	55	-	78	74	-
	Path B	55	44	35	30	-	62	61	-

Scheme 2. The synthesis of 2-amino-4*H*-pyran-3-carbonitriles



Scheme 3. Utilization of ethyl cyanoacetate **7** in the three-component interaction with 1,2-benzoxathiin-4(3*H*)-on 2,2-dioxide **1** and hetarencarbaldehydes **3**

3-carboxylates. According to the previous research data [6] the common conditions for similar reactions include reflux for 7 h in the presence of the catalytic amount of triethylamine. However, in this case the reaction selectivity significantly decreased and three types of products were obtained: the target 2-amino-3-ethoxycarbonyl-4*H*-pyrans 8a and 8f, α,β-unsaturated nitriles 10d and 10g and triethylammonium salts of bis(1,2-benzoxathiine-2,2-dioxo-4-ol-3-yl)(heteryl) methane 6c and 6h. The interaction with 4-pyridinecarbaldehyde 3b led to isolation of an unidentified product. Any product was not isolated for N-methylpyrrol-2-carbaldehyde 3e.

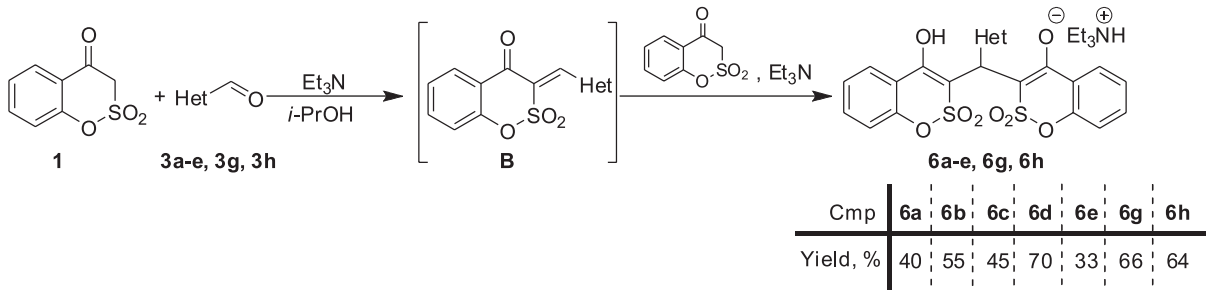
Formation of triethylammonium salts **6c** and **6h** can be explained by two possible reaction pathways. The first one presumes that Michael adduct **A** initially formed does not undergo the intramolecular *hetero*-Thorpe-Ziegler cyclization, but eliminates the molecule of ethyl cyanoacetate with formation of enone **B**. The latter as a Michael acceptor reacted with another molecule of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** with formation of the bis-adduct isolated as a triethylammonium salt. The second route supposed the direct interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** with hetarencarbaldehydes **3**.

Considering this probable reaction pathways we performed the same interaction for thiophen-2-carbaldehyde **3c** with the 3-fold and 7-fold excess of ethyl cyanoacetate **7** in order to shift the equilibrium towards 2-amino-4*H*-pyran **8c** and to avoid salt **6c** formation. However, according to ^{1}H NMR the mixture of the corresponding α,β-unsaturated nitrile and triethylammonium salt was obtained. Furthermore, application of the two-step approach as an interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** and Knoevenagel products for aldehydes **3c** and **3h** also resulted in forming triethylammonium salts **6c** and **6h**.

These results proved the proposed mechanism of *retro*-Michael cleavage for the two-component reaction described and gave the opportunity to consider the similar mechanism for the three-component reaction along with a direct interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** with hetarencarbaldehydes **3**.

It is interesting that the triethylammonium salt similar to **6c** was obtained in the previous studies of 1*H*-2,1-benzothiazin-4-one 2,2-dioxide in the same reaction [6]. Considering the assumption that formation of salt **6c** proceeds through enone **B** we tried to obtain the similar triethylammonium salts of bis-adducts by a direct interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** with hetarenecarbaldehydes **3** (Scheme 4). The reaction was performed in the molar ratio of compounds **1** and **3** 2:1 in propan-2-ol in the presence of the equimolar amount of triethylamine.

The structures of all compounds synthesized were confirmed by ^{1}H NMR-spectroscopy and elemental analysis. The ^{1}H NMR-spectra of compounds **4a-d, 4f, 4g** are characterized by the presence of the narrow high intensity singlet of a proton in position 4 of the 4*H*-pyran ring in the range of 4.85-5.32 ppm, the singlet of the 2-amino group can be observed in the spectral region of 7.30-7.63 ppm. In ^{1}H NMR-spectra of compounds **8a** and **8f** the singlet in position 4 of the 4*H*-pyran ring is situated at 4.83 ppm and 4.90 ppm, respectively, the signal of the 2-NH_2 group is shifted to downfield compared to **4a** and **4f**. The last fact can be explained by formation of the intramolecular hydrogen bond between NH_2 and carbonyl oxygen of the ester fragment. The ^{1}H NMR-spectra of bis-adducts **6a-d, 6g, 6h** are characterized by the presence of the singlets of the benzoxathiine OH-group at 16.78-17.53 ppm and the bridging CH-group at 5.45-5.84 ppm. There



Cmp	6a	6b	6c	6d	6e	6g	6h
Yield, %	40	55	45	70	33	66	64

Scheme 4. The synthesis of triethylammonium salts **6a-e, 6g, 6h**

are no signals of the triethylammonium NH-group in the ^{1}H NMR-spectra probably due to the fast deutero exchange.

The study of the antimicrobial activity of the compounds synthesized was performed according to the international standards [9, 10] by the agar diffusion method against the standard test-strains of gram-positive and gram-negative bacteria, as well as against fungi of *C. albicans*. The results showed a higher antimicrobial activity compared to the reference drugs (Table). The moderate activity revealed was higher in the case of gram-positive strains than for gram-negative bacteria and fungi. The most active were triethylammonium salts corresponding to 2-amino-4*H*-pyran-3-carbonitriles. Therefore, the synthesis of such ammonium salts with other amines may be considered as a promising way for further creation of antimicrobials with the narrow spectrum.

Experimental Chemical Part

Starting aldehydes and active methylene nitriles were obtained from commercial sources and used without further purification. Melting points were determined on a Gallenkamp melting point apparatus, Model MFB-595 in open capillary tubes. The ^{1}H NMR-spectra were recorded on a Varian WXR-400 spectrometer using DMSO-d_6 as a solvent and TMS as an internal standard. Elemental analyses were carried out using a Carlo Erba CHNS-O EA 1108 analyzer.

The general procedure for the synthesis of 2-amino-4-heteryl-4,6-dihydropyrano[3,2-*c*][2,1] benzoxathiin-3-carbonitrile 5,5-dioxides (4a-d, 4f, 4g).

Path A. The three-component one-pot procedure. To the solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** (0.198 g, 0.001 Mol), malononitrile **2** (0.066 g, 0.001 Mol) and the corresponding hetarenecarbaldehyde **3a-d, 3f, 3g** (0.001 Mol) in ethanol (5-10 mL) add the catalytic amount of triethylamine. Keep the mixture for 24 h at room temperature. Filter the precipitates of **4a-d, 4f, 4g** obtained, wash with ethanol and then dry in air.

Path B. The synthesis using intermediate acrylonitriles. Mix the solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** (0.198 g, 0.001 Mol) and the corresponding acrylonitrile **6a-d, 6f, 6g** (0.001 Mol) in ethanol (5-10 mL) for 10 min. Keep the resulting reaction mixtures for 24 h at room temperature. Filter the precipitates of **4a-d, 4f, 4g** obtained, wash with ethanol and then dry in air.

2-Amino-4-(pyrid-3-yl)-4,6-dihydropyrano [3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4a). A light gray powder. M. p. – 263-265 °C (EtOH); Anal. Calcd for $C_{17}H_{11}N_3O_4S$, %: C 57.78; H 3.14; N 11.89. Found, %: C 57.73; H 3.10; N 11.86; ^{1}H NMR (400 MHz,

Table

The antimicrobial activity of compounds **4a-d, 6-a,c,d**

No.	Diameter of the growth inhibition zones (the average for three experiments), mm					
	Gram-positive bacteria			*Gram-negative bacteria*		*Fungi*
	S. aureus	*E. coli*	*B. subtilis*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*
Metronidazole	14	14	16	0	0	14
Synthomycine	14	17	17	17	17	0
4a	20	18	20	16	17	17
4b	17	16	19	15	15	16
4c	20	19	21	16	16	17
4d	20	19	21	16	17	17
6a	21	19	21	16	16	18
6c	20	18	21	16	17	17
6d	21	18	22	16	17	17

DMSO-d_6): δ (ppm) 8.58 (s, 1H, Ar); 8.50 (d, *J* = 4.58 Hz, 1H, Ar); 7.89 (d, *J* = 7.63 Hz, 1H, Ar); 7.82 (d, *J* = 7.93 Hz, 1H, Ar); 7.62-7.75 (m, 1H, Ar); 7.45-7.60 (m, 4H, Ar, NH_2); 7.30-7.43 (m, 1H, Ar); 4.86 (s, 1H, CH).

2-Amino-4-(pyrid-4-yl)-4,6-dihydropyrano [3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4b. A light yellow powder. M. p. – 225-227 °C (EtOH); Anal. Calcd for $C_{17}H_{11}N_3O_4S$, %: C 57.78; H 3.14; N 11.89. Found, %: C 57.74; H 3.11; N 11.85; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.57 (d, *J* = 5.87 Hz, 2H, Ar); 7.90 (d, *J* = 7.83 Hz, 1H, Ar); 7.66-7.75 (t, 1H, Ar); 7.36-7.63 (m, 6H, Ar, NH_2); 4.85 (s, 1H, CH).

2-Amino-4-(2-thienyl)-4,6-dihydropyrano [3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4c). A light yellow powder. M. p. – 255-258 °C (EtOH); Anal. Calcd for $C_{16}H_{10}N_2O_4S_2$, %: C 53.62; H 2.81; N 7.82. Found, %: C 53.59; H 2.78; N 7.78; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.86 (d, *J* = 7.83 Hz, 1H, Ar); 7.64-7.73 (t, 1H, Ar); 7.42-7.58 (m, 5H, Ar, NH_2); 7.07 (d, *J* = 2.35 Hz, 1H, Ar); 6.89-6.99 (m, 1H, Ar); 5.12 (s, 1H, CH).

2-Amino-4-(2-furyl)-4,6-dihydropyrano[3,2-*c*] [2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4d). A light brown crystalline powder. M. p. – 260-262 °C (EtOH); Anal. Calcd for $C_{16}H_{10}N_2O_5S$, %: C 56.14; H 2.94; N 8.18. Found, %: C 56.11; H 2.91; N 8.13; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.86 (d, *J* = 7.83 Hz, 1H, Ar); 7.65-7.72 (t, 1H, Ar); 7.43-7.62 (m, 5H, Ar); 6.38 (s, 2H, NH_2); 4.92 (s, 1H, CH).

2-Amino-4-(N-phenyl-3-methyl-5-chloropyrazolyl)-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4f). A light yellow crystalline powder. M. p. – 165-167 °C (EtOH); Anal. Calcd for $C_{22}H_{15}ClN_4O_4S$, %: C 56.59; H 3.24; N 12.00. Found, %: C 56.43; H 3.17; N 11.87; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.86 (d, *J* = 7.63 Hz, 1H, Ar); 7.69 (d, *J* = 7.93 Hz, 1H, Ar); 7.38-7.59 (m, 9H, Ar, NH_2); 4.87 (s, 1H, CH); 2.18 (br. s., 3H, CH_3).

2-Amino-4-(ethyl-N-phenyl-4-pyrazolyl-3-carboxylate)-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4g). A yellow crystalline powder. M. p. – 175-178 °C (EtOH); Anal. Calcd for $C_{24}H_{18}N_4O_6S$, %: C 58.77; H 3.70; N 11.42. Found, %: C 58.62; H 3.61; N 11.34; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.85 (s, 1H, Ar); 7.81-7.92 (m, 3H, Ar); 7.64-7.73 (m, 1H, Ar); 7.46-7.59 (m, 4H, Ar, NH_2); 7.32-7.43 (m, 3H, Ar); 5.32 (br. s., 1H, CH); 4.27 (q, *J* = 6.41 Hz, 2H, CH_2); 1.25 (t, *J* = 7.02 Hz, 3H, CH_3).

The general procedure for the synthesis of ethyl 2-amino-4-heteryl-4,6-dihydropyrano[3,2-*c*] [2,1]benzoxathiin-3-carboxylate 5,5-dioxides (8a, 8f). To the solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** (0.198 g, 0.001 Mol), ethyl cyanoacetate **7** (0.11 mL, 0.001 Mol) and hetarencarbaldehyde **3a** or **3f** (0.001 Mol) in ethanol (5 mL) add the catalytic amount of triethylamine. Reflux the mixture for 7 h and cool to room temperature. Filter the precipitate of **8a** or **8f** obtained, wash with cold ethanol, dry in air and recrystallize from ethanol.

Ethyl 2-amino-4-(pyrid-3-yl)-4,6-dihydropyrano [3,2-*c*][2,1]benzoxathiin-3-carboxylate 5,5-dioxide (8a). A yellow powder. M. p. – 200-203 °C (EtOH); Anal. Calcd for $C_{19}H_{16}N_2O_6S$, %: C 56.99; H 4.03; N 7.00. Found, %: C 56.95; H 3.97; N 6.88; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.50 (s, 1H, Ar); 8.38-8.46 (m, 2H, Ar); 7.97 (d, *J* = 7.93 Hz, 1H, Ar); 7.93 (br. s., 2H, NH_2); 7.65-7.73 (m, 1H, Ar); 7.53-7.58 (m, 1H, Ar); 7.50 (d, *J* = 8.24 Hz, 2H, Ar); 4.83 (s, 1H, CH) 3.90-4.00 (m, 2H, CH_2); 1.03 (t, *J* = 7.17 Hz, 3H, CH_3).

Ethyl 2-amino-4-(N-phenyl-3-methyl-5-chloropyrazolyl)-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carboxylate 5,5-dioxide (8f). A brown powder. M. p. > 250 °C (EtOH); Anal. Calcd for $C_{24}H_{20}ClN_3O_6S$, %: C 56.09; H 3.92; N 8.18. Found, %: C 55.87; H 3.17; N 8.05; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.85-7.98 (m, 3H, Ar); 7.68 (t, *J* = 7.32 Hz, 1H, Ar); 7.34-7.60 (m, 7H, Ar, NH_2); 4.90 (s, 1H, CH); 3.96-4.11 (m, 2H, CH_2); 2.19 (br. s., 3H, CH_3); 1.09 (t, *J* = 7.02 Hz, 3H, CH_3).

The general procedure for the synthesis of triethylammonium3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)heteryl]-2,1-benzoxathiin-5-olat 2,2-dioxides (6a-e, 6g, 6h). To the solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **1** (0.198 g, 0.001 Mol) and the corresponding hetarenecarbaldehyde **3a-e, 3g, 3h** (0.0005 Mol) in propan-2-ol (10 mL) add triethylamine (0.13 mL, 0.001 Mol). Mix the solution for 1 h at room temperature. Filter the precipitates of **6a-e, 6g, 6h** obtained, wash with propan-2-ol and dry in air.

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(pyrid-3-yl)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide (6a). A gray powder. M. p. – 175-177 °C (EtOH); Anal. Calcd for $C_{28}H_{30}N_2O_8S_2$, %: C 57.32; H 5.15; N 4.77. Found, %: C 57.25; H 5.10; N 4.68; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 17.24 (br. s., 1H, OH); 8.43 (s, 1H, Ar); 8.35 (d, *J* = 4.58 Hz, 1H, Ar); 7.81 (d, *J* = 7.02 Hz, 2H, Ar); 7.58 (d, *J* = 7.93 Hz, 1H, Ar); 7.49-7.55 (m, 2H), Ar; 7.22-7.38 (m, 6H, Ar); 5.51 (s, 1H, CH); 3.04 (q, *J* = 7.22 Hz, 6H, $3CH_2$); 1.12 (t, *J* = 7.32 Hz, 9H, $3CH_3$).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(pyrid-4-yl)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide (6b). A yellow powder. M. p. – 125-127 °C (EtOH); Anal. Calcd for $C_{28}H_{30}N_2O_8S_2$, %: C 57.32; H 5.15; N 4.77. Found, %: C 57.28; H 5.11; N 4.72; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 17.18 (br. s., 1H, OH); 8.37-8.45 (m, 2H, Ar); 7.82 (d, *J* = 7.63 Hz, 2H, Ar); 7.45-7.57 (m, 2H, Ar); 7.29-7.38 (m, 4H, Ar); 7.23 (d, *J* = 5.19 Hz, 2H, Ar); 5.45 (s, 1H, CH); 3.04 (q, *J* = 7.32 Hz, 6H, $3CH_2$); 1.13 (t, *J* = 7.17 Hz, 9H, $3CH_3$).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(2-thienyl)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide (6c). A light brown powder. M. p. – 153-155 °C (EtOH); Anal. Calcd for

$C_{27}H_{29}NO_8S_3$, %: C 54.80; H 4.94; N 2.37. Found, %: C 54.77; H 4.91; N 2.32; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 17.53 (s, 1H, OH); 7.84 (d, *J* = 7.93 Hz, 2H, Ar); 7.51 (t, J = 7.78 Hz, 2H, Ar); 7.25-7.38 (m, 4H, Ar); 7.20 (d, *J* = 5.19 Hz, 1H, Ar); 6.77-6.86 (m, 1H, Ar); 6.74 (br. s., 1H, Ar); 5.63 (s, 1H, CH); 3.03 (q, *J* = 7.02 Hz, 6H, $3CH_2$); 1.12 (t, *J* = 7.17 Hz, 9H, $3CH_3$).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(2-furyl)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide (6d). A light brown powder. M. p. – 160-162 °C (EtOH); Anal. Calcd for $C_{27}H_{29}NO_9S_2$, %: C 56.33; H 5.08; N 2.43. Found, %: C 56.31; H 5.05; N 2.41; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 17.38 (br. s., 1H, OH); 7.84 (d, *J* = 7.93 Hz, 2H, Ar); 7.47-7.56 (m, 2H, Ar); 7.41 (s, 1H, Ar); 7.20-7.36 (m, 4H, Ar); 6.27 (br. s., 1H, Ar); 6.06 (br. s., 1H, Ar); 5.45 (s, 1H, CH); 3.05 (q, *J* = 7.32 Hz, 6H, $3CH_2$); 1.13 (t, *J* = 7.17 Hz, 9H, $3CH_3$).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(2-N-methylpyrryl)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide (6e). A yellow powder. M. p. – 150-152 °C (EtOH); Anal. Calcd for $C_{28}H_{32}N_2O_8S_2$, %: C 57.13; H 5.48; N 4.76. Found, %: C 57.05; H 5.39; N 4.68; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm). 17.27 (br. s., 1 H, OH); 7.86 (d, *J* = 7.02 Hz, 2H, Ar); 7.45-7.52 (m, 2H, Ar); 7.32 (t, *J* = 7.63 Hz, 2H, Ar); 7.26 (d, *J* = 7.93 Hz, 2H, Ar); 6.47 (br. s., 1H, Ar); 5.96 (br. s., 1H, Ar); 5.75 (t, *J* = 3.05 Hz, 1H, Ar); 5.41 (s, 1H, CH); 3.43 (s, 3H, CH_3); 3.02 (q, *J* = 7.32 Hz, 6H, $3CH_2$); 1.10 (t, *J* = 7.32 Hz, 9H, $3CH_3$).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(ethyl N-phenyl-4-pyrazolyl-3-carboxylate)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide (6g). A light brown powder. M. p. – 128-130 °C (EtOH); Anal. Calcd for $C_{35}H_{37}N_3O_{10}S_2$, %: C 58.08; H 5.15; N 5.81. Found, %: C 57.97; H 5.04; N 5.69; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm). 16.78-17.15 (m, 1H, OH); 8.20 (s, 1H, Ar); 7.83 (d, *J* = 6.71 Hz, 2H, Ar); 7.70 (d, *J* = 7.63 Hz, 2H, Ar); 7.42-7.51 (m, 4H, Ar); 7.23-7.36 (m, 5H, Ar); 5.84 (s, 1H, CH); 4.18 (q, *J* = 7.02 Hz,2H, CH_2); 3.03 (q, *J* = 7.12 Hz, 6H, $3CH_2$); 1.06-1.19 (m, 12H, $4CH_3$).

Triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(3-indolyl)methyl]-2,1-benzoxathiin-5-olate 2,2-dioxide (6h). A yellow powder. M. p. – 135-137 °C (EtOH); Anal. Calcd for $C_{31}H_{32}N_2O_8S_2$, %: C 59.60; H 5.16; N 4.48. Found, %: C 59.44; H 5.06; N 4.32; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm). 17.47 (br. s., 1H, OH); 10.71 (br. s., 1H, NH); 7.81 (d, *J* = 7.63 Hz, 2H, Ar); 7.59 (d, *J* = 7.93 Hz, 1H, Ar); 7.43-7.52 (m, 2H, Ar); 7.24-7.33 (m, 5H, Ar); 7.03 (s, 1H, Ar); 6.94 (t, *J* = 7.32 Hz, 1H, Ar); 6.81 (t, *J* = 7.48 Hz, 1H, Ar); 5.70 (s, 1H, CH); 3.01 (q, *J* = 7.32 Hz, 6H, $3CH_2$); 1.10 (t, *J* = 7.17 Hz, 9H, $3CH_3$).

Experimental Microbiological Part

According to the WHO recommendations [9] the following test-strains were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 4636, *Candida albicans* ATCC 653/885. The inoculum suspension was prepared using a Densi-La-Meter apparatus (made by PLIVA-Lachema, Czech Republic; 540-nm wavelength).

The suspension was prepared according to the manual for the device and the information sheet No. 163-2006 "Standardization for preparation of microbial suspensions" (Kyiv) concerning innovations in the healthcare system. The inoculum density was 10^7 cells in 1 mL of the medium, and it was determined by comparing with McFarland standard [11]. The 18 to 24-hour old cultures of the microorganisms were used for the test. For the antimicrobial evaluation the Mueller-Hinton agar was used, for *Candida albicans* Strain the Sabouraud agar was taken. The compounds were introduced into agar by the diffusion method [9]. The antibacterial activity was assessed by measuring zones of inhibition of the corresponding microorganism and was compared with those for the reference antimicrobial drugs.

Conclusions

1. The three-component interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with different hetarenecarbaldehydes and active methylene nitriles have been studied and described.

2. The utilization of malononitrile led to formation of 2-amino-4*H*-pyran-3-carbonitriles in moderate and high yields. It has been proven that the multicomponent format for this interaction is more favorable than the stepwise approach. When using ethyl cyanoacetate three different types of products depending on the initial hetarenecarbaldehyde were obtained, namely the expected ethyl 2-amino-4*H*-pyran-3-carboxylates, the triethylammonium salts of bis-adduct or ethyl 2-cyanoacrylates.

3. The synthesis of the triethylammonium salts was performed by the two-component interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with hetarenecarbaldehydes in the presence of triethylamine.

4. The study of the antimicrobial properties of the compounds synthesized was performed and revealed a higher activity than in the reference drugs, especially against gram-positive bacteria and fungi. The most active compounds, which may be used in further biological studies, are triethylammonium salts **6a** and **6d**.

Conflict of Interests: authors have no conflict of interests to declare.

References

1. Смит, В. Органический синтез. Наука и искусство / В. Смит, Л. Бочков, Р. Кейпл. – М. : Мир, 2001. – 573 с.
2. Woodward, R. B. The total synthesis of vitamin B_{12} / R. B. Woodward // Pure Appl. Chem. – 1973. – Vol. 33, Issue 1. – P. 145–178. doi: 10.1351/pac197333010145
3. Tietze, L. F. Domino-Reactions in Organic Synthesis / L. F. Tietze // Chem. Rev. – 1996. – Vol. 96. – P. 115–136.
4. Synthesis and identification of β–aryloxyquinolines and their pyrano[3,2–*c*]chromene derivatives as a new class of antimicrobial and antituberculosis agents / D. C. Mungra, M. P. Patel, D. P. Rajani, R. G. Patel // Eur. J. of Med. Chem. – 2011. – Vol. 46, Issue 9. – P. 4192–4200. doi: 10.1016/j.ejmech.2011.06.022
5. Anticancer activities of some newly synthesized pyridine, pyrane, and pyrimidine derivatives / A. E. Amr, A. M. Mohamed, S. F. Mohamed et al. // Bioorg. & Med. Chem. – 2006. – Vol. 14, Issue 16. – P. 5481–5488. doi: 10.1016/j.bmc.2006.04.045
6. Lega, D. A. The study of the three–component interaction between 1–ethyl–1*H*–2,1–benzothiazin–4(*H*)–one 2,2–dioxide, heterylcarbaldehydes and active methylene nitriles / D. A. Lega, V. P. Chernykh, L. A. Shemchuk // Журн. орг. та фармац. хімії. – 2016. – Т. 14, Вип. 1 (53). – С. 6–16. doi: 10.24959/ophcj.16.876
7. Brahmachari, G. Facile and one–pot access to diverse and densely functionalized 2–amino–3–cyano–4*h*–pyrans and pyran–annulated heterocyclic scaffolds via an eco–friendly multicomponent reaction at room temperature using urea as a novel organo–catalyst / G. Brahmachari, B. Banerjee // ACS Sustainable Chem. Eng. – 2013. – Vol. 2, Issue 3. – P. 411–422. doi: 10.1021/sc400312n
8. Shaterian, H. R. Domino Knoevenagel condensation, Michael addition, and cyclization using ionic liquid, 2–hydroxyethylammonium formate, as a recoverable catalyst / H. R. Shaterian, M. Arman, F. Rigi // J. Mol. Liq. – 2011. – Vol. 158, Issue 2. – P. 145–150. doi: 10.1016/j.molliq.2010.11.010
9. Coyle, M. B. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology / M. B. Coyle. – Washington : American Society for Microbiology, 2005. – 236 p.
10. Performance Standards for Antimicrobial Susceptibility Testing; Twenty–Second Informational Supplement. Document M100–S22 // CLSI, Wayne, PA. – 2012. – Vol. 32, Issue 3.
11. McFarland, J. The nephelometer : an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines / J. McFarland // J. of the American Med. Association. – 1907. – Vol. 49, Issue 14. – 1176 p. doi: 10.1001/jama.1907.25320140022001f

References

1. Smit, V., Bochkov, L., Keipl, R. (2001). *Organicheskii sintez. Nauka i iskusstvo.* Moscow: Mir, 573.
2. Woodward, R. B. (1973). The total synthesis of vitamin B12. *Pure and Applied Chemistry, 33 (1).* doi: 10.1351/pac197333010145
3. Tietze, L. F. (1996). Domino Reactions in Organic Synthesis. *Chem. Rev., 96,* 115–136.
4. Mungra, D. C., Patel, M. P., Rajani, D. P., Patel, R. G. (2011). Synthesis and identification of β–aryloxyquinolines and their pyrano[3,2–c]chromene derivatives as a new class of antimicrobial and antituberculosis agents. *European Journal of Medicinal Chemistry, 46 (9),* 4192–4200. doi: 10.1016/j.ejmech.2011.06.022
5. Amr, A.–G. E., Mohamed, A. M., Mohamed, S. F., Abdel–Hafez, N. A., Hammam, A. E.–F. G. (2006). Anticancer activities of some newly synthesized pyridine, pyrane, and pyrimidine derivatives. *Bioorganic & Medicinal Chemistry, 14 (16),* 5481–5488. doi: 10.1016/j.bmc.2006.04.045
6. Lega, D. A., Chernykh, V. P., Shemchuk, L. A. (2016). The study of the three–component interaction between 1–ethyl–1H–2,1–benzothiazin–4(3H)–one 2,2–dioxide, heterylcarbaldehydes and active methylene nitriles. *Žurnal Organìčnoï Ta Farmacevtičnoï Hìmìï, 14 (1 (53)),* 6–16. doi: 10.24959/ophcj.16.876
7. Brahmachari, G., Banerjee, B. (2013). Facile and one–pot access to diverse and densely functionalized 2–amino–3–cyano–4h–pyrans and pyran–annulated heterocyclic scaffolds via an eco–friendly multicomponent reaction at room temperature using urea as a novel organo–catalyst. *ACS Sustainable Chemistry & Engineering, 2 (3),* 411–422. doi: 10.1021/sc400312n
8. Shaterian, H. R., Arman, M., Rigi, F. (2011). Domino Knoevenagel condensation, Michael addition, and cyclization using ionic liquid, 2–hydroxyethylammonium formate, as a recoverable catalyst. *Journal of Molecular Liquids, 158 (2),* 145–150. doi: 10.1016/j.molliq.2010.11.010
9. Coyle, M. B. (2005). Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology. *American Society for Microbiology,* 236.
10. Performance Standards for Antimicrobial Susceptibility Testing; Twenty–Second Informational Supplement. Document M100–S22 (2012). *CLSI, Wayne, PA, 32 (3).*
11. McFarland, J. (1907). The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *JAMA: The Journal of the American Medical Association, XLIX (14),* 1176. doi: 10.1001/jama.1907.25320140022001f

Надійшла до редакції 12.12.2017 р.