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Synthesis of 1,2-benzoxathiine 2,2-dioxide derivatives using aliphatic aldehydes and assessment of their antimicrobial activity

Nowadays the problem of the antimicrobial resistance promotes the search of new chemical core-structures with the antimicrobial properties.

Aim. To study the interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with active methylene nitriles and aliphatic aldehydes and assess the antimicrobial activity of the compounds obtained.

Results and discussion. 1,2-Benzoxathiin-4(3*H*)-one 2,2-dioxide as a structural analog of 1,3-dicarbonyl compounds was used in the three-component interaction with aliphatic aldehydes and active methylene nitriles. In the case of malononitrile the target compounds were formed. When using ethyl cyanoacetate the only isolated product was triethylammonium salt that could be also obtained by the two-component reaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with aliphatic aldehydes. The study of the antimicrobial properties showed the higher activity of the compounds studied than in the reference drugs, especially against gram-positive strains.

Experimental part. The series of 2-amino-4-alkyl-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)alkyl]-1,2-benzoxathiin-4-olate 2,2-dioxides was synthesized. The antimicrobial activity of the compounds obtained was determined by the agar “well” diffusion method.

Conclusions. It has been shown that 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide as a structural analog of 1*H*-2,1-benzothiazin-4-one 2,2-dioxide can be used in similar three- and two-component reactions, but its reactivity is less due to the replacement of the 1-N-R-group with an O-atom. The novel compounds obtained exceeded the antimicrobial activity of the reference drugs, and were more active against gram-positive bacteria in contrast to isosteric derivatives of 1*H*-2,1-benzothiazin-4-one 2,2-dioxide that were active against gram-negative strains and fungi.

Key words: 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide; 2-amino-4*H*-pyran; three-component interaction; ammonium salt; antimicrobial activity

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Синтез похідних 1,2-бензоксатіїн 2,2-діоксиду з використанням аліфатичних альдегідів та дослідження їх антимікробної активності

На сучасному етапі проблема антимікробної резистентності сприяє пошуку нових молекулярних структур з антимікробними властивостями.

Метою даної роботи було дослідити взаємодію 1,2-бензоксатіїн-4(3*H*)-он 2,2-діоксиду з метиленактивними нітрилами та аліфатичними альдегідами та антимікробні властивості синтезованих сполук.

Результати та їх обговорення. 1,2-Бензоксатіїн-4(3*H*)-он 2,2-діоксид як структурний аналог 1,3-дикарбонільних сполук було використано в трикомпонентній взаємодії з аліфатичними альдегідами та метиленактивними нітрилами. У випадку малонодинітрилу утворювалися цільові похідні. При використанні етилціаноацетату єдиним ізольованим продуктом була триетиламонієва сіль, одержання якої можливе також у випадку двокомпонентної взаємодії. Вивчення антимікробних властивостей показало вищу активність, ніж у препаратів порівняння, особливо проти грампозитивних штамів мікроорганізмів.

Експериментальна частина. Було синтезовано ряд 2-аміно-4-алкіл-4,6-дигідропірано[3,2-*c*][2,1]бензоксатіїн-3-карбонітрил 5,5-діоксидів та 3-[1-(4-гідрокси-2,2-діоксидо-1,2-бензоксатіїн-3-іл)алкіл]-1,2-бензоксатіїн-4-олат 2,2-діоксидів. Для одержаних сполук було проведено визначення антимікробної активності методом дифузії в агар.

Висновки. 1,2-Бензоксатіїн-4(3*H*)-он 2,2-діоксид як структурний аналог 1*H*-2,1-бензотіазин-4-он 2,2-діоксиду був використаний у три- та двокомпонентних реакціях, але його реакційна здатність виявилась меншою за рахунок заміни 1-N-R-групи на атом кисню. Одержані сполуки за антимікробною активністю перевищили препарати порівняння та виявились більш активними щодо грампозитивних штамів мікроорганізмів на відміну від ізостерних похідних 1*H*-2,1-бензотіазин-4-он 2,2-діоксиду, антимікробні властивості яких були пов’язані з інгібуючим впливом на грамнегативні штами та гриби.

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

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Синтез производных 1,2-бензоксатиин 2,2-диоксида с использованием алифатических альдегидов и оценка их антимикробной активности

На современном этапе проблема антимикробной резистентности способствует поиску новых молекулярных структур с антимикробными свойствами.

Целью данной работы было изучить взаимодействие 1,2-бензоксатиин-4(3*H*)-он 2,2-диоксида с метиленактивными нитрилами и алифатическими альдегидами и оценить антимикробную активность полученных соединений.

Результаты и их обсуждение. 1,2-Бензоксатиин-4(3*H*)-он 2,2-диоксид как структурный аналог 1,3-дикарбонильных соединений был использован в трехкомпонентном взаимодействии с алифатическими альдегидами и метиленактивными нитрилами. В случае малонодинитрила образовывались целевые производные. При использовании этилцианацетата единственным изолированным продуктом была триэтиламмониевая соль, получение которой возможно также в случае двухкомпонентного взаимодействия. Изучение антимикробных свойств показало более высокую активность, чем у препаратов сравнения, особенно в отношении грамположительных штаммов.

Экспериментальная часть. Был синтезирован ряд 2-амино-4-алкил-4,6-дигидропирано[3,2-*c*][2,1] бензоксатиин-3-карбонитрил 5,5-диоксидов и 3-[1-(4-гидрокси-2 2-диоксидо-1,2-бензоксатиин-3-ил)алкил]-1,2-бензоксатиин-4-олат 2,2-диоксидов. Для полученных соединений было проведено определение антимикробной активности методом диффузии в агар.

Выводы. 1,2-Бензоксатиин-4(3*H*)-он 2,2-диоксид как структурный аналог 1*H*-2,1-бензотиазин-4-он 2,2-диоксида был использован в трех- и двухкомпонентных реакциях, но его реакционная способность оказалась меньше за счет замены 1-N-R-группы на атом кислорода. Полученные соединения по антимикробной активности превысили препараты сравнения и проявили активность в отношении грамположительных бактерий в отличие от изостерных производных 1*H*-2,1-бензотиазин-4-он 2,2-диоксида, антимикробные свойства которых были связаны с ингибирующим влиянием на грамотрицательные штаммы и грибы.

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

The results of the studies in different countries all over the world indicate the growth of the antimicrobial resistance (AR) and, particularly the multiple drug resistance in numerous microorganisms that are the main cause of growth of different infectious sickness rate [1, 2, 3]. The obvious consequences of this process include an increase in morbidity and mortality, prolongation of the disease time and a greater risk of complications [4]. AR becomes also the cause for the greater economic burden for the population due to decrease in their labor productivity and increase of the costs for diagnosis and treatment of such disease type [5]. Altogether the impact of AR on the health and economic system can be estimated as extremely negative. Therefore, the synthesis of new efficient biologically active compounds with the promising antimicrobial properties still remains one of the topical issues in development of new drugs [6]. The World Health Organization is also encouraging works in this direction. According to this the Global Strategy on Containment of Antimicrobial Resistance (2001) [7] was worked out. It contains a complete list of recommendations for AR combating. In particular, the strategy to promote the creation of new drugs and vaccines with the necessary properties was proposed, especially with novel chemical core-structures, which were not earlier utilized as antimicrobial substances.

In our previous works we used 1*H*-2,1-benzothiazin-4-one 2,2-dioxide as a core-structure to obtain its new pyran-annulated derivatives, as well as novel ammonium salts and consequently to assess their antimicrobial activity [8, 9]. These studies allowed us to find the substances with a moderate activity against *P. aeruginosa* and *C. albicans*. In this regard, aiming to obtain new effective antimicrobial agents we continued our investigations in this field by modifying of the abovementioned core-structure *via* isosteric replacement of the 1-N-R group with an O-atom, resulting in 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide (Fig.).

This idea has marked the beginning of new research of our scientific group dedicated to revealing the synthetic and pharmacological potential of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide. It is also targeted on determination of the general regularities on the structure-bioactivity relationships in series of SO_2-containing heterocycles and subsequently on purposeful construction of drugs with a desired activity.

Therefore, the present article describes the synthesis of new 2-amino-4-alkyl-4*H*-pyran-3-carbonit-

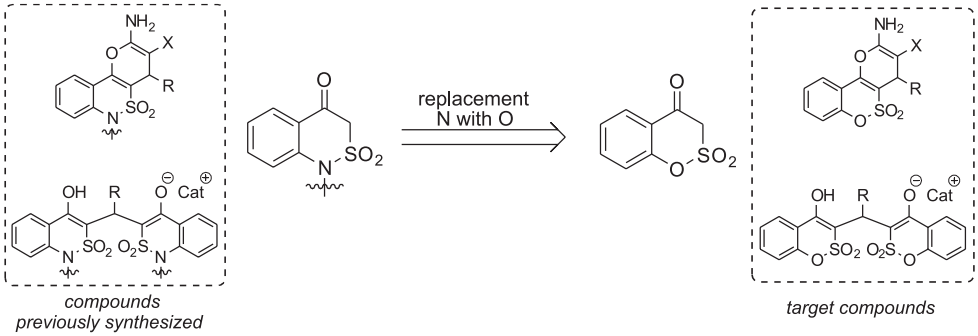
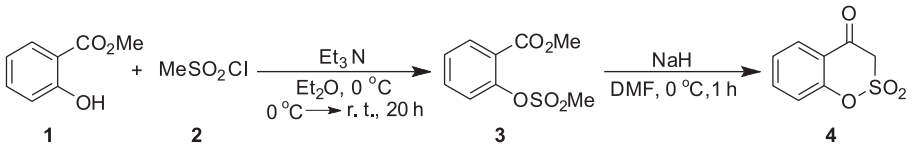


Fig. The isosteric relationships of 1*H*-2,1-benzothiazin-4-one 2,2-dioxide and 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide



Scheme 1. The synthesis of the starting 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4**

riles and triethylammonium 3-[1-(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)alkyl]-1,2-benzoxathiin-4-olate 2,2-dioxides based on 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide.

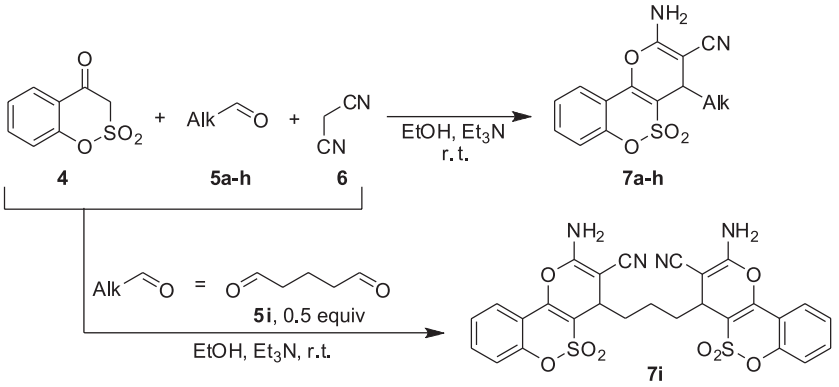
The starting 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** was synthesized according to Scheme 1. At the first stage methyl salicylate **1** reacted with methanesulfonyl chloride **2** yielding methanesulfonate **3**. The latter was next cyclized under the action of sodium hydride in DMF solution at 0 °C producing sultone **4** [10, 11].

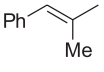
It is well known that 2-amino-4*H*-pyrans can be easily obtained *via* three-component domino-type interaction of 1,3-dicarbonyl compounds with aldehydes and active methylene nitriles using a wide range of bases as a catalyst [12]. Since 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** can be considered as a structural analog of 1,3-dicarbonyl compounds, according to the task set it was introduced into the three-component interaction with aliphatic aldehydes **5** and malononitrile **6** (Scheme 2) which resulted in formation of the target 2-amino-4-alkyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides **7** as precipitates that did not require further purification. The reaction readily proceeded when using equimolar quantities of the initial reagents in ethanol in the presence of the catalytic amount of triethylamine to promote the interaction. As it was additionally determined, this three-component reaction did not require heating and proceeded smoothly under the room temperature. It is in a full agreement with the previously regularities found [8]. Performing the reaction under reflux affected neither the product obtained nor the yield.

The yields of 2-amino-4*H*-pyrans **7** (Tab. 1) in the range from formaldehyde to butyraldehyde were average. However, in the case of valeric aldehyde the yield of **7e** turned out to be poor – only approximately 10 %. The solvent replacement of ethanol to methanol gave its increase up to 45 %, which might be due to lower solubility of the product. It is interesting that the fused derivative **7h** was obtained for α-methylcinnamaldehyde, whereas despite of our efforts, involvement of cinnamaldehyde often used in the 2-amino-4*H*-pyrans synthesis [13] was not successful. Presumably, the α-methylgroup in this case may act as a steric hindrance and avoids formation of undesirable by-products.

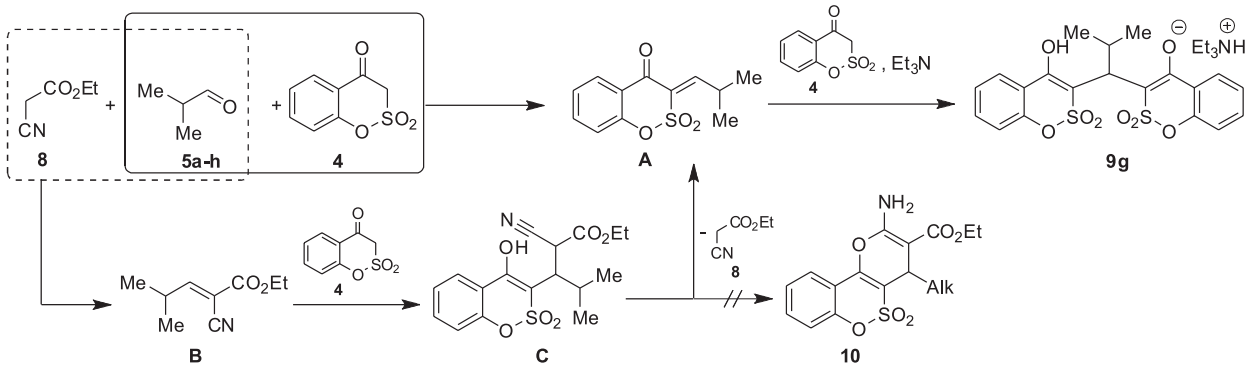
In the three-component reaction glutaraldehyde **5i** as a bifunctional representative was also introduced. This gives a chance to obtain two types of 2-amino-4*H*-pyrans depending on the number of aldehyde equivalents applying in the reaction, namely 2-amino-4*H*-pyran with a free aldehyde group and the corresponding bis-derivative of 2-amino-4*H*-pyran. Thus, using glutaraldehyde in the amount of 1 equiv in the three-component interaction did not result in the desired reaction product. At the same time, application of 0.5 equiv of **5i** led to isolation of bis-2-amino-4*H*-pyran **7i**, but unfortunately, in an extremely poor yield (Scheme 2).

In continuation of the current study we then applied other possible representative of active methylene nitrile – ethyl cyanoacetate **8** – in the three-component interaction with a view to introduce the ester group in position 3 of the 2-amino-4*H*-pyran ring. Nevertheless our efforts appeared to be unsuccessful since any desired ethyl 2-amino-4*H*-pyran-3-carboxylate was not isolated during these attempts. At the same time, the corresponding triethylammonium salt of bis-1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide derivative **9g** was isolated in 6% yield as the single



5a Alk = H; **5b** Alk = Me; **5c** Alk = Et; **5d** Alk = *n*-Pr; **5e** Alk = *n*-Bu; **5f** Alk = *i*-Pr; **5g** Alk = *i*-Bu; **5h** Alk = 

Scheme 2. The synthesis of 2-amino-4*H*-pyran-3-carbonitriles **7a-i**



Scheme 3. Two possible ways towards triethylammonium salt **9g**

product when isobutyric aldehyde was applied in the three-component interaction (Scheme 3). Taking into account the known mechanism of 2-amino-4*H*-pyran formation [14] and the regularities previously found [8] this fact can be explained by two ways depicted in Scheme 3. The first way implies the direct interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** with aldehyde **5g** with formation of a highly reactive intermediate **A**. Then this intermediate reacts not with ethyl cyanoacetate **8**, but with the second molecule of **4** forming a symmetrical bis-derivative isolated in the form of triethylammonium salt **9g**. Besides this, we can assume the second way involving the yield is primary Knoevenagel condensation between ester **8** and aldehyde **5g** resulted in intermediate **B**. The subsequent Michael addition of the latter to 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** gives adduct **C**, which looses the molecule of ester **8** and is converted into enone **A**. Thereafter, enone **A** reacts as described above giving salt **9g**. In our opinion, both of these routes are equiprobable.

Triethylammonium salts similar to **9** are new derivatives of 1,2-benzoxathiine 2,2-dioxide. In this regard, we set the task of the purposeful obtaining of salts **9** based on the two-component approach described previously on the example of 1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide [15]. According to this procedure the target salts **9** were obtained by the interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** with aliphatic aldehydes in the molar ratio of 2:1 in the presence of the equimolar amount of triethylamine in *i*-PrOH for 1 hour (Scheme 4).

The data for the compounds obtained are presented in Tab. 1 and 2. The ^{1}H NMR data are given in Tab. 3.

The study of the antimicrobial properties of the compounds obtained was performed according to the international standards, 16] by the agar "well" diffusion method against the standard test-strains of gram-positive and gram-negative bacteria and fungi. The results revealed the higher antimicrobial activity than those of the reference drugs. The activity against the gram-positive strains was a little higher than moderate compared to gram-negative bacteria and fungi. The most active were samples with propyl, isopropyl and butyl substituents in position 4 of the pyran core, the activity increased along with the prolongation of the chain. Furthermore, triethylammonium salts corresponding to 2-amino-4*H*-pyran-3-carbonitriles showed higher antimicrobial properties. Thereby, utilization of long-chain aliphatic aldehydes along with the synthesis of the corresponding triethylammonium salts may be considered as a promising way for further construction of the narrow spectrum antibiotics.

For similar derivatives of 1*H*-2,1-benzothiazin-4(3*H*)-one 2,2-dioxide the moderate activity against *P. aeruginosa* and *C. albicans* was revealed [8]. Thus, the isosteric replacement of the 1-N-R-group to O-atom caused alteration of the antimicrobial activity.

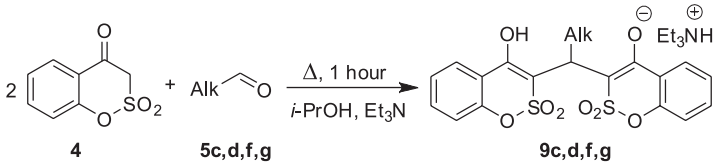
The results of studying the antimicrobial properties are presented in Tab. 4.

Experimental Part

Chemistry

The 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.

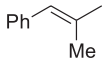
The procedure for the synthesis of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide 4. To the ethereal solution (200 mL) of methylsalicylate **1** (25.8 mL, 0.2 mol) and triethylamine (31 mL, 0.22 mol) add methane-



Scheme 4. The synthesis of triethylammonium salts **9c,d,f,g**

Table 1

Melting points, elemental analysis and yields for 2-amino-4-alkyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides **(7a-i)**

No.	Alk	M. p., °C	Molecular formula, M. m.	Elemental analysis, % calc / exp			Yield, %
				C	H	N	
7a	H	258-260	$C_{12}H_8N_2O_4S$ 276.27	52.15 / 52.17	2.89 / 2.92	10.10 / 10.14	55
7b	Me	241-243	$C_{13}H_{10}N_2O_4S$ 290.29	53.75 / 53.79	3.44 / 3.47	9.61 / 9.65	36
7c	Et	217-219	$C_{14}H_{12}N_2O_4S$ 304.32	55.21 / 55.25	3.95 / 3.97	9.18 / 9.21	48
7d	*n*-Pr	208-209	$C_{15}H_{14}N_2O_4S$ 318.35	56.55 / 56.59	4.41 / 4.43	8.76 / 8.80	75
7e	*n*-Bu	222-224	$C_{16}H_{16}N_2O_4S$ 332.37	57.79 / 57.82	4.83 / 4.85	8.41 / 8.43	45
7f	*i*-Pr	201-203	$C_{15}H_{14}N_2O_4S$ 318.35	56.55 / 56.59	4.41 / 4.43	8.76 / 8.80	46
7g	*i*-Bu	205-207	$C_{16}H_{16}N_2O_4S$ 332.37	57.79 / 57.82	4.81 / 4.85	8.41 / 8.43	39
7h		230-233	$C_{21}H_{16}N_2O_4S$ 392.43	64.24 / 64.27	4.08 / 4.11	7.11 / 7.14	46
7i	$(CH_2)_3$	257-259	$C_{27}H_{20}N_4O_8S_2$ 592.60	54.70 / 54.72	3.38 / 3.40	9.42 / 9.45	6

sulfonyl chloride **2** (15.4 mL, 0.22 mol). Stir the reaction mixture for 18 h when cooling. Then wash it with the saturated solution of sodium carbonate (50 mL) and water (100 mL). Evaporate the ethereal layer after drying with sodium sulfate to give a light yellow solid; the yield is 39.0 g (85 %). M. p. – 45-50 °C.

Dissolve methansulfonate **3** (39.0 g, 0.17 mol) in dry DMF (100 mL), and cool the solution to 0 °C. Then add it to the cooled suspension of sodium hydride (19.55 g, 0.51 mol) in dry DMF (100 mL) and mix for 1 h at 0-5 °C. Pour the reaction mixture into ice, acidify with diluted HCl to pH 3-4, filter the white precipitate obtained and recrystallize from ethanol; the yield is 22.0 g (67 %). M. p. – 80-85 °C.

The general procedure for the synthesis of 2-amino-4-alkyl-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides (7a-h). To the solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** (0.198 g, 0.001 mol), malononitrile **6** (0.066 g, 0.001 mol) and the appropriate aliphatic aldehyde **5a-h** (0.001 mol) in ethanol (5-10 mL) add the catalytic amount of triethylamine. Allow the mixture to stand for 24 h at the room temperature. Filter the resulting precipitates of **7a-h**, wash with ethanol and then dry on air.

Table 2

Melting points, elemental analysis and yields for triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)alkyl]-2,1-benzoxathiin-5-olat 2,2-dioxides **(5c,d,f,g)**

No.	Alk	M. p., °C	Molecular formula, M. m.	Analysis, % calc / exp			Yield, %
				C	H	N	
9c	Et	151-153	$C_{25}H_{31}NO_8S_2$ 537.65	55.81 / 55.85	5.75 / 5.81	2,57 / 2.61	37
9d	*n*-Pr	130-132	$C_{26}H_{33}NO_8S_2$ 551.67	56.57 / 56.61	6.01 / 6.03	2.51 / 2.54	45
9f	*i*-Pr	188-190	$C_{26}H_{33}NO_8S_2$ 551.67	56.58 / 56.61	6.01 / 6.03	2.49 / 2.54	69
9g	*i*-Bu	153-155	$C_{27}H_{35}NO_8S_2$ 565.70	57.31 / 57.33	6.21 / 6.24	2.45 / 2.48	45

Table 3

The ^{1}H NMR spectral data (δ, ppm; *J*, Hz) of the compounds obtained

7a	7.78 (d, *J* = 7.93 Гц, 1H, Ar); 7.60-7.70 (m, 1H, Ar); 7.46-7.57 (m, 2H, Ar); 7.35 (br. s., 2H, NH_2); 3.33 (s, 2H, CH_2 pyran)
7b	7.77-7.82 (m, 1H, Ar); 7.61-7.73 (m, 1H, Ar); 7.45-7.55 (m, 2H, Ar); 7.32 (s, 2H, NH_2); 3.59 (3.59 (q, *J* = 6.70 Hz, 1H, CH pyran); 1.37 (d, *J* = 6.26 Hz, 3H, CH_3)
7c	7.76-7.91 (m, 1H, Ar); 7.62-7.72 (m, 1H, Ar); 7.44-7.56 (m, 2H, Ar); 7.37 (br. s., 2H, NH_2); 3.69 (t, *J* = 3.91 Hz, 1H, CH pyran); 1.69 (dt, *J* = 7.43, 3.72 Hz, 2H, CH_2CH_3); 0.83 (t, *J* = 7.43 Hz, 3H, CH_2CH_3)
7d	7.79 (d, *J* = 7.83 Hz, 1H, Ar); 7.61-7.71 (m, 1H, Ar); 7.44-7.55 (m, 2H, Ar); 7.36 (br. s., 2H, NH_2); 3.64 (t, *J* = 4.11 Hz, 1H, CH pyran); 1.57-1.72 (m, 2H, $CH_2CH_2CH_3$); 1.20-1.37 (m, 2H, $CH_2CH_2CH_3$); 0.86 (t, *J* = 1.00 Hz, 3H, $CH_2CH_2CH_3$)
7e	7.79 (d, *J* = 7.83 Hz, 1H, Ar); 7.61-7.70 (m, 1H, Ar); 7.46-7.55 (m, 2H, Ar); 7.36 (br. s., 2H, NH_2); 3.66 (t, *J* = 1.00 Hz, 1H, CH pyran); 1.66 (m, 2H, $CH_2CH_2CH_2CH_3$); 1.25 (m, 4H, $CH_2CH_2CH_2CH_3$); 0.81 (t, *J* = 1.00 Hz, 3H, $CH_2CH_2CH_2CH_3$)
7f	7.81 (d, *J* = 7.83 Hz, 1H, Ar); 7.66 (d, *J* = 8.22 Hz, 1H, Ar); 7.49-7.57 (m, 2H, Ar); 7.45 (s, 2H, NH_2); 3.50 (d, *J* = 2.74 Hz, 1H, CH pyran); 2.04 (d, *J* = 2.35 Hz, 1H, $CH(CH_3)_2$); 1.01 (d, *J* = 7.04 Hz, 3H, $CH(CH_3)_2$); 0.77 (d, *J* = 7.04 Hz, 3H, $CH(CH_3)_2$)
7g	7.81 (d, *J* = 7.83 Hz, 1H, Ar); 7.59-7.72 (m, 1H, Ar); 7.46-7.55 (m, 2H, Ar); 7.39 (s, 2H, NH_2); 3.55 (t, *J* = 5.87 Hz, 1H, CH pyran); 1.87 (m, 1H, $CH_2CH(CH_3)_2$); 1.56 (t, *J* = 6.26 Hz, 2H, $CH_2CH(CH_3)_2$); 0.91 (d, *J* = 6.26 Hz, 3H, $CH_2CH(CH_3)_2$); 0.85 (d, *J* = 6.65 Hz, 3H, $CH_2CH(CH_3)_2$)
7h	7.82 (d, *J* = 7.43 Hz, 1H, Ar); 7.64-7.72 (m, 1H, Ar); 7.43-7.57 (m, 4H, Ar, NH_2); 7.30-7.37 (m, 2H, Ar); 7.21-7.29 (m, 3H, Ar);6.55 (s, 1H, CH); 4.32 (s, 1H, CH pyran); 1.76 (s, 3H, CH_3)
7i	7.59-7.72 (m, 4H, Ar); 7.40-7.48 (m, 4H, Ar); 7.30-7.35 (m, 4H, NH_2, NH_2); 3.62 (t, *J* = 1.00 Hz, 2H, CH, CH); 1.59-1.74 (m, 6H, $(CH_2)_3$)
9c	17.61 (br. s., 1H, OH); 7.86 (d, *J* = 7.63 Hz, 2H, Ar); 7.41-7.52 (m, 2H, Ar); 7.26-7.34 (m, 2H, Ar); 7.22 (d, *J* = 8.24 Hz, 2H, Ar); 4.00 (t, *J* = 8.24 Hz, 1H, CH); 3.02 (q, *J* = 7.12 Hz, 6H, $N(CH_2CH_3)_3$); 2.17 (quin, *J* = 7.48 Hz, 2H, CH_2CH_3); 1.12 (t, *J* = 7.32 Hz, 9H, $N(CH_2CH_3)_3$); 0.83 (t, *J* = 7.17 Hz, 3H, CH_2CH_3)
9d	17.60 (br. s., 1H, OH); 7.85 (d, *J* = 7.63 Hz, 2H, Ar); 7.39-7.50 (m, 2H, Ar); 7.26-7.35 (m, 2H, Ar); 7.21 (d, *J* = 7.93 Hz, 2H, Ar); 4.12 (t, *J* = 7.93 Hz, 1H, CH); 3.04 (q, *J* = 7.32 Hz, 6H, $N(CH_2CH_3)_3$); 2.13 (q, *J* = 7.83 Hz, 2H, $CH_2CH_2CH_3$); 1.19-1.30 (m, 2H, $CH_2CH_2CH_3$); 1.13 (t, *J* = 7.32 Hz, 9H, $N(CH_2CH_3)_3$); 0.83 (t, *J* = 7.32 Hz, 3H, $CH_2CH_2CH_3$)
9f	17.67 (s, 1H, OH); 7.84 (d, *J* = 7.63 Hz, 2H, Ar); 7.40-7.51 (m, 2H, Ar); 7.29 (t, *J* = 7.17 Hz, 2H, Ar); 7.21 (d, *J* = 7.93 Hz, 2H, Ar); 3.63 (d, *J* = 10.99 Hz, 1H, CH); 3.05 (q, *J* = 7.32 Hz, 7H, $CH(CH_3)_2$, $N(CH_2CH_3)_3$); 1.13 (t, *J* = 7.32 Hz, 9H, $N(CH_2CH_3)_3$); 0.89 (d, *J* = 6.41 Hz, 6H, $CH(CH_3)_2$)
9g	17.54 (br. s., 1H, OH); 7.86 (d, *J* = 7.32 Hz, 2H, Ar); 7.42-7.49 (m, 2H, Ar); 7.30 (t, *J* = 7.63 Hz, 2H, Ar); 7.21 (d, *J* = 8.24 Hz, 2H, Ar); 4.23 (t, *J* = 7.93 Hz, 1H, CH); 3.03 (q, *J* = 7.12 Hz, 6H, $N(CH_2CH_3)_3$); 2.04 (t, *J* = 7.32 Hz, 2H, $CH_2CH(CH_3)_2$); 1.39-1.49 (m, 1H, $CH_2CH(CH_3)_2$); 1.12 (t, J = 7.17 Hz, 9H, $N(CH_2CH_3)_3$); 0.85 (d, *J* = 6.41 Hz, 6H, $CH_2CH(CH_3)_2$)

The procedure for the synthesis of 1,3-bis(2-amino-4,6-dihydropyrano[3,2-*c*][2,1]benzoxathiine-3-carbonitrile-4-yl 5,5-dioxide)propan (7i). To the solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** (0.198 g, 0.001 mol), malononitrile **6** (0.066 g, 0.001 mol) and 50 % solution of glutaraldehyde **5i** (0.100 g, 0.0005 mol) in ethanol (5-10 mL) add the catalytic amount of triethylamine. Allow the mixture to stand for 24 h at the room temperature. Filter the resulting precipitate of **7i**, wash with ethanol and then dry on air.

The general procedure for the synthesis of triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)alkyl]-2,1-benzoxathiin-5-olat 2,2-dioxides (9c,d,f,g). To the solution of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide **4** (0.198 g, 0.001 mol) and the appropriate aliphatic aldehyde **3c,d,f,g** (0.0005 mol) in propan-2-ol (10 mL) add triethylamine (0.13 mL, 0.001 mol). Mix the solution for 1 h under reflux. After cooling filter the resulting precipitates of **9c,d,f,g**, wash with propan-2-ol and dry on air.

Microbiology

According to the WHO recommendations 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 suspen-

Table 4

The antimicrobial activity of compounds **7b**-**7h**, **9c**-**9g**

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
7b	13	13	15	growth	growth	16
7c	17	16	18	14	14	15
7d	**21**	19	**22**	15	16	15
7e	**21**	19	**23**	16	15	16
7f	18	18	**20**	17	16	17
7g	18	16	18	18	18	17
7h	16	17	19	17	16	16
9c	20	19	**21**	17	17	18
9d	18	18	**21**	16	17	18
9f	**21**	**20**	**22**	17	18	17
9g	19	19	**22**	18	18	18

sions" (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. The 18 to 24-hour old culture of the microorganism was 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 "wells" method. The antibacterial activity was assessed by measuring the inhibition zones of the corresponding microorganism and was compared to those for the reference antimicrobial drugs.

Conclusions

1. The series of 2-amino-4-alkyl-4,6-dihydropyrano [3,2-*c*][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides has been synthesized based on the three-component interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide with malononitrile and aliphatic aldehydes.

2. Using ethyl cyanoacetate in the same interaction did not lead to the desired ethyl 2-amino-4*H*-pyran-3-carboxylates and in the case of isobutyric aldehyde resulted in formation of triethylammonium salt. It is explained by two equiprobable reaction pathways.

3. By means of the two-component approach based on the interaction of 1,2-benzoxathiin-4(3*H*)-one 2,2-dioxide and aliphatic aldehydes the series of triethylammonium 3-[1-(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)alkyl]-1,2-benzoxathiin-4-olate 2,2-dioxides has been synthesized.

4. The assessment of the antimicrobial properties of the compounds obtained has revealed the higher activity than those of the reference drugs, especially against the gram-positive strains, and the activity against gram-negative bacteria and fungi is slightly less.

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