CYCLOALKANECARBALDEHYDES IN SYNTHESIS OF NOVEL 1,2-BENZOXATHIIN-4(3H)-ON 2,2-DIOXIDE DERIVATIVES AND STUDY OF THE ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS

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1. Introduction

Nowadays the variety of organic compounds is absolutely tremendous. In 2015 the world’s largest database of unique chemical substances CAS registered the 100 millionth chemical substance [1], and thousands of new molecules are still generated by scientists all over the world each year. Among this number the compounds with prospective biological properties are of the greatest interest, because they usually give rise to the development of novel medicines. 2-Amino-4H-pyrans, that were for the first time synthesized nearly 60 years ago, can be considered as such group, because of the known antimicrobial [2] and antitumor [3] activities for this class of compounds through applying of wide range of initial components. 2-Amino-4H-pyran represents the three-component reaction of active methylene nitriles A, carbonyl compounds B and enolnucleophiles C (Fig. 1) and includes the Thorpe-Ziegler domino type reaction. According to the recent literature data such interaction proceeds perfectly as multicomponent reaction, when adducts D are generated in situ from the mixture of starting compounds. Formation of D and heterocyclization processes occurs sequentially and is considered as Knoevenagel-Michael-hetero-Thorpe-Ziegler domino type reaction.

Fig. 1. The general route towards 2-amino-4H-pyrans synthesis
Among carbonyl compounds cycloalkanecarbaldehydes were not previously widely used in such interactions type. Utilization of latter along with 1,2-benzoazathiin-3(4H)-on 2,2-dioxide as enolnucleophile gives the possibility to obtain novel condensed 2-amino-4H-pyrans, the evaluation of the antimicrobial activity of which are of the greatest interest due to the current necessity of the search of new core-structures with such properties.

3. Analysis of recent studies and publications in which a solution of the problem is found and which draws on the author

The structural diversity of 2-amino-4H-pyrans depends on the initial compounds that are used in their synthesis: active methylene nitriles, carbonyl compounds and enolnucleophiles.

The most well-known active methylene nitriles applied in the interaction described above are malononitrile A1 and esters of cyanoacetic acid A2. Cyanacetamide A3 and thiocyanacetamide A4 are not so widely used. As for carbonyl compounds they are represented with different types of aldehydes, the most common of which are aromatic B1 and hetarencarbaldehydes B2. In the case of ketones (isatins B5 and ningidrin B6) the products of the interaction are spirocondensed compounds. Enolnucleophiles is the widest group in the 2-amino-4H-pyrans synthesis and is represented with diketones C1, ketoesters C2-C4, cyanoketones C5-C6, nitroketones C7 and many other related compounds that are shown on Fig. 2.

![Fig. 2. The variety of components in 2-amino-4H-pyrans synthesis](image-url)

Corresponding multicomponent interactions generally proceed under reflux in polar solvents medium in the presence of basic catalysts, such as triethylamine, morpholine, piperidine [4].

Current investigations in this synthetic area are mainly focused on the selection of new reaction conditions and catalysts, and to search of some novel suitable components, that could replace well-known ones in order to expand the existing 2-amino-4H-pyrans diversity.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

As it was mentioned above, the huge variety of enolnucleophiles have been already used in multicomponent 2-amino-4H-pyrans synthesis. That is why searching of some new compounds of this group is challenging and interesting at the same time.

Previously we reported the utilization of 1H-2,1-benzothiazin-4-on 2,2-dioxide in 2-amino-4H-pyran synthesis [5, 6]. This “newcomer” turned out to be the prospective core structure for such interactions due to the presence of SO₂CH₂CO moiety. Moreover the series of obtained compounds were screened for antibacterial, antifungal, analgesic and anti-inflammatory activities [7], and among them some promising substances with moderate to high levels of them were found.

As the next consequential step of the research we drew our attention to the other almost unknown enolnucleophile – 1,2-benzoazathiin-4(3H)-on 2,2-dioxide, that is an isostere of 1H-2,1-benzothiazin-4-on 2,2-dioxide. This approach gave us the opportunity not only to compare the chemical properties of such close related compounds, but also to investigate the structure/bioactivity relationships for the obtained substances.
We also decided to use cycloalkanecarbaldehydes as a carbonyl component for the 2-amino-4H-pyran synthesis, because this group was not broadly employed in such interactions before.

5. Formulation of goals (tasks) of the article

According to the information given above we aimed to synthesize 2-amino-4H-pyran with the use of cycloalkanecarbaldehydes, 1,2-benzoxathiin-4(3H)-on 2,2-dioxide and active methylene nitriles three-component interaction, to determine the most suitable reaction conditions and to evaluate the antimicrobial properties of the obtained compounds.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

Considering the previous research of the interaction of 1H-2,1-benzothiazin-4-on 2,2-dioxide with active methylene nitriles and cycloalkanecarbaldehydes [6] we began our investigation from the studying of the reaction of 1,2-benzoxathiin-4(3H)-one 2,2-dioxide I with malononitrile 2 and cyclopropanecarbaldehyde 3a. It was discovered, that as in the previous case, the reaction proceeded under room temperature in ethanol with the presence of catalytic amount of triethylamine and resulted in the formation of 2-amino-4-cyclopentyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide 4a in 55% yield (Scheme 2). The same reaction conditions were successfully applied for cyclopentane- and cyclohexanecarbaldehydes 3b and 3c respectively.

Inspired by such encouraging results we continued the research and replaced malononitrile with ethyl cyanoacetate, which is also often used in such interactions type. The reaction was carried out with equimolar quantities of all reagents in the same conditions as previous one, but unfortunately gave no rise to the desired products. We did not succeed in isolation of any product at all in this case. Simultaneously the performance of the reaction at 60–70 °C in the case of aldehyde 3e allowed us to isolate a crystalline powder that appeared to be the triethylammonium 3-[(4-hydroxy-2,2-dioxido-2,1-benzoxathiin-3-yl)(cyclohexane)methyl]-2,1-benzoxathiin-5-olato 2,2-dioxide 5e.

This unexpected result can be explained by two ways (Fig. 3). The first one comprises the formation of Michael adduct F which losses the molecule of ethyl cyanoacetate and is converted into enone G, that gives triethylammonium salt according to what was reported previously [6]. The second possible way involves the formation of enone G by direct interaction of 1,2-benzoxathiin-4(3H)-on 2,2-dioxide I with aldehyde 3c, that next reacts with the second molecule of I forming symmetrical bis-derivative isolated as triethylammonium salt 5c. On the current stage of the research these two ways can be considered as equiprobable and further investigations are needed to disclose finally the mechanism of salts 5 formation via applying of multicomponent format of the reaction.

Since no traces of triethylammonium salts were found for three-component reaction of 1,2-benzoxathiin-4(3H)-on 2,2-dioxide with malononitrile and cycloalkanecarbaldehydes, we can assume that 2-amino-4H-pyran-3-carbonitriles 4 are more stable in the applied conditions than the corresponding salts 5. Considering the possibility of the enones G to be formed into the reaction mixture we tried to convert triethylammonium salt 5e into 2-amino-3-carbonitrile-4H-pyran 4c by treating the former with malononitrile. In spite of this the attempt appeared to be unsuccessful and only initial material was recovered after the reaction. In this way, we can suppose that such triethylammonium salts 5 are rather stable compounds and the \textit{retro}-Michael cleavage reaction with formation of the enones G is not typical for them.

Formation of such pharmacoologically interesting compounds type as triethylammonium salts and previously reported results devoted to the two-component

\[\text{Fig. 3. Synthesis of 2-amino-4-cycloalkyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides}\]
reaction of 1H-2,1-benzothiazin-4-on 2,2-dioxide with cycloalkanecarbaldehydes [6] encouraged us to continue these investigations and to study similar interaction for 1,2-benzoxathiin-4(3H)-on 2,2-dioxide.

So the reaction between 1,2-benzoathiin-4(3H)-on 2,2-dioxide 1 and appropriate cycloalkanecarbaldehydes 3a-c was carried out applying conditions found before [6] and the solution of starting compounds in i-PrOH was mixed for 1 h at 60-70°C in the presence of equivolmar quantity of triethylamine. The obtained white crystalline substances appeared to be the expected triethylammonium 3a-[(4-triethylamine. The obtained white crystalline substances was isolated for aldehyde 3c in the case of piperidine, morpholine, 1,2,3,4-tetrahydroisoquinoline and dimethylamine (Fig. 5).

The reaction proceeded under gently heating and mixing for 1 h in i-PrOH with equivolmar quantity of the corresponding amine.

![Fig. 4. The formation of triethylammonium salts 5a-c](image)

![Fig. 5. Synthesis of the ammonium salts 6c, 7c, 8c, 9c](image)

The antimicrobial properties of the obtained compounds was studied according to the international standards [8, 9] by the agar “well” diffusion method against the standard test-strains of gram-positive and gram-negative bacteria and fungi. The results revealed higher antimicrobial activity than for the reference drugs against S. aureus and E.coli. The compound 4e appeared to be the most active among all tested samples. The diameters of its growth inhibition zones were 21 mm for S. aureus, 19 mm for E. coli, 17 mm for C. albicans and 16 mm for B. subtilis, P. aeruginosa and P. vulgaris. The results for the reference drugs Synthomycine and Metronidazole did not exceed 17 mm.

There was no significant distinction of antimicrobial properties of triethylammonium salts corresponding to 2-amino-4H-pyran 4 synthesis (Fig. 4).

In regard to expand the range of such ammonium salts we also examined the possibility of secondary amines utilization in this reaction. The desired products 6c, 7c, 8c, 9c were isolated for aldehyde 3c in the case of piperdine, morpholine, 1,2,3,4-tetrahydroisoquinoline and dimethylamine (Fig. 5).

The screening of antimicrobial activities of the obtained substances revealed generally higher activity than for the reference drugs against the gram-positive strains. The 2-amino-4-cyclohexyl-4,6-dihydropyran 6c as the lead compound may be proposed for further investigations in this area.

### 7. Findings from the research and prospects of further development of this area

Series of 2-amino-4-cycloalkyl-4,6-dihydropyranosynthese 6(10)2017 1108 analyzer.

In 2-amino-4-cycloalkyl-4,6-dihydropyrano [3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides and ammonium salts were synthesized based on cycloalkanecarbaldehydes and 1,2-benzoxathiin-3(4H)-on 2,2-dioxide. The screening of antimicrobial properties of the obtained substances revealed generally higher activity than for the reference drugs against the gram-positive strains. The 2-amino-4-cyclohexyl-4,6-dihydropyranosynthese 6(10)2017 1108 analyzer.

Experimental chemical part

Initial 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. 1H NMR spectra were recorded on Varian WXR-400 spectrometer using DMSO-d6 as solvent and TMS as an internal standard. Elemental analyses were carried out using Carlo Erba CHNS-O EA 1108 analyzer.
General procedure for the synthesis of 2-amino-4-cycloalkyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-dioxides (4a-c)

To a solution of 1,2-benzoxathiin-4(3H)-one 2,2-dioxide 1 (0.198 g, 0.001 mol), malononitrile 2 (0.066 g, 0.001 mol) and appropriate alicyclic aldehyde 3a-c (0.001 mol) in ethanol (5-10 mL), catalytic amount of triethylamine was added. The mixture stayed for 24 h at room temperature. In the cases of 3a, c, the products 4a, 4c were obtained; the products 4b were filtered off, washed with ethanol and dried on air. For 4b the solvent was evaporated under reduced pressure and the residue was treated with methanol producing light yellow crystalline powder of 4b.

2-Amino-4-cyclopropyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4a)

Yellow powder; mp 255-258°C (from EtOH); Anal. Calcld: for C_{17}H_{16}N_{2}O_{4}S, %: C 56.95; H 3.82; N 8.86. Found, %: C 56.91; H 3.79; N 8.84. 1H NMR (400 MHz, DMSO-d6): δ (m.n.) 7.97 (d, J = 7.83 Hz, 1H, Ar); 7.60-7.70 (t, 1H, Ar); 7.44-7.55 (m, 2H, Ar); 7.32 (s, 2H, NH_{2}); 2.89 (d, J = 9.00 Hz, 1H, CH); 0.97-1.11 (m, 1H, CH); 0.36-0.62 (m, 4H, CH$_2$ cyclopropane).

2-Amino-4-cyclopropyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4b)

Light yellow crystalline powder; mp > 250°C (from EtOH); Anal. Calcld: for C_{17}H_{16}N_{2}O_{4}S, %: C 59.29; H 4.68; N 8.13. Found, %: C 59.25; H 4.63; N 8.09. 1H NMR (400 MHz, DMSO-d6): δ (m.n.) 7.81 (d, J = 7.63 Hz, 1H, Ar); 7.62-7.72 (m, 1H, Ar); 7.49-7.57 (m, 2H, Ar); 7.43 (s, 2H, NH$_2$); 3.63 (d, J = 3.66 Hz, 1H, CH); 2.27 (br. s., 1H, CH); 1.36-1.77 (m, 8H, CH$_2$ cyclopropyl).

2-Amino-4-cyclohexyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-dioxide (4c)

White crystalline powder; mp > 250°C (from EtOH); Anal. Calcld: for C_{28}H_{35}N_{2}O_{8}S, %: C 60.32; H 5.06; N 7.82. Found, %: C 60.29; H 5.01; N 7.78. 1H NMR (400 MHz, DMSO-d6): δ (m.n.) 7.80 (d, J = 7.83 Hz, 1H, Ar); 7.66 (d, J = 8.22 Hz, 1H, Ar); 7.47-7.57 (m, 2H, Ar); 7.43 (s, 2H, NH$_2$); 3.46 (s, 1H, CH); 1.77-2.80 (m, 11H, cyclohexyl).

General procedure for the synthesis of triethylammonium 3-[(4-hydroxy-2,2-dioxido-1,2-benzoxathiin-3-yl)cylohexyl]methyl]-1,12-benzoxathiin-5-olate 2,2-dioxide (6c, 7c, 8c, 9c)

To a solution of 1,2-benzoxathiin-4(3H)-one 2,2-dioxide 1 (0.198 g, 0.001 mol) and cyclohexanecarboxaldehyde 3c (0.0005 mol) in propanol-2 (10 mL) the equimolar amount of corresponding amine was added. The solution was mixed for 1 h at 60-70°C. The obtained precipitates of 6c, 7c, 8c, 9c were filtered off, washed with propanol-2 and dried on air.

Piperidinium 3-[(4-hydroxy-2,2-dioxido-1H,1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,12-benzoxathiin-5-olate 2,2-dioxide (6d, 7d, 8d, 9d)

White crystalline powder; mp 138-140°C (from EtOH); Anal. Calcld: for C_{26}H_{31}N_{2}O_{8}S, %: C 58.21; H 6.11; N 2.42. Found, %: C 58.18; H 6.07; N 2.38. 1H NMR (400 MHz, DMSO-d6): δ (m.n.) 17.75 (s, 1H, OH); 7.84 (d, J = 7.63 Hz, 2H, Ar); 7.39-7.49 (m, 2H, Ar); 7.29 (t, J = 7.48 Hz, 2H, Ar); 7.20 (d, J = 8.24 Hz, 2H, Ar); 3.78 (d, J = 11.29 Hz, 1H, CH); 3.44 (br. s., 1H, CH$_2$ cyclohexyl); 2.98-3.11 (m, 6H, N(CH$_2$CH$_3$)$_3$); 1.22-1.64 (m, 8H, CH$_2$ cyclohexyl); 1.14 (t, J = 6.41 Hz, 9H, N(CH$_2$CH$_3$)$_3$).

Morpholinium 3-[(4-hydroxy-2,2-dioxido-1H,1,2-benzoxathiin-3-yl)(cyclohexyl)methyl]-1,12-benzoxathiin-5-olate 2,2-dioxide (6e, 7e, 8e, 9e)

White crystalline powder; mp 128-130°C (from EtOH); Anal. Calcld: for C_{38}H_{39}NOS, %: C 56.14; H 5.41; N 2.42. Found, %: C 56.12; H 5.38; N 2.41. 1H NMR (400 MHz, DMSO-d6): δ (m.n.) 17.63-17.70 (m, 11H, CH$_2$ cyclohexyl); 7.82 (d, J = 6.41 Hz, 2H, Ar); 7.40-7.49 (m, 2H, Ar); 7.29 (s, 2H, Ar); 7.21 (d, J = 7.93 Hz, 2H, Ar); 4.33 (d, J = 1.00 Hz, 1H, CH); 3.66-3.77 (m, 4H, CH$_2$ cyclohexyl).
The compounds were introduced into agar by the "wells" method [8]. The antibacterial activity was evaluated by measuring zones of inhibition of the corresponding microorganism and was compared with those for reference antimicrobial drugs.

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IDENTIFICATION AND QUANTITATIVE DETERMINATION OF STEROIDAL COMPOUNDS IN THE PLANT MATERIAL OF CABBAGE

M. Kuznetsova, O. Kyslychenko, I. Zhuravel

The study of crops as sources of medicinal preparations obtaining on their basis is actual for pharmacy nowadays. Cabbage (Brassica oleracea L.) is a member of the family Brassicaceae (or Cruciferae). In Ukraine it is grown as a vegetable culture, which has a sufficient raw material base and a big number of varieties. The complex of compounds contained in cabbage gives it many pharmacotherapeutic properties.

In folk medicine of the West and the East cabbage has long been used against various diseases. The leaves, roots, stumps, and seeds of the plant are use [1]. Cabbage juice is prescribed for gastritis and peptic ulcer of the stomach and duodenum, in ulcerative colitis, as well as in a mixture with honey in lung tuberculosis, in liver disorder. Roots and stumps are considered as an antitumor agent [2, 3]. The decoction of the seeds is used for gout, pain in the joints, as an antiseptic and a diuretic. Folk medicine recommends that fresh leaves of cabbage can be applied to purulent wounds and ulcers, to the mamma glands during mastopathy. Cabbage leaves are also be applied to purulent wounds and ulcers, to the mamma glands during mastopathy.}

1. Introduction

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