The synthesis of 2-amino-4-aryl-4H-pyran[3,2-c][1,2]benzoxathiine-3-carbonitrile 5,5-dioxides and the study of their effect on the blood coagulation process

To date, coumaric oral anticoagulants are the worldwide standard for thrombosis treatment. However, representatives of this group also possess a number of undesirable side effects; therefore, the search for novel anticoagulants are still in progress.

**Aim.** To synthesize 2-amino-4-aryl-4H-pyran[3,2-c][1,2]benzoxathiine-3-carbonitrile 5,5-dioxides and study their effect on the blood coagulation process.

**Results and discussion.** Reflux of equimolar quantities of 1,2-benzoxathiin-4(3H)-one 2,2-dioxide with malononitrile and arencarbaldehydes for 1 h in ethanol with the catalytic amount of triethylamine led to formation of 2-amino-4-aryl-4H-pyran[3,2-c][1,2]benzoxathiine-3-carbonitrile 5,5-dioxides. A wide range of substituted aromatic aldehydes was used for further study of the "structure – biological activity" relationship. Among the compounds synthesized substances with anticoagulant and hemostatic properties were found.

**Experimental part.** A series of 2-amino-4-aryl-4H-pyran[3,2-c][1,2]benzoxathiine-3-carbonitrile 5,5-dioxides was synthesized. The effect of the compounds obtained on the blood coagulation process was studied in vitro by the Burker method.

**Conclusions.** The target 2-amino-4-aryl-4H-pyran[3,2-c][1,2]benzoxathine-3-carbonitrile 5,5-dioxides can be easily obtained with moderate to high yields in the three-component interaction of 1,2-benzoxathin-4(3H)-one 2,2-dioxide, malononitrile and arencarbaldehydes. According to the in vitro studies both anticoagulant and hemostatic substances with relatively high levels of the activities were found among this novel heterocyclic group of compounds.

**Key words:** 1,2-benzoxathin-4(3H)-one 2,2-dioxide; aromatic aldehydes; malononitrile; 4H-pyran; anticoagulant activity; hemostatic activity
The process of creating new drugs is closely connected with the search of core-structures with the necessary pharmacological activity. However, the existing or novel core-structures usually cause undesirable side effects, have imperfect pharmacokinetic characteristics, and, therefore, they require further structural optimization. The synthetic modification of the primary structure of biologically active substances is aimed to increase its activity, improve selectivity and reduce toxicity. One of the powerful tools in this regard is the concept of isosterism [1].

For the first time it was formulated by I. Langmuir in 1919 [2]. Later G. Erlenmeyer gave the following definition for the term “isostere”: “atoms, ions, or molecules, in which the outer electron shells can be considered as identical” [3]. Comparing the properties of isosteres he considered not only their physical, chemical properties and reactivity, but also the biological activity.

As for the term “bioisosterism”, it was proposed in 1951 by G. Friedman [4], who named as bioisosteres all compounds (atoms and molecules) that satisfied the definition of isosteres and had the same biological activity. It is worth mentioning that G. Friedman noted that bioisosteres affected the same biological target, but assumed that their activity could be either similar or antagonistic.

Further development of the bioisosterism concept belongs to C. Hansch [5, 6], one of the founders of the QSAR (quantitative structure-activity relationships) methodology. He defined bioisosteres as “compounds causing an identical biochemical or pharmacological response in a standard system, such as a cell membrane, enzyme, receptor, experimental animal”.

In case of a bioisosteric replacement (i.e. changing of a structural part of a molecule with another one that is structurally close to it), one or more of the following molecule characteristics are changed: size, shape, hydrophobicity, solubility, pKₐ, reactivity, etc. These alterations give the possibility to increase the selectivity of the compound action, reduce its side effects, improve pharmacokinetic parameters, and the increase metabolic stability.

According to the above information 1,2-benzoxathiin-4(3H)-one 2,2-dioxide I can be considered as an isostere of such famous pharmacophore as 4-hydroxycoumarin core A (Fig.). The derivatives of the latter revealed a pronounced anticoagulant effect; they reduce blood coagulation by inhibiting vitamin K [7]. On their basis anticoagulant drugs for the treatment and prevention of thrombosis were created. Despite their undisputable effectiveness, coumaric oral anticoagulants have a narrow therapeutic index and are associated with a high risk of major bleeding [8]. Therefore, the task of searching for novel anticoagulants of the similar structure considering isosteric principles remains important.

Previously, we reported that the three-component interaction of 1,2-benzoxathiin-4(3H)-one 2,2-dioxide I with malononitrile 2 and benzaldehydes 3 led to formation of 2-amino-4-aryl-4H-pyrano[3,2-c][1,2]benzoxathiine-3-carbonitrile 5,5-dioxides 4a-e (Scheme) [9]. This interaction proceeded smoothly under reflux of equimolar quantities of reagents in ethanol for 1 h; it was also found that the most suitable catalyst for the reaction was triethylamine.

The aim of the current research was to synthesize a series of new 2-amino-4-aryl-4H-pyrano[3,2-c][1,2]...
benzoxathiine-3-carbonitrile 5,5-dioxides and study their
effect on the blood coagulation process.

Materials and methods

Experimental chemical part

The starting 1,2-benzoxathiin-4(3H)-one 2,2-dioxide 1 was synthesized according to the procedure [10].
The starting aromatic aldehydes and malononitrile 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 of the com-
ounds synthesized were recorded on a Varian WXR-400
spectrometer using DMSO-d6 as a solvent and TMS as
an internal standard. Elemental analysis was carried out
using a Carlo Erba CHNS-O EA 1108 analyzer.

The general procedure for the synthesis of 2-ami-
no-4-aryl-4H-pyrano[3,2-c][1,2]benzoxathiin-3-car-
bonitrile 5,5-dioxides (4f-m).

To the 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 the correspon-
ding aromatic aldehyde 3f-m (0.001 mol) in ethanol
(5-10 mL) add the catalytic amount of triethylamine.
Reflex the mixture for 1 h. Filter the precipitates of com-
ounds 4f-m obtained, wash with ethanol and then dry
on air.

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

Caled for C19H16N2O2S, %: C 55.89; H 2.87; N 7.24.
Found, %: C 55.71; H 3.03; N 7.46; 1H NMR (400 MHz,
DMSO-d6): δ (ppm) 7.87-7.94 (m, 2H, Ar); 7.65-7.73
(m, 1H, Ar); 7.55 (t, J = 7.78 Hz, 1H, Ar); 7.50 (d,
J = 8.24 Hz, 1H, Ar); 7.46 (br.s., 2H, NH2); 7.42 (s, 1H, Ar);
7.28-7.38 (m, 2H, Ar); 4.80 (s, 1H, CH).

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

A white fine-crystalline powder. M. p. – 258-260 °C
N 7.56. Found, %: C 58.26; H 2.87; N 7.41; 1H NMR
(400 MHz, DMSO-d6): δ (ppm) 7.88 (d, J = 7.32 Hz,
1H, Ar); 7.65-7.71 (m, 1H, Ar); 7.53-7.57 (m, 1H, Ar);
7.47-7.53 (m, 1H, Ar); 7.42 (br.s., 2H, NH2); 7.38 (dd,
J = 8.24, 5.49 Hz, 2H, Ar); 7.16 (t, J = 8.70 Hz, 2H, Ar)
4.76 (s, 1H, CH).

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

Caled for C21H17FN2O2S, %: C 58.37; H 2.99; N 7.56.
Found, %: C 58.25; H 3.08; N 7.41; 1H NMR (400 MHz,
DMSO-d6): δ (ppm) 7.88 (d, J = 7.63 Hz, 1H, Ar); 7.65-7.72
(m, 1H, Ar); 7.53-7.58 (m, 1H, Ar); 7.50 (d, J = 8.24 Hz,
1H, Ar); 7.45 (br.s., 2H, NH2); 7.38 (d, J = 6.10 Hz, 1H, Ar);
7.30 (d, J = 8.24 Hz, 1H, Ar); 7.16-7.24 (m, 1H, Ar);
7.10 (br.s., 1H, Ar); 4.79 (s, 1H, CH).

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

for C19H16O2N2S, %: C 58.69; H 3.28; N 7.60. Found, %:
C 58.81; H 3.12; N 7.82; 1H NMR (400 MHz, DMSO-d6):
δ (ppm) 9.14 (s, 1H, OH); 7.86 (d, J = 7.93 Hz, 1H, Ar);
7.64-7.71 (m, 1H, Ar); 7.51-7.56 (m, 1H, Ar); 7.49 (d,
J = 8.24 Hz, 1H, Ar); 7.31 (s, 2H, NH2); 7.09 (d, J = 8.55 Hz,
2H, Ar); 6.70 (d, J = 8.55 Hz, 2H, Ar); 4.57 (s, 1H, CH).

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

for C19H16O2N2S, %: C 58.69; H 3.28; N 7.60. Found, %:
C 58.53; H 3.41; N 7.44; 1H NMR (400 MHz, DMSO-d6):
δ (ppm) 9.40 (br.s., 1H, OH); 7.88 (d, J = 7.93 Hz, 1H, Ar);
7.63-7.72 (m, 1H, Ar); 7.47-7.57 (m, 2H, Ar); 7.31-7.44
(m, 2H, NH2); 7.05-7.19 (m, 2H, Ar); 6.59-6.78 (m, 2H, Ar);
4.59 (s, 1H, CH).

2-Amino-4-(4-trifluoromethylphenyl)-4,6-dihydropyrano-
[3,2-c][2,1]benzoxathiin-3-carbonitrile 5,5-di-
oxide (4k).

A white fibrous precipitate. M. p. > 250 °C
(EtOH). Anal. Caled for C22H10F3N2O2S, %: C 54.29;
H 2.64; N 6.67. Found, %: C 54.43; H 2.81; N 6.53;
1H NMR (400 MHz, DMSO-d6); δ (ppm) 7.90 (d, J = 8.55 Hz,
1H, Ar); 7.67-7.75 (m, 3H, Ar); 7.58 (d, J = 8.24 Hz,
2H, NH2); 7.47-7.53 (m, 4H, Ar); 4.90 (s, 1H, CH).

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

A yellow fibrous precipitate. M. p. > 250 °C
(EtOH). Anal. Caled for C22H13N2O2S2, %: C 57.27;
H 3.54; N 7.03. Found, %: C 57.43; H 3.77; N 7.08;
1H NMR (400 MHz, DMSO-d6); δ (ppm) 7.83-7.91 (m,
1H, Ar); 7.64-7.72 (m, 1H, Ar); 7.53-7.57 (m, 1H, Ar);
7.47-7.53 (m, 1H, Ar); 7.39 (s, 2H, NH2); 7.17-7.28 (m,
4H, Ar); 4.68 (s, 1H, CH); 2.46 (br. s., 3H, CH3).

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

A yellow fine-crystalline powder. M. p. > 250 °C (EtOH).
Anal. Caled for C19H13N2O2S, %: C 60.65; H 2.83; N 11.13;
1H NMR (400 MHz,
The procedure for the synthesis of 2-amino-4-(4-carboxy)-phenyl-4H-pyran-[3,2-c][1,2]benzoxathine-3-carbonitrile 5,5-dioxide 4n. Reflux the solution of 1,2-benzoxathin-4(3H)-one 2,2-dioxide 1 (0.198 g, 0.001 mol), malononitrile 2 (0.066 g, 0.001 mol) and 4-formaldehyde acid 3n (0.001 mol) in ethanol (5-10 mL) for 1 h. Filter the precipitate of compound 4n obtained, wash with ethanol and then dry on air.

4-(2-amino-3-cyano-5,5-dioxido-4H-pyrano-[3,2-c][1,2]benzoxathine-4yl)benzoic acid (4n). A white fibrous precipitate. M. p. > 250 °C (EtOH). Anal. Calcd for C_{19}H_{12}N_{2}O_{6}S, %: C 57.57; Н 3.05; N 7.07. Found, %: C 57.42; Н 3.19; N 7.25;

Results and discussion

In order to broaden the number of the compounds studied in current paper we expanded the range of arene-carbaldehydes 3 and synthesized the corresponding 2-amino-4H-pyrano-3-carbonitriles 4. Thus, different substituted benzaldehydes 3f–n were used in the abovementioned reaction conditions, in case of 4-formaldehyde acid 3n the reaction readily proceeded without addition of triethylamine. The corresponding target compounds 4f–n were successfully obtained with moderate to high yields (Scheme). The structures of all compounds synthesized were confirmed by $^1$H NMR-spectroscopy and elemental analysis.

The results of studying the effect of 2-amino-4-aryl-4H-pyran-[3,2-c][1,2]benzoxathine-3-carbonitrile 5,5-dioxides on blood coagulation are shown in Table. According to the results of the study it was found that compounds 4g and 4f in the concentration of 1 mg/ml significantly increased the time of blood clotting by 1.6 and 1.9 times, respectively, compared to control, indicating their anticoagulant properties. Additionally, these compounds in the concentration of 3 mg/ml showed less pronounced anticoagulant effect, i.e. no dose-dependent features were revealed.

Surprisingly enough the hemostatic activity was found for compounds 4a, b, c, d, e, l, m; it revealed in a significant decrease in the blood coagulation time compared to control. The most pronounced effect was demonstrated by compound 4a, which significantly reduced the time of coagulation by 1.6 times in the concentration of 3 mg/ml.
The effect of 2-amino-4-aryl-4H-pyran[3,2-c][1,2] benzoxathine-3-carbonitrile 5,5-dioxides on the coagulation time (n=4)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration, mg/ml</th>
<th>Coagulation time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>143.75 ± 4.79</td>
</tr>
<tr>
<td>Aminocaproic acid</td>
<td>1</td>
<td>34.00 ± 1.41*</td>
</tr>
<tr>
<td>Heparin</td>
<td>1</td>
<td>578.75 ± 50.55*</td>
</tr>
<tr>
<td>4a</td>
<td>1</td>
<td>42.25 ± 2.50***</td>
</tr>
<tr>
<td>4a</td>
<td>3</td>
<td>21.00 ± 2.58***</td>
</tr>
<tr>
<td>4b</td>
<td>1</td>
<td>95.50 ± 4.65***</td>
</tr>
<tr>
<td>4b</td>
<td>3</td>
<td>96.25 ± 5.68***</td>
</tr>
<tr>
<td>4c</td>
<td>1</td>
<td>75.75 ± 4.35***</td>
</tr>
<tr>
<td>4c</td>
<td>3</td>
<td>84.00 ± 3.37***</td>
</tr>
<tr>
<td>4d</td>
<td>1</td>
<td>80.25 ± 4.11***</td>
</tr>
</tbody>
</table>

Comparison to the reference drug – aminocaproic acid, and at the same time, its solution in the concentration of 1mg/ml showed the activity at the level of the reference drug. Thus, the data obtained indicate a direct dose-dependent effect on blood coagulation in this case.

Therefore, among 1,2-benzoxathin-4(3H)-one 2,2-dioxide derivatives compounds with both anticoagulant and hemostatic properties were revealed. Considering a relatively high level of these activities the effect of 2-amino-4-aryl-4H-pyran[3,2-c][1,2]benzoxathine-3-carbonitrile 5,5-dioxides on the blood coagulation requires further detailed study.

Conclusions

1. A series of 2-amino-4-aryl-4H-pyran[3,2-c][1,2] benzoxathine-3-carbonitrile 5,5-dioxides was synthesized in the course of the three-component interaction of 1,2-benzoxathin-4(3H)-one 2,2-dioxide, malononitrile and substituted benzaldehydes.

2. The study of the effect of the compounds obtained on the blood coagulation process revealed both anticoagulant and hemostatic substances. Namely, 2-amino-4-(4-fluoro)-phenyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathin-3-carbonitrile 5,5-dioxide and 2-amino-4-(3-chloro)-phenyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathin-3-carbonitrile 5,5-dioxide significantly increased the time of blood clotting without dose-dependent changes in activity. However, for the majority of compounds the hemostatic activity with a direct dose-dependent effect was determined; it was the highest for 2-amino-4-phenyl-4,6-dihydropyrano[3,2-c][2,1]benzoxathin-3-carbonitrile 5,5-dioxide 4a.

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

References
