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SYNTHESIS, ANTIAGGREGATION AND ANTITROMBOTIC ACTIVITIES OF NEW DERIVATIVES OF HYDROXYBENZOIC ACIDS WITH TAURIC FRAGMENT

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A high prevalence of thrombotic disorders, insufficient effectiveness or safety of antithrombotic therapy is an urgent problem of modern healthcare. The main means of preventing thrombosis is acetylsalicylic acid. Despite its long history, aspirin attracts researchers in the fields of medicinal chemistry, biology, and medicine. The development of new antiplatelet agents, including chemical modification of the acetylsalicylic acid molecule, remains relevant. Modification of the acetylsalicylic acid molecule using amino acids and obtaining their salt forms makes it possible to maintain antiplatelet or antithrombotic properties, as well as to impart additional pharmacodynamic effects. In modern science, a lot of attention is paid to the sulfur-containing amino acid taurine. An analysis of modern scientific literature revealed the protective effect of taurine in diabetes mellitus and cardiovascular diseases, liver dysfunction, gastrointestinal tract, and kidney diseases.

The aim of the article is to study synthesis of new compounds, determination of their physical characteristics and assessment of their antiplatelet and antithrombotic activities *in vitro* and *in vivo*.

Materials and methods. To confirm the structure of the synthesized new derivatives of hydroxybenzoic acids with a taurine fragment by the acelation method, thin layer chromatography and NMR spectra were used. *In vitro* studies were carried out on the model of ADP-induced platelet aggregation according to the Born G. methods modified by V.A. Gabbasov. *In vivo*, the studies were carried out on the model of arterial thrombosis induced by the application of iron chloride in the following groups of animals: intact, with experimental diabetes mellitus and three-year-olds; the rate of bleeding from the tail vein was also evaluated.

Results. New compounds – derivatives of ortho-, meta- and para-hydroxybenzoic acids with a taurine residue – were synthesized. A procedure for the preparation of N-hydroxybenzoyl taurine compounds and their salt forms have been described; their spectral characteristics and melting points have been determined. The synthesized compounds are superior to acetylsalicylic acid in solubility and are not inferior to it in antiplatelet and antithrombotic activities. The results of the *in vitro* antiplatelet activity assessment in a wide concentration range from 10^{-4}M to 10^{-8}M, are presented. It has been revealed that the dipotassium salt of N-(2-hydroxybenzoyl)taurine exhibits a less antiplatelet activity than the dipotassium salt of N-(3-hydroxybenzoyl)taurine. The most pronounced antiplatelet activity is exhibited by the compound N-(4-hydroxybenzoyl) taurine. In *in vivo* experiments on the model of arterial thrombosis in 3-year-olds or animals with experimental diabetes mellitus, carotid artery thrombosis occurred faster than in young or intact animals. A single preliminary oral administration of the test compounds prolonged the time of the thrombus formation, which makes it possible to conclude that they have an antithrombotic effect. In this study, the dipotassium salt of N-(3-hydroxybenzoyl)taurine exhibits a more pronounced activity than that of acetylsalicylic acid.

Conclusion. Against the background of the modeled pathologies, the studied drugs showed the expected antithrombotic activity, in terms of the severity not inferior to that found in acetylsalicylic acid.

Keywords: antiplatelet agents; antiplatelet activity; antithrombotic activity; acetylsalicylic acid; platelet aggregation; taurine

Abbreviations: ESC – European Society of Cardiology; DMSO-d_6 – dimethyl sulfoxide-d6; GAPDH – glyceraldehyde-3-phosphate dehydrogenase (glyceraldehyde-3-phosphate dehydrogenase); HUVECs – Human Umbilical Vein Endothelial Cells (human endothelial cells); ADP – adenosine diphosphoric acid; ASA – acetylsalicylic acid; ROS – reactive oxygen species; NSAIDs – non-steroidal anti-inflammatory drugs; DM – diabetes mellitus; CVD – cardiovascular disease; COX – cyclooxygenase; GI tract – gastrointestinal tract

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СИНТЕЗ, АНТИАГРЕГАЦИОННАЯ И АНТИТРОМБОТИЧЕСКАЯ АКТИВНОСТИ НОВЫХ ПРОИЗВОДНЫХ ГИДРОКСИБЕНЗОЙНЫХ КИСЛОТ С ТАУРИНОВЫМ ФРАГМЕНТОМ

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Высокая распространенность тромботических нарушений, недостаточная эффективность или безопасность антитромботической терапии является актуальной проблемой современного здравоохранения. Основным средством профилактики тромбоза является ацетилсалициловая кислот. Несмотря на многолетнюю историю аспирин привлекает исследователей в области медицинской химии, биологии и медицины. Разработка новых антиагрегантов, в том числе и химической модификацией молекулы ацетилсалициловой кислоты остается актуальной. Модификация молекулы ацетилсалициловая кислот с использованием аминокислот и получением их солевых форм, позволяет сохранять антиагрегантные или антитромботические свойства, а также сообщить дополнительные фармакодинамические эффекты. В современной науке уделяется немало внимания серосодержащей аминокислоте таурин. При анализе современной научной литературы обнаружено протективное действие таурина при сахарном диабете и сердечно-сосудистых заболеваниях, дисфункции печени, желудочно-кишечного тракта, заболеваниях почек.

Цель. Синтез новых соединений, определение их физических характеристик и оценка антиагрегантной и антитромботической активности *in vitro* и *in vivo.*

Материалы и методы. Для подтверждения структуры, синтезированных новых производных гидроксибензойных кислот с тауриновым фрагментом методом ацелирования, использовали тонкослойню хроматографию, ЯМР спектры. Исследования *in vitro* проводили на модели АДФ-индуцированной агрегации тромбоцитов по методике Born G. в модификации Габбасова В.А. Исследования *in vivo* проводили на модели артериального тромбоза, индуцированного аппликацией хлоридом железа на следующих группах животных: интактные, с экспериментальным сахарным диабетом и трех годовалые, так же была проведена оценена скорость кровотечения из хвостовой вены.

Результаты. Были синтезированы новые соединения, представляющие собой производные орто-, мета- и пара-гидроксибензойных кислот с остатком таурина. Описана методика получения соединений N-гидроксибензоил таурина и их солевых форм, определены спектральные характеристики и температура плавления. Синтезированные соединения по растворимости превосходят ацетилсалициловую кислоту, не уступают ей в антиагрегантной и антитромботической активности. Представлены результаты оценки антиагрегантной активности *in vitro* в широком диапазоне концентраций от 10^{-4}М до 10^{-8}М. Выявлено, что дикалиевая соль N-(2-гидроксибензоил)таурина проявляет меньшую антиагрегантную активность, чем дикалиевая соль N-(3-гидроксибензоил)таурина. Наиболее выраженную антиагрегантную активность проявляет соединение N-(4-гидроксибензоил)таурин. В экспериментах *in vivo* на модели артериального тромбоза у 3-летних или животных с экспериментальным сахарным диабетом, тромбоз сонной артерии происходил быстрее, чем у молодых или интактных. Однократное предварительное пероральное введение исследуемых соединений пролонгировало время образования тромба, что позволяет сделать заключение о наличии у них антитромботического действия. Дикалиевая соль N-(3-гидроксибензоил)таурина в проведённом исследовании проявляет более выраженную чем у ацетилсалициловой кислоты активность.

Заключение. На фоне моделируемых патологий, исследуемые препараты проявили ожидаемую антитромботическую активность, по выраженности не уступающую выявленной у ацетилсалициловой кислоты.

Ключевые слова: антиагреганты; антиагрегантная активность; антитромботическая активность; ацетилсалициловая кислота; агрегация тромбоцитов; таурин

Список сокращений: ESC – европейская ассоциация кардиологии; DMSO-d_6 – диметилсульфоксид-d6; GAPDH – глицеральдегид-3-фосфатдегидрогеназа; HUVECs – эндотелиальные клетки человека; АДФ – аденозинди дифосфорная кислота; АСК – ацетилсалициловая кислота; АФК – активные формы кислорода; НПВП – нестероидные противовоспалительные препараты; СД – сахарный диабет; ЭСД – экспериментальный сахарный диабет; ССЗ – сердечно-сосудистые заболевания; ЦОГ – циклооксигеназа; ЖКТ – желудочно-кишечный тракт

INTRODUCTION

Cardiovascular diseases occupy a leading position in mortality statistics in most developed countries [1–4] and are the main cause of death in most European countries [5]. For 70 years, the main aim of the European Society of Cardiology (ESC) has been to improve the standards of diagnosis and treatment of cardiovascular diseases (CVDs), including the optimization of antiplatelet and

antithrombotic therapy [6]. First of all, this is a decrease in the number of thromboses, arising from the imbalance in the hemostasis system, being the main cause of strokes, myocardial infarction and limb amputations [7, 8].

Cardiovascular diseases are the cause of mortality for more than 50% of people in middle-income countries and <30% of people in high-income countries [9]. The observed difference arose due to the effective implementation of preventive measures and high-tech medical care, optimization of the use and improvement of the drugs quality, which in conjunction led to a decrease in mortality from CVDs [10].

The key factor determining the prognosis of most diseases of the cardiovascular system is the problem of the low efficiency of preventing arterial thrombosis. Timely implementation of preventive measures increases the life expectancy, improves its quality, and reduces the cost of treatment and rehabilitation of patients.

Antiplatelet agents, suppressing the functional activity of platelets, prevent intravascular hemocoagulation; their use has been proven to reduce the risk of thromboembolism in many socially significant diseases [11].

Attempts to improve the pharmacodynamic or pharmacokinetic properties of the known drugs are a common approach to the development of new ones.

Innovative pharmaceuticals should inhibit the functional activity of platelets more effectively and safely than the known antiplatelet agents and, at the same time, exert pleiotropic effects. Acetylsalicylic acid, blockers of ADP – P2Y12 receptors (ticlopidine, clopidogrel, prasugrel, ticangrelor, cangrelor), antagonists of glycoproteins IIb/IIIa have high efficacy and safety rates [12], but at the same time, the problem of thrombotic complications has not been fully resolved.

Acetylsalicylic acid continues to be the most often prescribed antiplatelet agent. A number of common diseases such as diabetes mellitus, metabolic syndrome, obesity are considered as independent factors of a high risk of cardiovascular complications. In the latest recommendations [13] on the primary prevention of atherosclerotic complications of diabetes mellitus (DM), low doses of acetylsalicylic acid are indicated as a means of basic therapy. This indicates the undeniable recognition of this drug merits, its therapeutic margin and availability. Chemical modification of molecules with a pronounced pharmacological activity continues to be one of the methods of drug development. Derivatization of the ASA molecule and the addition of amino acid taurine to its structure, in conjunction with the production of salt forms, makes it possible to enhance the antiplatelet effect or reduce the severity of side effects, provide an additional pharmacological action, increase the efficiency of synthesis and/or facilitate its production [14–16]. Throughout the history of the acetylsalicylic acid use, and especially intensively in the last two decades, chemists have been trying to modify its molecule in order to impart new, mainly pharmacodynamic effects to it, such as vasodilating, or the ability to generate reactive oxygen species (ROS) in tumor cells, exhibit antibacterial and antiproliferative and/or antitumor activities. So, anhydride conjugates of aspirin with fatty acids pass through cell membranes more easily, and cause a more pronounced dose-dependent platelet aggregation. Aspirin-lipid conjugates act by inhibiting the cyclooxygenase (COX)-thromboxane synthase (TXAS) pathway. All conjugates are hydrolyzed up to the parent aspirin and fatty acid molecules in a controlled manner. Aspirin-fatty acid anhydrides have a greater bioavailability (the free carboxyl group in aspirin remains an ionized species at physiological pH and is poorly absorbed through cell membranes) and an antiplatelet activity (one of the reasons for creating a hybrid "codrug" is that the prodrug aspirin-fatty acid- anhydride is hydrolyzed with the release of not one, but two active molecules, which independently inhibit COX), and are less ulcerogenic [17].

Acetylsalicylic acid and analogs of short chain fatty acids such as butyryl salicylic acid, exhibit a pronounced antimicrobial activity against *Salmonella Typhimurium.* [18].

The number of works revealing the antitumor activity of non-steroidal anti-inflammatory drugs (NSAIDs) and, in particular, ASA, continues to increase [19, 20]. Thus, aspirin derivatives based on cinnamaldehyde, are being studied as a potential agents for the treatment of colorectal cancer [21–23].

The inclusion of a metal ion in the acetylsalicylic acid molecule can impart additional pharmacodynamic properties to it. That makes it possible to retain the ability to inhibit COX. However, the ability to generate ROS by the metal part of the conjugate appears, can help overcome the resistance of tumor cells or microorganisms. The development of ASA organometallic derivatives is one of the areas of bioorganic chemistry and has become a powerful alternative to traditional approaches in the development of bioactive compounds [24]. For example, new derivatives of aspirin, which include nitric oxide (NO-aspirins) in their molecule, are safer in relation to the mucous membrane of the gastrointestinal (GI) tract, and have pronounced cytotoxic effects in relation to lung cancer [25]. IPA/NO-aspirins are prodrugs that are safer and have more pronounced pharmacodynamic effects, probably due to the improved cellular uptake and delivery. The aspirin compounds containing nitric oxide, were non-toxic to normal endothelial cells

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(HUVECs; do not affect viability up to 100 μM), but were toxic to some cancer cell lines, indicating cancer-specific sensitivity that holds promise for chemotherapy or chemoprophylaxis. The selective cytotoxicity of prodrugs based on NO-aspirin, may be associated with their effect on the activity of the glycolytic protein GAPDH (the thiol group of GAPDH is suppressed by HNO donors, which are formed during the hydrolysis of IPA/NO-aspirin in the body), the activity of which determines the rate of glycolysis in tumor cells. The use of aspirin compounds containing nitric oxide (IPA/NO-aspirin) increased the function of murine cardiomyocytes *in vivo*. This confirms that HNO donors are positive inotropic/lusitropic agents and increase transients in the Ca^{2+} channels of cardiomyocytes. Thus, prolonged forms of drugs containing aspirin and HNO or NO can have a wide therapeutic use as anti-inflammatory, antitumor, and cardioprotective agents [26].

Taurine is an organic osmolyte involved in the regulation of cell metabolism and provides a substrate for the formation of bile salts. It plays an important role in modulating the concentration of intracellular free calcium, and although it is one of the amino acids not included in proteins, taurine is one of the most abundant amino acids in the brain, retina, muscle tissue and organs [27]. A taurine derivative stimulates the formation of less toxic amyloid-β fibrils, which leads to the prevention of cognitive deficits in an acute experimental model of Alzheimer's disease in mice [28]. Taurine improves insulin secretion and decreases insulin resistance. Taurine treatment reduced the severity of oxidative stress in the brain, diabetic hepatotoxicity, the severity of vascular diseases and heart traumas in diabetes [29, 30]. The literature describes the effect of taurine supplementation on insulin resistance; the balance of iron, zinc and copper; parameters of oxidative stress in control animals and rats with a high-fat diet [31].

Thus, the synthesis and preclinical study of new derivatives of hydroxybenzoic acids is a promising and urgent problem for modern pharmacology.

THE AIM of the article is to synthesize new compounds, determine their physical characteristics and evaluate antiplatelet and antithrombotic activities *in vitro* and *in vivo.*

MATERIALS AND METHODS

Synthesis and determination of physical characteristics

The general procedure for the synthesis of dipotassium salts of N-(hydroxybenzoyl)taurines had been described in detail by the authors before [32, 33]. A solution of 2-aminoethanesulfonic acid(taurine) in 25.00 ml of water was placed in a reactor equipped with a stirrer, and 6 N. sodium hydroxide solution. Hydroxybenzoic acid chloride was added dropwise to the solution for 1.5 h under cooling. Then the reaction mixture was stirred for another 1.5 h (under cooling), controlling the pH of the medium (pH>7). The resulting mixture was poured into ice and acidified with hydrochloric acid to pH = 5, the precipitated crystals were recrystallized from isopropanol, filtered and dried. The characteristics of the compounds are presented in Table 1. Then, 100 mmol of potassium ethylate, 100.00 ml of benzene and 50 mmol of N-(hydroxybenzoyl) taurine were loaded into a 3-necked reactor equipped with a stirrer, a reflux condenser and a thermometer, and stirred at the temperature of 100°C for 30 minutes. After cooling, the product was separated by filtration, washed with a small amount of an alcoholic alkali solution and dried.

Melting points were determined by the capillary method on a Stuart SMP-30 device (Great Britain) at the heating rate of 10°C/min. The purity and individuality of the compounds were confirmed by thin-layer chromatography on Silufol UV-254 plates, the mobile phase was in an n-butanol:ethanol:water ratio of 5:2:1, the development was in iodine vapor and UV light.

^{1}H-NMR spectra of derivatives in DMSO-d_6 were recorded on a Bruker DRX500 spectrometer (Bruker, Germany) with the internal standard of hexamethyldisiloxane 500 MHz. The spectra were interpreted using a licensed software product from Advanced Chemistry Development Inc. by the trade name of ACD/HNMR Predictor Pro v. 3.

The studied N-derivatives of taurine were synthesized by the acylation reaction of taurine with an equimolar amount of 2-, 3-, or 4-hydroxybenzoic acid chloride. Then hydroxybenzoyltaurines were converted into a water-soluble form by obtaining dipotassium salts (Fig. 1).

In vitro studies

The study of the effect of substances on the functional activity of platelets *in vitro* was carried out according to the Born G. methods modified by V.A. Gabbasov (1989) [34] on a two-channel laser analyzer of platelet aggregation "Biola" 220LA (Russia). The studies were carried out on platelet-rich rat plasma according to the method described by V.A. Lyusov, Yu.B. Belousov (1971) [35]. The blood was obtained from anesthetized (chloral hydrate, 400 mg/kg, i.p., Organic, Russia) animals from the abdominal aorta [36], stabilized with a 3.8% sodium citrate solution (Reakhim, Russia) in the ratio of 9:1, then centrifuged for 10 min at 1000 rpm on a CM-6m centrifuge (ELMI, Latvia). The device was calibrated using distilled water, according to the instructions, the light transmission of distilled water was taken as 100%.

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To obtain a control sample, 300 µL of platelet-rich plasma was added to the glass cuvette of the aggregometer; after the recording of the aggregatogram had been turned on, at the 10th second of the registration process, ADP (Sigma Aldrich, USA) was added to the cuvette, at the final concentration of 5 µM [37].

To study the antiplatelet activity of the compounds under study, 30 µl of the solution of the test sample at a certain concentration was added to the cuvette with 270 µl of platelet-rich plasma. The sample was incubated in thermostated cells of the aggregometer (at 37°C for 3 minutes), after which the sample was transferred to a recording cell and the aggregatogram was recorded for 5 minutes.

Hydroxybenzoic acids derivatives and acetylsalicylic acid (the reference drug) were studied in the concentration range of 10^{-4}–10^{-8}M.

In vivo studies

The compounds were administered intragastrically once via a gastric tube. 60 minutes after the administration, the animals were anesthetized (chloral hydrate at the dose of 400 mg/kg, intraperitoneally), the left common carotid artery was isolated, and the model of intravascular thrombosis was simulated [38]. Parafilm was placed under the carotid artery. The blood flow velocity in the carotid artery had been recorded by ultrasound Doppler until it stopped completely as a result of thrombosis, initiated by applying a cotton swab moistened with a 50% solution of iron (III) chloride on the vessel.

To determine the bleeding time, the animal was cut off 5 mm from the tip of the tail, which was then placed in a flask with saline (t = 37°C) and the time until the end of the bleeding was recorded [37, 39].

Study design

Pharmacological studies of compounds in *in vitro* and *in vivo* models were performed according to the study design (Fig. 2).

Statistical processing of results

Statistical analysis of the data obtained was performed using the Microsoft Excel statistical software package and the Prism 6.0 software (Graph Pad Software Inc., USA). The data were presented as the arithmetic mean and its mean error. The comparison of the mean data of the independent samples with a normal distribution of the variant in the data set (sample), was calculated using the Student's t-test. When the variant distribution in the sample was different from the normal, the Mann-Whitney U-test (when comparing two groups) and the Kruskal-Wallis test (when comparing more than two groups) were used. A significant level of differences was considered a probability of at least 95% ($p < 0.05$).

Compliance with ethical standards

The experiments were carried out in accordance with the methodological guidelines and regulatory documents GOST ISO/IEC 17025-2009, GOST R ISO 5725-2002 and the rules of laboratory practice for preclinical studies in the Russian Federation in accordance with the Principles of Good Laboratory Practice (GOST R 33044-2014, 2015) and "On the approval of the rules of good laboratory practice" (Ministry of Health of the Russian Federation, Order No. 199n dated April 1, 2016), in compliance with Directive 2010/63 / EU of the European Parliament and the Council of the European Union dated September 22, 2010 on the protection of animals used in scientific purposes.

On compliance with ethical standards, an expert opinion from the Local Ethics Committee of Volgograd State Medical University of the Ministry of Health of the Russian Federation (registration number IRB 00005839 IORG 0004900 (OHRP)) was received.

The euthanasia of the animals was carried out in compliance with the requirements set out in the "International Recommendations for Biomedical Research Using Animals" (1997). For 24 hours before the start of the experiments, all the animals were in complete food deprivation with a free access to water.

RESULTS AND DISCUSSION

The addition of compound C-60 (dipotassium salt of N-(3-hydroxybenzoyl)taurine) to the blood plasma of the laboratory animals at the concentrations of 10^{-7} and 10^{-8} M significantly reduces the degree of platelet aggregation by 38% and 37% in comparison with the control group of the animals, and by 19% and 26% compared with the reference drug. The introduction of compound C-61 (dipotassium salt of N-(4-hydroxybenzoyl)taurine) into the platelet-rich blood plasma of the laboratory animals in all the studied concentrations, reduces the degree of platelet aggregation. At the concentrations of 10^{-4} and 10^{-5} M, the degree of aggregation decreased by 46% and 44% in comparison with the control group. When the test compound was added to the platelet-rich plasma at the concentrations of 10^{-6} and 10^{-7} M, the degree of aggregation decreased by about 10 times. At the concentration of 10^{-8}M, no statistically significant decrease in aggregation was observed.

A more pronounced antiplatelet effect of the studied compounds in comparison with ASA may be associated with the formation of a covalent bond of taurine with thiol and thioether groups of atoms or disulfide bridges in molecular targets. That had been justified for chloraminic and chlorimine derivatives of taurine [40]. The compound under the laboratory code of C-59-N-(2-hydroxybenzoyl)taurine showed its antiplatelet activity at the level of the reference drug.

The addition of compounds C-60 and C-61 at the concentrations of 10^{-5}–10^{-7} M to the blood plasma of healthy laboratory animals to the maximum and more pronounced than the reference preparation ASA, limited the development of platelet aggregation. The addition

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of ASA had an antiplatelet effect only at the concentrations of 10^{-5} and 10^{-6} M, which is confirmed by the results of numerous studies of this drug.

Compounds C-59, C-60, and C-61 are, like ASA, derivatives of hydroxybenzoic acids; therefore, there is a reason to believe that the main mechanism of the antiplatelet action is realized due to the irreversible inactivation of cyclooxygenase-1 after acetylation of the serine residue in the area of the active site. As a result, the synthesis of thromboxane A_2 is blocked, and thus the secondary activation of platelets is prevented [40].

The compounds under study are the salt form of hydroxybenzoic acids derivatives and contain potassium, which does not only improve their solubility in water, but also exhibits its own antiplatelet properties. These data are consistent with the literature data [41].

When analyzing the data obtained (Fig. 3) in the conducted experiments, it can be concluded that the most pronounced antiplatelet effect in *in vitro* studies is revealed by compound C-61, significantly superior to the similar effect of acetylsalicylic acid.

One of the important factors determining the outcomes of the cardiovascular system diseases, is the problem of arterial thrombosis [42], which is inextricably linked with the state of the hemostasis system. Thrombosis is often the cause of sudden death, myocardial infarction, vascular complications of diabetes mellitus, and limbs amputation. Therefore, the search and study of the atrombogenic effect of potential antithrombotic agents is relevant. Preclinical studies of the antithrombotic effect of new potential molecules should be evaluated not only in healthy and young animals, but also in those simulated in the state as close as possible to the clinical conditions of the antiplatelet administration. In the work by JP Garner, the following was concluded: if scientists want animal models to correspond to the pathophysiology of human diseases and be equally susceptible to the effect of the tested drugs, then the experiments should be performed on animals as if they were carried out on humans [43]. In the present work, the studies of the antithrombotic effect of new derivatives of hydroxybenzoic acids were carried out on young and healthy animals, elderly and healthy, elderly and with diabetes mellitus, which corresponds to a frequent clinical situation in which therapy with such drugs is carried out. The study of the antithrombotic activity of new derivatives of hydroxybenzoic acids was carried out on a model of arterial thrombosis induced by the application of a 50% solution of iron (III) chloride to the carotid artery of healthy animals, three-year-old (elderly) rats, and the animals with experimental pathology (diabetes mellitus, study of the bleeding time from the tail vein). The study of the antithrombotic activity of new derivatives of hydroxybenzoic acids in a model of arterial thrombosis induced by the application of a 50% solution of iron (III) chloride in healthy animals, was carried out.

In the control group of the animals, administrated with a solvent (saline), the average time of the complete occlusion of the carotid artery was 14.8±0.77 minutes.

The reference drug, acetylsalicylic acid at the dose of 36 mg/kg, increased the occlusion time by 69% ($p<0.05$).

In comparison with the control group, the compounds under study with a taurine residue under the laboratory code of C-59 at the equimolar concentration with the reference drug, prolonged the time of thrombus formation by 61%. In this experimental model of thrombosis, compound C-60 demonstrated an antithrombogenic activity and exceeded the reference drug by 3% in the time of complete occlusion and in relation to the control group, by 73% ($p<0.05$). The investigated compound C-61 increased the time of vascular occlusion twice compared with the control group ($p<0.05$), the data with the reference drug ASA did not differ. The results are presented (Fig. 4).

Study of antithrombotic effect of hydroxybenzoic acids new derivatives in model of arterial thrombosis induced by application of 50% ferric chloride solution in 3-year-old (elderly) animals

In 3-year-old (elderly) animals, the antithrombotic effect of the compounds under study was assessed in a single intragastric administration of the substances shown in Fig. 5, to the rats.

The average time of complete occlusion of the carotid artery in the animals of the control group was 16.50±0.89 minutes. The reference drug, acetylsalicylic acid, in an effective therapeutic dose increased the occlusion time to 20.14±0.55 minutes, which was 22% longer than the control group of animals. The data are statistically significant.

The compound C-60 at the dose of 18 mg/kg prolonged the time of thrombus formation to 20.67±1.20 minutes ($p<0.05$), which was 25% slower than in the group that had received saline, and 3% slower than in the group of the animals that had been administrated with the reference drug. When the compound C-61 was administered at the dose of 23 mg/kg, the time of complete occlusion was 18.17±1.56 minutes, which was 10% slower compared to the control group, and 10% slower than the group administered with the reference drug.

Thus, in the model of arterial thrombosis of the carotid artery of 3-year-old (elderly) rats, compound C-60 demonstrated the antithrombotic effect; complete occlusion of the carotid artery proceeded slower than effected by acetylsalicylic acid.

Study of platelet effect of leading compounds in model of arterial thrombosis in animals with experimental diabetes mellitus

The antithrombogenic effect of the most active compounds (C-60, C-61) was studied in the animals with streptozotocin-nicotinamide-induced diabetes mellitus.

As a result of the experiment, it was revealed that in the control group of animals, thrombus formation occurred at the 10.3th±0.78 minutes (Fig. 6).

Table 1 – Characteristics of obtained N-(hydroxybenzoyl)-substituted taurines N-(4-hydroxybenzoyl)taurine

Compound	X	Efficiency, %	Melting point, °C	Rf*	1H-NMR spectra (DMSO-d6), δ, ppm
N-(2-hydroxybenzoyl)taurine	2-OH	63.5	158–160	0.664	6.88–7.78 (4H, м, C_6H_4), 8.03–8.05 (1H, м, NH), 10.70 (1H, с, OH and SO_2OH), 2.78–3.60 (4 H, м, C_2H_4)
N-(3-hydroxybenzoyl)taurine	3-OH	63.8	199–201	0.636	6.95–7.33 (4H, м, C_6H_4), 7.50–7.83 (1H, м, NH), 9.81 (1H, с, OH and SO_2OH), 2.67–3.02 (4 H, м, C_2H_4)
N-(4-hydroxybenzoyl)taurine	4-OH	64.2	204–206	0.753	6.76–7.75 (4H, м, C_6H_4), 7.96–8.23 (1H, м, NH), 10.19 (1H, с, OH and SO_2OH), 2.23–3.34 (4 H, м, C_2H_4)

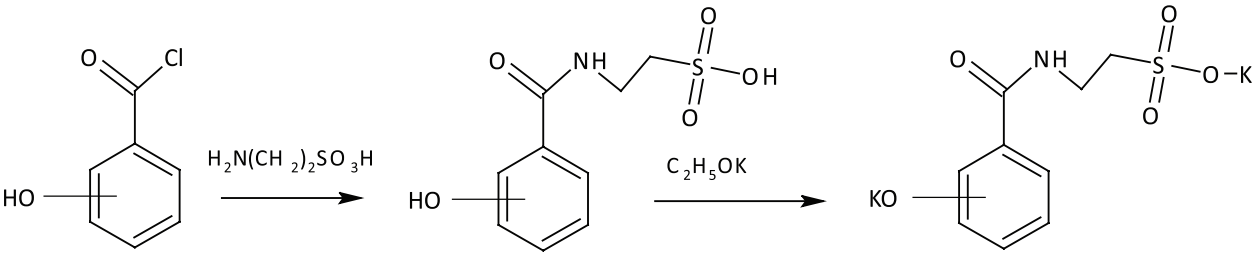

Figure 1 – Taurine acylation reaction

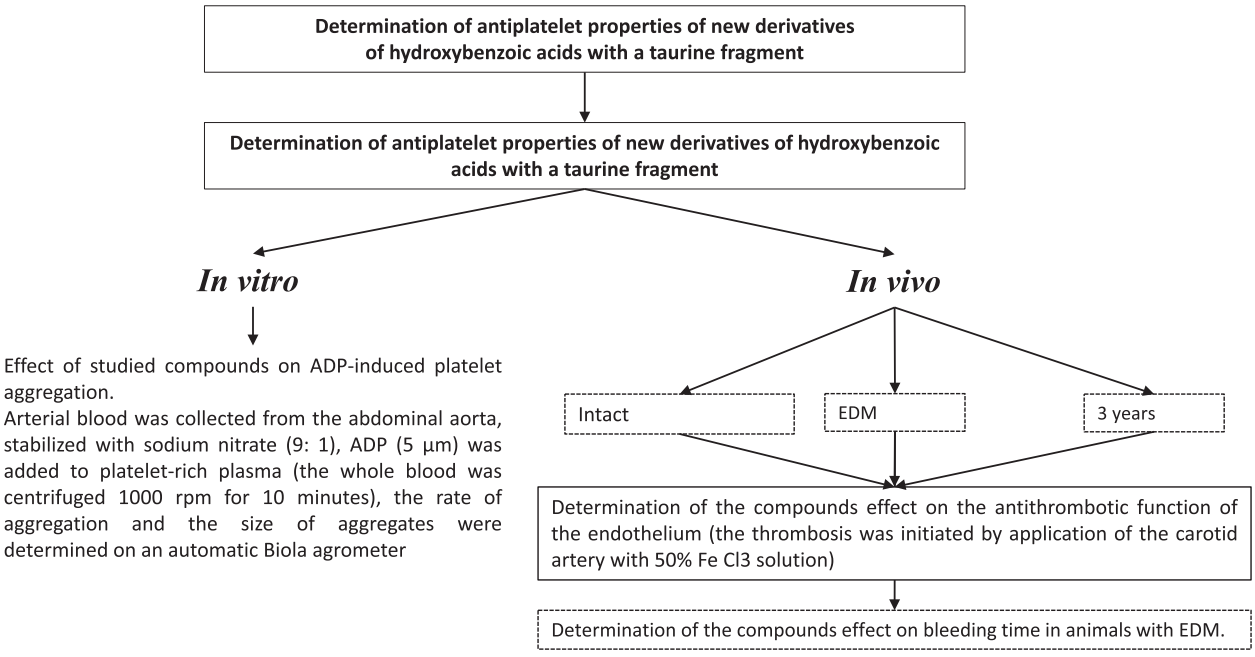

Figure 2 – Study design

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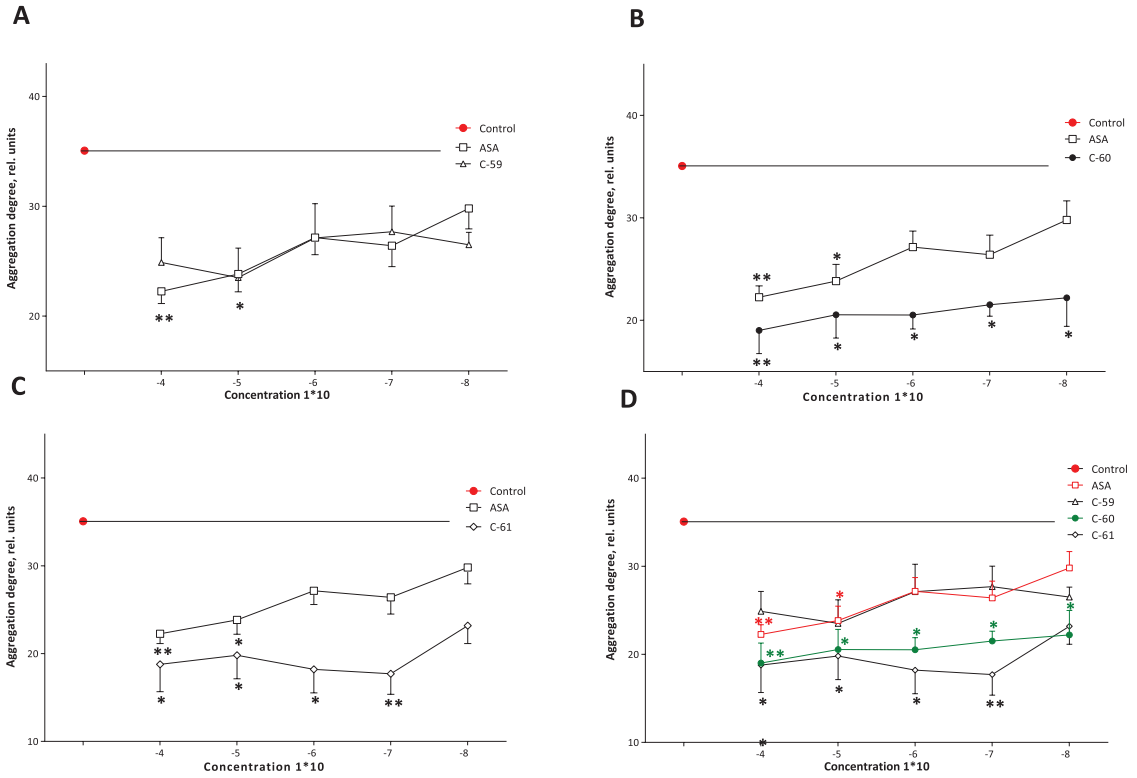

Figure 3 – Antiplatelet activity of compounds in the model of ADP-induced platelet aggregation *in vitro*

Note: A – antiplatelet activity of compound C-59; B – antiplatelet activity of compound C-60; C – antiplatelet activity of compound C-61; D – the combined scheme of the investigated compounds, reference preparation and control; * – p<0.05; ** – p<0.01 changes are statistically significant in relation to the control Student's test with Bonferroni correction.

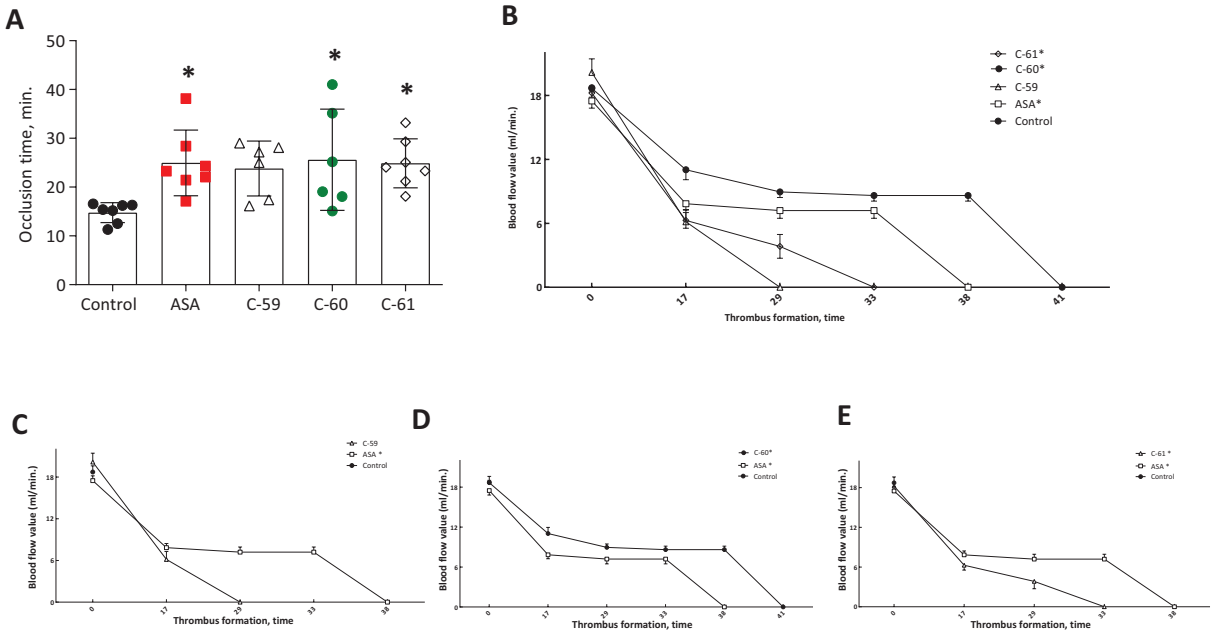

Figure 4 – Time of thrombus formation after application of 50% solution of iron chloride to carotid artery of healthy animals

Note: A – combined scheme of dynamics of thrombus formation of studied compounds, reference and control drugs; B – time of vessels complete occlusion of the studied compounds, reference and control drugs; C – dynamics of thrombus formation of the C-59 compound; D – dynamics of thrombus formation of the C-60 compound; E – dynamics of thrombus formation of the C-61 compound; * – p <0.05 changes are statistically significant in relation to the control Student's test with Bonferroni correction

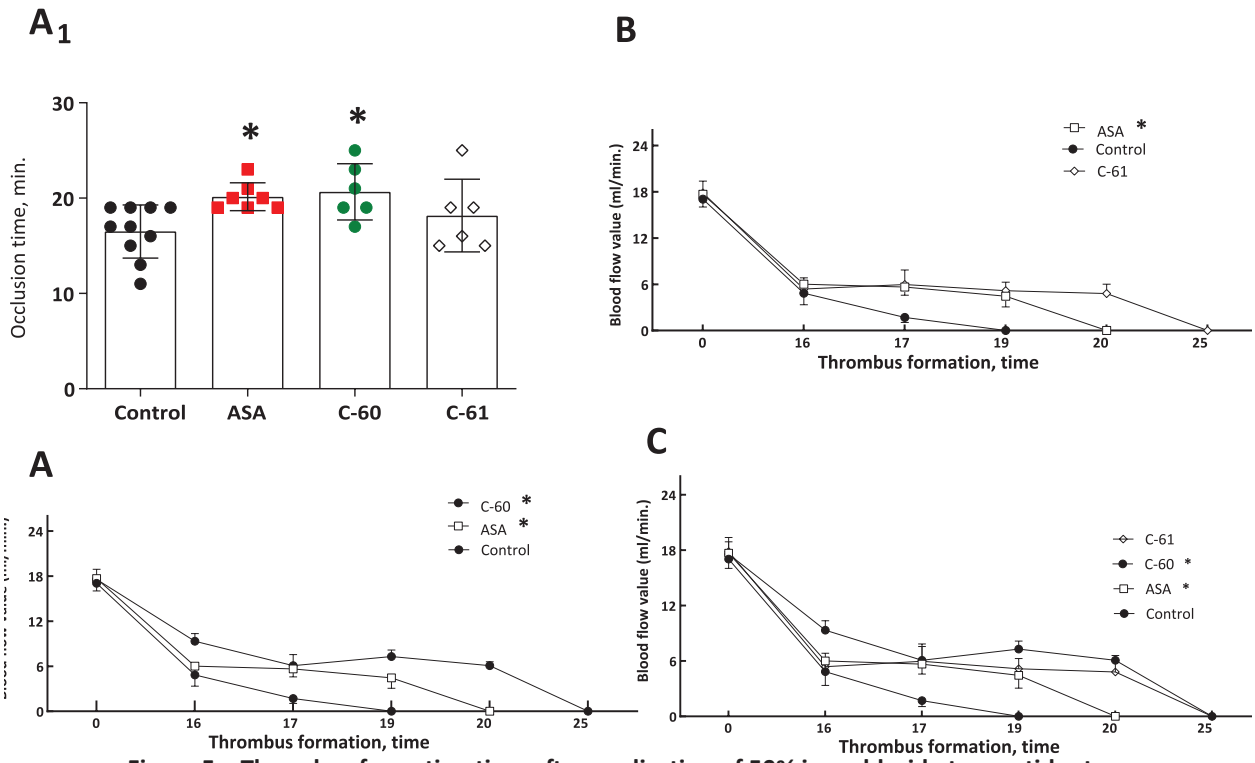

Figure 5 – Thrombus formation time after application of 50% iron chloride to carotid artery in 3-year-old (elderly) animals

Note: A – dynamics of thrombus formation of the C-60 compound; A1 is the time of complete occlusion of vessels of test compounds, reference and control drugs; B – dynamics of thrombus formation of C-61 compound; C – combined scheme of dynamics of thrombus formation of studied compounds, reference and control drugs; * – p <0.05 changes are statistically significant in relation to the control Student's test with Bonferroni's correction

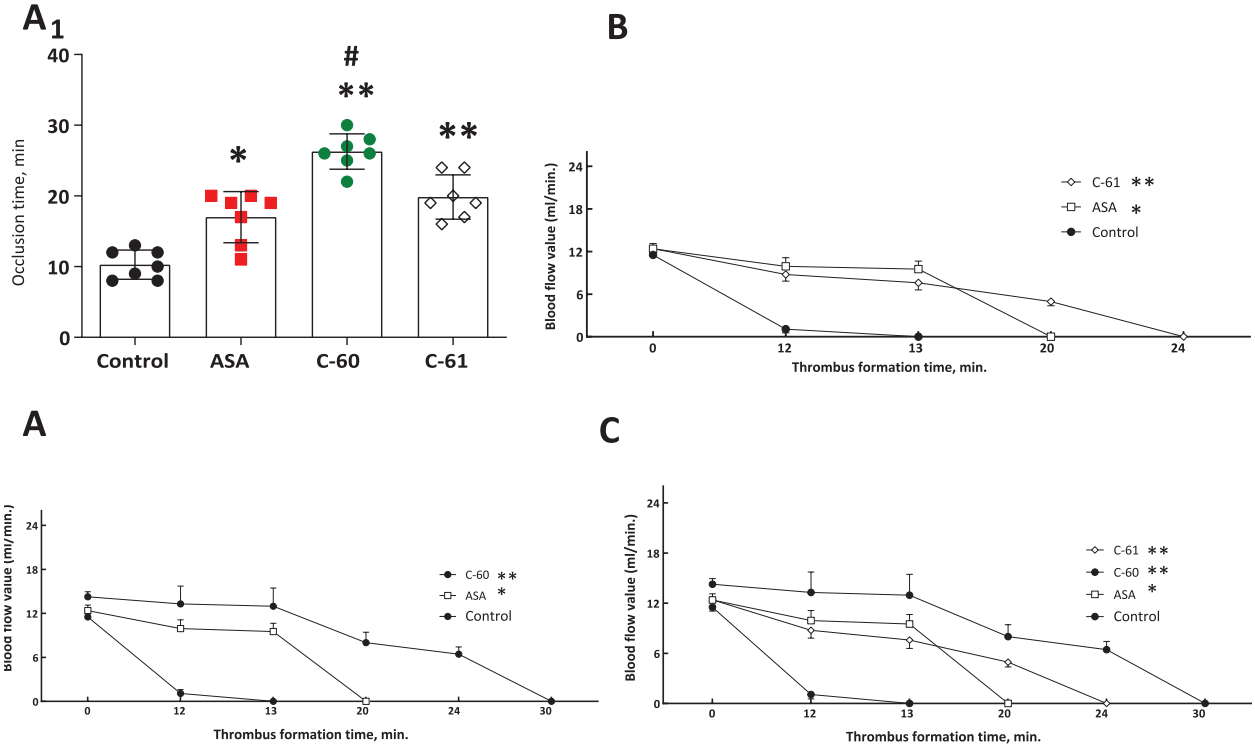

Figure 6 – Thrombus formation time after application of 50% iron chloride to the carotid artery of animals with diabetes

Note: A – dynamics of thrombus formation of C-60 compound; A1 is the time of complete occlusion of vessels of test compounds, reference and control drugs; B – dynamics of thrombus formation of C-61 compound; C – combined scheme of dynamics of thrombus formation of studied compounds, reference and control drugs; * – p <0.05, ** – p <0.01 changes are statistically significant in relation to control Student's test with Bonferroni's correction, # – p <0.05 changes are statistically significant in relation to effect of reference drug, Student's test with Bonferroni's correction

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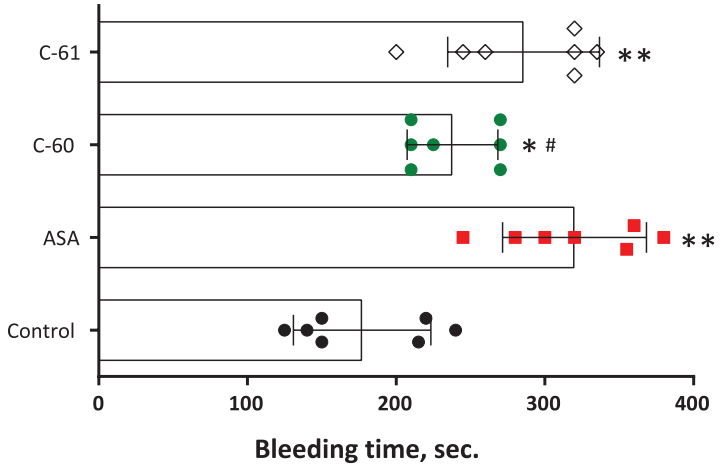

Figure 7 – Study of bleeding time in animals with diabetes mellitus
Note: * – p<0.05, ** – p<0.01 changes are statistically significant in relation to control Student's test with Bonferroni correction, # – p <0.05 changes are statistically significant in relation to effect of reference drug, Student's test with Bonferroni's correction

In an effective therapeutic dose, acetylsalicylic acid prolonged the time of complete occlusion to 17.0±1.36 minutes, which exceeded the indicators of the control group by 65%.

Compound C-60 at the dose of 18 mg/kg significantly prolonged the time of thrombus formation to 26.3±0.94 min, which was 156% slower relative to the control values and 55% slower relative to the group of the animals administered with the reference drug (p <0,01).

In the rats' group, which were administered with compound C-61 at the dose of 23 mg/kg per os, the time of complete occlusion of the carotid artery was 19.9 ± 1.18 minutes, which was 93% slower than in the control group (p <0.01) and 17% slower than in the group of the animals administered with the reference drug.

Thus, in the single oral administration to the animals with experimental diabetes mellitus in the doses taken at equimolar concentrations, the compounds under study have a pronounced antithrombotic effect and are superior to the reference drug, acetylsalicylic acid.

Investigation of leading compounds and acetylsalicylic acid effect on bleeding time from tail vein of animal with diabetes mellitus

In the control group of the animals, the bleeding time was 177.1±17.45 seconds. The studied derivatives in the doses taken in equimolar concentrations, prolonged the bleeding time. The reference drug increased the bleeding time by 81%; it was 320.0±18.29 seconds.

In the group of the animals administered with the compound under the laboratory code of C-60, the bleeding time was prolonged up to 237.9±11.54 seconds, which was 34% slower than in the control group, and 26% faster than in the group of the animals administered with the reference drug.

Compound C-61 increased bleeding time up to 285.7±19.29 seconds; which was 61% slower than in the animals administered with saline, and 11% faster than in the group of the animals administered with acetylsalicylic acid. The results are shown in Fig. 7.

CONCLUSION

According to the results of the study *(in vitro),* it was found out that the compound under the laboratory code of C-60 exhibits a pronounced antiplatelet activity, in *in vivo* studies it prolongs a thrombus formation. Compound C-60 has a more pronounced antiplatelet and antithrombotic effect than the reference drug. The safety of new derivatives of hydroxybenzoic amino acids, in terms of the effect on the bleeding rate, is comparable to the ASA indicator.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

CONTRIBUTION OF AUTHORS

A.K. Brel, Y.N. Budaeva, S.V. Lisin – methodology for synthesis of compounds, interpretation of results, text writing; N.V. Atapina, S.S. Tsaruk – research methodology, statistical processing of results, interpretation of results, text writing; D.V. Kurkin – work on the concept and design of research, research methodology, interpretation and of results visualization, text writing; I.N. Tyurenkov – work on the concept and design of research, interpretation and visualization of the results.

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