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*RESEARCH ARTICLE*

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# ANTIMICROBIAL ACTIVITY OF WATER-ETHANOLIC EXTRACTIONS FROM *QUERCUS ROBUR* L. LEAVES AND BUDS

**N.A. Ryabov, V.M. Ryzhov, V.A. Kurkin, S.D. Kolpakova, A.V. Zhestkov, A.V. Lyamin**

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The problem of finding new antimicrobial drugs based on medicinal plant raw materials in modern pharmaceutical practice, is still relevant. There are interesting plant objects that have an antimicrobial action due to the content of a complex of biologically active substances in them. *Quercus robur* L. is a promising plant object, medicinal plant raw materials of which can be used for the development of new antimicrobial drugs.

**The aim** of the study is screening of the antimicrobial activity of water-ethanolic extractions from *Quercus robur* L. leaves and buds.

**Materials and methods.** The determination of the minimum inhibitory concentration was carried out by the method of double serial dilutions in Mueller-Hinton nutrient broth (Bio-Rad, USA). As test cultures, strains of microorganisms of the American Type Culture Collection (ATCC) were used: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), as well as *Candida albicans* (a clinical strain). The incubation was carried out at the temperature of 35°C for 24 hours. Simultaneously, an experiment was carried out to establish a "negative" control. The results were assessed visually by the presence / absence of the growth of microorganisms in test tubes with the corresponding dilutions of the test samples.

**Results.** In the course of the study, it was found out that-water-ethanolic extractions of *Quercus robur* L. leaves have the greatest antimicrobial effect against strains of *Staphylococcus aureus* and *Escherichia coli*. The water-ethanolic extractions of *Quercus robur* L. buds exhibit a pronounced antimicrobial activity against *Pseudomonas aeruginosa* and *Candida albicans* strains.

It was revealed that the preparation of *Quercus robur* L. leaves tincture in the raw material:extractant ratio of 1:5 has a pronounced antimicrobial effect on the strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and with a higher multiplicity of dilution – on the strains of *Escherichia coli* and *Candida albicans*. The drug tincture of *Quercus robur* L. buds in the raw material:extractant ratio of 1:5 has a pronounced antimicrobial effect on the strains of microorganisms *P. aeruginosa, S. aureus, E. coli* and *C. albicans* in an eight-fold dilution. With respect to *P. aeruginosa* strains, antimicrobial activity was observed in 16-fold dilutions. The most pronounced antimicrobial effect was recorded against the *C. albicans* strain in a 32-fold dilution.

As a result of the study, it can be concluded that to obtain the antimicrobial drugs – tincture of *Quercus robur* L. leaves and buds – is advisable to use the optimal extractant – 70% alcohol in a raw material:extractant ratio of 1:5. With these parameters of extraction, the greatest antimicrobial effect is observed in relation to the studied strains of the microorganisms. 70% alcohol has also a better penetrating ability into the deep layers of the epidermis in comparison with higher concentrations.

**Conclusion.** The results of the screening analysis of the antimicrobial activity will be used as a justification for the introduction of antimicrobial drugs based on the leaves and buds of the *Quercus robur* L. in a medical and pharmaceutical practice.

**Key words:** English oak; *Quercus robur* L.; leaves; buds; water-ethanolic extractions; tincture; minimum inhibitory concentration; antimicrobial activity

**List of abbreviations:** ATCC – American Type Culture Collection; MRSA – Methicillin-resistant Staphylococcus aureus; CLSI – Clinical and Laboratory Standards Institute; MRS strains – Methicillin-resistant Staphylococcus; MIC – Minimum inhibitory concentration; SP RF – State Pharmacopoeia of the Russian Federation; CFU / ml – Colony forming units / ml; SMR-1547 – Index Herbarium of the Samara State University, Department for Ecology, Botany and Nature Protection Faculty of Biology; G, MUK – "Guidelines", "Methodical instructions"; *Q.* – *Quercus* L. (eg, *Q. robur*). – English oak

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# АНТИМИКРОБНАЯ АКТИВНОСТЬ ВОДНО-СПИРТОВЫХ ИЗВЛЕЧЕНИЙ ЛИСТЬЕВ И ПОЧЕК ДУБА ЧЕРЕШЧАТОГО (*QUERCUS ROBUR* L.)

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Проблема поиска новых противомикробных препаратов на основе лекарственного растительного сырья в современной фармацевтической практике является по-прежнему актуальной. Интерес представляют растительные объекты, обладающие антимикробным действием благодаря содержанию в них комплекса биологически активных веществ. Дуб черешчатый – *Quercus robur* L. является перспективным растительным объектом, лекарственное растительное сырье которого может быть использовано при разработке новых антимикробных препаратов.

**Цель.** Проведение скрининга антимикробной активности водно-спиртовых извлечений листьев и почек дуба черешчатого.

**Материалы и методы.** Определение минимальной ингибирующей концентрации проводилось методом двойных серийных разведений в питательном бульоне Мюллера-Хинтона (Bio-Rad, США). В качестве тестовых культур были использованы штаммы микроорганизмов Американской коллекции типовых культур (АТСС): *Staphylococcus aureus* (АТСС 29213), *Escherichia coli* (АТСС 25922), *Pseudomonas aeruginosa* (АТСС 27853), а также *Candida albicans* (клинический штамм). Инкубацию проводили при температуре 35°С в течение 24 часов. Параллельно проводился опыт для постановки «отрицательного» контроля. Оценку результатов проводили визуально по наличию/отсутствию роста микроорганизмов в пробирках с соответствующими разведениями исследуемых образцов.

**Результаты.** В ходе проведенного исследования установлено, что водно-спиртовые извлечения листьев дуба черешчатого оказывают наибольший антимикробный эффект в отношении штаммов *Staphylococcus aureus* и *Escherichia coli.* Водно-спиртовые извлечения почек дуба черешчатого проявляют выраженную антимикробную активность в отношении штаммов *Pseudomonas aeruginosa* и *Candida albicans.*

Выявлено, что препарат настойка листьев дуба черешчатого в соотношении «сырье – экстрагент» (1:5) обладает выраженным антимикробным эффектом на штаммы *Pseudomonas aeruginosa, Staphylococcus aureus,* а при большей кратности разведения на штаммы *Escherichia coli* и *Candida albicans.* Препарат настойка почек дуба черешчатого в соотношении «сырье – экстрагент» (1:5) обладает выраженным антимикробным эффектом в отношении штаммов микроорганизмов *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* и *Candida albicans* при восьмикратном разведении. В отношении штаммов *Pseudomonas aeruginosa* антимикробная активность наблюдалась при 16 кратном разведении. Максимально выраженный антимикробный эффект был зафиксирован в отношении штамма *Candida albicans при 32-кратном разведении*.

В результате проведенного исследования можно сделать вывод о том, что для получения противомикробных препаратов – настойки листьев и почек дуба черешчатого, целесообразно использовать в качестве оптимального экстрагента спирт 70% в соотношении «сырье – экстрагент» (1:5). При данных параметрах экстракции отмечается наибольший антимикробный эффект в отношении изучаемых штаммов микроорганизмов. Также спирт 70% обладает лучшей проникающей способностью в глубокие слои эпидермиса по сравнению с более высокими концентрациями.

**Заключение.** Полученные результаты скринингового анализа антимикробной активности будут использованы в качестве обоснования для внедрения антимикробных препаратов на основе листьев и почек дуба черешчатого в медицинскую и фармацевтическую практику.

***Ключевые слова:*** Дуб черешчатый; *Quercus robur* L.; листья; почки; водно-спиртовые извлечения; настойка; минимальная ингибирующая концентрация; антимикробная активность

**Список сокращений:** АТСС – Американская коллекция типовых культур (American Type Culture Collection); MRSA – Метициллинрезистентный золотистый стафилококк (Methicillin-resistant *Staphylococcus aureus*); CLSI – Clinical and Laboratory Standards Institute; MRS-штаммы – Метициллин-резистентные стафилококки (Methicillin-resistant *Staphylococcus*); МИК – Минимальная ингибирующая концентрация; ГФ РФ – Государственная Фармакопея Российской Федерации; КОЕ/мл – Колониеобразующие единицы / мл; SMR-1547 – Индекс гербария Самарского государственного университета, кафедра экологии, ботаники и охраны природы, биологического факультета; МУК – Методические указания; *Q.* – *Quercus* L. (н-р, *Q. robur*)

## INTRODUCTION

Currently, obtaining new antimicrobial drugs based on plant raw materials is an urgent task of modern pharmacy. The spread of antimicrobial resistance poses a serious danger, which reduces the effectiveness of measures for the prevention and treatment of human infectious diseases [1, 2].

In terms of the search for new antimicrobial drugs, a promising object is a representative of the genus *Quercus* L. – English oak (*Q. robur* L.). In the *Quercus* L. genus, there are more than 500 species from the temperate and subtropical regions of the Northern Hemisphere. In Russia, 19 species grow in the wild, about 50 species have been introduced [3, 4]. *Quercus* L. is one of the most

important forest-forming species in Europe and the European part of Russia [3, 4]. *Q. robur* L. is rather widely used in folk medicine as a remedy for the prevention and treatment of diseases of the gastrointestinal tract, gynecological diseases, as well as for otorhinolaryngological and dermatological diseases. An official classic medicinal product of *Q. robur* L. is a decoction of the bark as an agent with astringent and anti-inflammatory properties [5–7]. Recently, a large number of investigations have been carried out to study the antimicrobial properties of *Q. robur* L. barks, as well as the preparations based on it [8–16]. *Q. robur* L. bark is a part of many complex preparations, such as «Stomatofit», «Tonsilgon N», «Dentos», etc., and it is also used to obtain extracts for medical and cosmetic purposes[1]. In addition to tannins, *Q. robur* L. bark contains triterpenes (Fridelin, Fridelinol, 3-Fridelanol), flavonoids such as quercetin, quercitrin, leukoanthocyanidin, etc. [13, 17–19]. As a group of biologically active compounds, flavonoids have a number of valuable pharmacological properties, such as anti-inflammatory, diuretic, choleretic, antispasmodic, antiviral, antimicrobial, etc.[2] [18–20]. Earlier, in order to search for new antibacterial agents, a study of *Quercus incana* species was carried out. During it, two substances were isolated: 4-hydroxydecanoic acid and 4-hydroxy-3-(hydroxymethyl) pentanoic acid. The isolated compounds were tested for the antifungal activity against *Aspergillus niger* and *Aspergillus favus*. The antibacterial activity of the isolated compounds was determined by diffusion into agar wells. The compound 4-hydroxydecanoic acid exhibited a great antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus* (gram-positive).

The obtained compounds showed an antibacterial activity against *Staphylococcus aureus* with an inhibition zone of 16 mm and 13 mm. The compound 4-hydroxy-3-(hydroxymethyl) pentanoic acid was moderately active against *Bacillus subtilis* and *Micrococcus luteus* with the inhibition zone of 5 mm and 9 mm. Both compounds were inactive against *Escherichi coli* and *Shigella fexneri* [11].

A profile of polyphenols in extracts obtained from the bark of *Q. robur*, *Q. macrocarpa* and *Q. Acutissima* was studied. As a result of it, antioxidant, antibacterial, antifungal and antitumor activities were revealed. In comparison with extracts of other species, *Q. robur* exhibited a significant antimicrobial activity against *Pseudomonas aeruginosa* [13].

A study to determine the antimicrobial activity of a *Q. robur* bark methanol extract (a solution of 80% methanol in water) was carried out. It was tested by diffusion in agar for *Staphylococcus aureus, Enterobacter aerogenes,* and *Candida albicans* strains [14]. As a result of the study, the possibility of using oak bark extracts as a bactericidal agent against *Staphylococcus aureus* strains and a bacteriostatic agent against *Enterobacter aerogenes* was determined [14]. Lipophilic extracts were active against the strains of *Staphylococcus aureus, Enterobacter aerogenes*, and *Candida albicans* [14].

In addition to water-ethanolic extractions of the *Q. robur* L. barks, water-ethanolic extractions from other types of this plant raw materials such as oak leaves and buds, are of interest. At the moment, a sufficient number of studies have been carried out to investigate *Q. robur* L. leaves by both Russian and foreign scientists, whose attention was mainly focused on the study of morphological and anatomical features, a chemical composition and antimicrobial properties [21–24]. The leaves of the *Quercus* representatives are promising objects for obtaining drugs with antimicrobial properties [21–24]. In the authors' opinion, the study of *Q. robur* L. buds, along with leaves, is also an urgent direction, because the buds of some plants are capable of exhibiting an antimicrobial activity and have a valuable chemical composition [25, 26].

One of the serious factors in the successful treatment of the infectious diseases, is a decrease in the resistance of pathogenic microorganisms to antimicrobial drugs [9, 27]. Staphylococci or methicillin-resistant strains (MRS strains), which are the cause of nosocomial and community-acquired infections, are of particular interest. Among the MRS strains, *Staphylococcus aureus* (MRSA) is most often found, its strains are resistant to many members of the β-lactam antibiotics group, including penicillins, cephalosporins, monobactams, carbapenems, etc. [9, 27]. The gram-negative bacterium *E. coli,* which is present in the human intestine and can cause various infectious diseases of the gastrointestinal tract and genitourinary system, is not a less dangerous strain [27, 28]. The study of the antimicrobial properties of water-ethanolic extractions and preparations based on the leaves and buds of the *Q. robur* L. will expand the spectrum of *Q. robur* pharmacological activities, and assess the possibilities of using this object in the creation of drugs in antibacterial therapy.

For an objective assessment of the antimicrobial activity of the studied raw materials, it is necessary to conduct a screening analysis of water-ethanolic extractions and determine the minimum inhibitory concentration (MIC) in relation to the main clinically significant strains of the microorganisms.

**THE AIM** of the study was to screen the antimicrobial activity of water-ethanolic extractions from the leaves and buds of *Quercus robur* L.

The research tasks included.

1. Screening analysis of the antimicrobial activity of water-ethanolic extractions of *Q. robur* L. leaves and buds;

2. Determination of the optimal concentration of

[1] State Register of Medicines. Available online: https://www.rlsnet.ru/tn_index_id_6283.htm

[2] Assessment report on Quercus robur L., Quercus petraea (Matt.) Liebl., Quercus pubescens Willd., cortex EMA/HMPC/3206/2009. Available online: https://www.ema.europa.eu/en

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the extractant and the conditions for the extraction of raw materials for the creation of preparations based on *Q. robur* L. plant raw materials.

## MATERIALS AND METHODS

The objects of the study were water-ethanolic extractions of *Q. robur* L. leaves and buds at various concentrations of a chemically pure ethanol grade (40%, 70%, 80%, 96%) (alcohol 96%, ZAO "Hippocrat", Russia, Samara, series: 360917). The preparations of *Q. robur* L. leaves tincture and *Q. robur* L. buds tincture 70% alcohol in the raw materials: extractant ratio of 1:5, were also obtained by the method of fractional percolation. The required alcohol concentration was obtained by diluting 96% alcohol according to Table No. 5 of the appendix to the State Pharmacopoeia of the Russian Federation of the XIV edition [7]. For most flavonoid-containing plants, the optimal extractant is 70% ethanol, since this concentration of ethyl alcohol allows the maximum amount of flavonoids in the plant to be extracted and has a better penetrating ability into the deep layers of the epidermis compared to higher concentrations [7, 18, 19].

### Analyzed samples of raw materials

The leaves of *Q. robur* L. were harvested from May to July 2020 (Samara region, Pohvistnevsky district, Pervomaiskaya Str., 2020). The buds of *Q. robur* L. were harvested from March to April 2020 (Samara region, Pohvistnevsky district, Pervomaiskaya Str., 2020).

The species specificity of the analyzed objects was confirmed with the help of the identifiers of the Russia central zone [3, 4]. In addition to the identifiers, the method of comparison with reliably known samples of the herbarium fund of Samara University was used. The inventory number of the main herbarium specimen is SMR-1547 (Herbarium Department for Ecology, Botany and Nature Protection Faculty of Biology, Samara University, 2021)[3].

### Test cultures

The strains of the American Type Cultures Collection (ATCC) were used as test cultures: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), as well as *Candida albicans* (clinical strain).

### Research methods

The MIC was determined by the method of double serial dilutions in broth (a tube test, a macro method) in accordance with the methods described in "Guidelines" (G) 4.2.1890-04 [27, 29]. The method of double serial dilutions in comparison with diffusion method allows a qualitative assessment of the presence of the antimicrobial effect by visual assessment in comparison with the standard, and determination of the minimum inhibitory concentration of the studied sample, which slows down the growth of the studied strains of the microorganisms [27, 29]. Mueller-Hinton nutrient broth (Bio-Rad, USA) was used as a nutrient medium [29].

### Methods

Testing of the studied samples was carried out in the volume of 1 ml of each sample dilution in water-ethanolic extractions.

### Preparation of working solution

To determine the sensitivity, the nutrient broth was poured 0.5 ml into each tube. In addition to the number of tubes required to dilute the sample, one tube was used to set up a "negative" control. A working solution of a test sample was prepared from a stock solution using a liquid nutrient medium (Mueller-Hinton nutrient broth). The concentration of the working solution was calculated based on the required maximum concentration in a series of dilutions, taking into account the dilution factor of the drug during the subsequent inoculation [27].

Using a micropipette with a sterile tip, the working solution in the amount of 0.5 ml was introduced into the first tube containing 0.5 ml of broth. Then it was thoroughly mixed, and with a new sterile tip, 0.5 ml of the test solution in broth was transferred into the second tube containing initially 0.5 ml of broth. The procedure had been repeated until the entire required dilution series was prepared. 0.5 ml of broth was removed from the last test tube. Thus, a number of test tubes with the solutions of the tested samples of the water-ethanolic extractions from *Q. robur* L. leaves and buds were obtained, the concentrations of which differed in the adjacent test tubes by a factor of 2. Simultaneously, additional series of serial sample dilutions were prepared for testing control strains [27].

### Inoculum preparation

For inoculation, a standard microbial suspension was used. It was equivalent to 0.5 according to McFarland's standard, diluted 100 times in nutrient broth. After that, the concentration of the microorganism in it would be approximately $10^6$ CFU/ml. 0.5 ml of inoculum was introduced into each tube containing 0.5 ml of the corresponding dilution of the test sample, and into one tube with 0.5 ml of nutrient broth without a sample ("negative" control). The final concentration of the microorganism in each tube reached the required concentration of about $5\times10^5$ CFU/ml. The inoculum was introduced into test tubes with sample dilutions not later than 15–30 min from the moment of its preparation [27].

[3] Herbarium Department for Ecology, Botany and Nature Protection Faculty of Biology, Samara University. SMR-1547. Available from: http://sweetgum.nybg.org/science/vh/collection-index/collection-index-details/?irn=124749.

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**Table 1 – Results of testing extracts from Q. robur L. leaves and buds**

| Object / Microorganism | Dilution ratio * | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| Pseudomonas aeruginosa | | | | | | | |
| *Q. robur* L. leaves 40% | – | – | + | + | + | + | + |
| *Q. robur* L. leaves 70% | – | – | – | + | + | + | + |
| *Q. robur* L. leaves 80% | – | – | – | – | + | + | + |
| *Q. robur* L. leaves 96% | – | – | – | + | + | + | + |
| *Q. robur* L. buds 40% | – | – | – | + | + | + | + |
| *Q. robur* L. buds 70% | – | – | – | – | + | + | + |
| *Q. robur* L. buds 80% | – | – | – | – | – | + | + |
| *Q. robur* L. buds 96% | – | – | – | – | – | + | + |
| Tincture of *Q. robur* L. leaves 70% | – | – | – | – | + | + | + |
| Tincture of *Q. robur* L. buds 70% | – | – | – | – | – | – | + |
| Staphylococcus aureus | | | | | | | |
| *Q. robur* L. leaves 40% | – | – | – | + | + | + | + |
| *Q. robur* L. leaves 70% | – | – | – | – | – | + | + |
| *Q. robur* L. leaves 80% | – | – | – | – | + | + | + |
| *Q. robur* L. leaves 96% | – | – | – | – | + | + | + |
| *Q. robur* L. buds 40% | – | – | – | + | + | + | + |
| *Q. robur* L. buds 70% | – | – | – | + | + | + | + |
| *Q. robur* L. buds 80% | – | – | – | – | + | + | + |
| *Q. robur* L. buds 96% | – | – | – | – | + | + | + |
| Tincture of *Q. robur* L. leaves 70% | – | – | – | – | + | + | + |
| Tincture of *Q. robur* L. buds 70% | – | – | – | – | + | + | + |
| Escherichia coli | | | | | | | |
| *Q. robur* L. leaves 40% | – | – | – | + | + | + | + |
| *Q. robur* L. leaves 70% | – | – | + | + | + | + | + |
| *Q. robur* L. leaves 80% | – | – | – | – | – | – | + |
| *Q. robur* L. leaves 96% | – | – | – | – | – | + | + |
| *Q. robur* L. buds 40% | – | – | – | + | + | + | + |
| *Q. robur* L. buds 70% | – | – | – | + | + | + | + |
| *Q. robur* L. buds 80% | – | – | – | – | – | + | + |
| *Q. robur* L. buds 96% | – | – | – | – | + | + | + |
| Tincture of *Q. robur* L. leaves 70% | – | – | – | – | – | + | + |
| Tincture of *Q. robur* L. buds 70% | – | – | – | – | – | + | + |
| Candida albicans | | | | | | | |
| *Q. robur* L. leaves 40% | – | – | – | + | + | + | + |
| *Q. robur* L. leaves 70% | – | – | – | + | + | + | + |
| *Q. robur* L. leaves 80% | – | – | – | – | + | + | + |
| *Q. robur* L. leaves 96% | – | – | – | – | + | + | + |
| *Q. robur* L. buds 40% | – | – | – | – | – | + | + |
| *Q. robur* L. buds 70% | – | – | – | – | – | + | + |
| *Q. robur* L. buds 80% | – | – | – | – | + | + | + |
| *Q. robur* L. buds 96% | – | – | – | – | – | + | + |
| Tincture of *Q. robur* L. leaves 70% | – | – | – | – | – | – | + |
| Tincture of *Q. robur* L. buds 70% | – | – | – | – | – | + | + |

Note: + presence of microorganism growth; absence of microorganism growth

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**Table 2 – Minimum inhibitory concentrations of ethanol ("negative" control)**

| Object / Microorganism | Dilution ratio * | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 |
| Pseudomonas aeruginosa | | | | | | | |
| Ethanol 40% | – | – | + | + | + | + | + |
| Ethanol 70% | – | – | + | + | + | + | + |
| Ethanol 80% | – | – | – | + | + | + | + |
| Ethanol 96% | – | – | + | + | + | + | + |
| Staphylococcus aureus | | | | | | | |
| Ethanol 40% | – | – | – | + | + | + | + |
| Ethanol 70% | – | – | – | + | + | + | + |
| Ethanol 80% | – | – | – | + | + | + | + |
| Ethanol 96% | – | – | + | + | + | + | + |
| Escherichia coli | | | | | | | |
| Ethanol 40% | – | – | – | + | + | + | + |
| Ethanol 70% | – | – | – | – | + | + | + |
| Ethanol 80% | – | – | – | + | + | + | + |
| Ethanol 96% | – | – | + | + | + | + | + |
| Candida albicans | | | | | | | |
| Ethanol 40% | – | – | – | + | + | + | + |
| Ethanol 70% | – | – | – | + | + | + | + |
| Ethanol 80% | – | – | – | + | + | + | + |
| Ethanol 96% | – | – | – | + | + | + | + |

Note: + presence of microorganism growth; – absence of microorganism growth

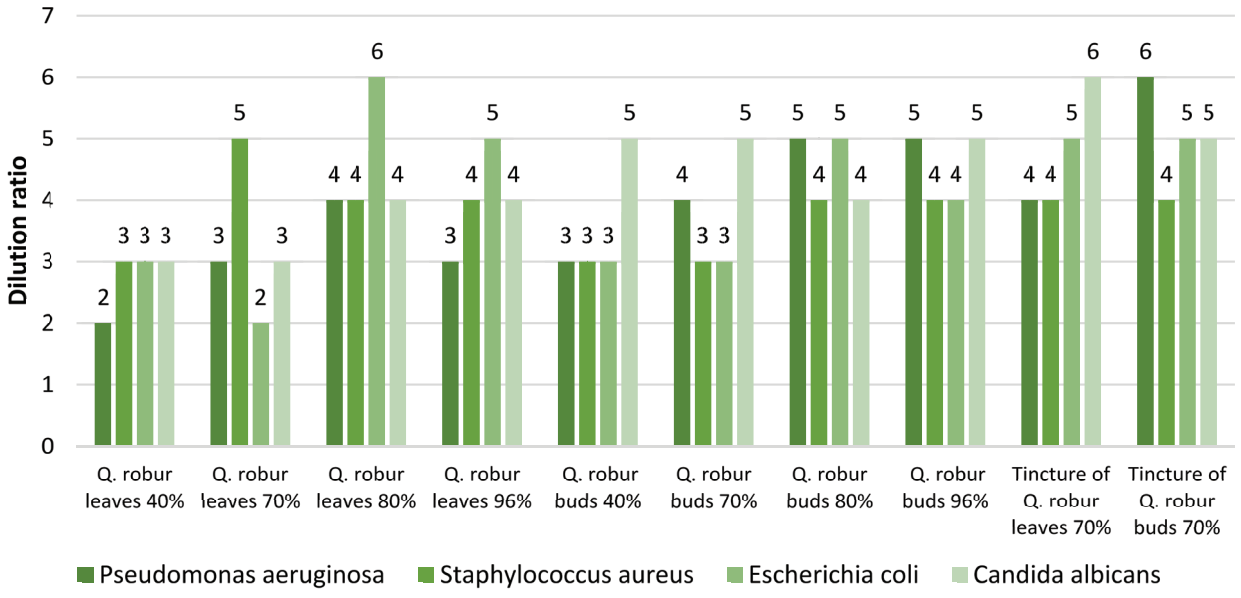


**Figure 1 – Comparative diagram of antibacterial activity of water-ethanolic extractions of *Q. robur* L. leaves and buds (the abscissa is the serial number of the dilution ratio)**

The tubes were closed with sterile gauze and cotton stoppers, and all tubes with the tested strains, except for the tube with the "negative" control, were incubated at 35℃ for 20–24 hours. The tube with the "negative" control was placed in a refrigerator at 4°C, and stored until the results were taken into account [27].

### Assessment of microorganisms growth

To determine the presence of the microorganism growth, the test tubes with crops were viewed in transmission. The growth of the culture in the presence of the test sample was carried out by comparison with the tube of the "negative" control containing the original inoculum and stored in the refrigerator. MIC was determined by the lowest concentration of the test sample, which suppresses the visible growth of the microorganism [27].

### Assessment of experimental results

The results were assessed visually by the presence/absence of the microorganisms growth of in test tubes with the appropriate dilutions of the test samples [27]. The minimum inhibitory concentration was the lowest concentration of the studied sample, which completely suppressed the growth of the microorganisms strain. At the same time, according to the requirements of the "Guidelines" (G 4.2.1890-04)[4] for determining the sensitivity of microorganisms to the antibacterial drugs, as well as the recommendations of the Performance Standard for Antimicrobial Susceptibility Tests (Clinical And Laboratory Standards Institute (CLSI))[5], the presence of turbidity, and the detection of a small number of the microorganisms (one colony) were not taken into account when registering the experimental result. The experiment was repeated three times [27, 29].

## RESULTS AND DISCUSSIONS

During the screening of the antimicrobial activity of the extracts from *Q. robur* L. leaves and buds, the following results were obtained.

When testing 40% water-ethanolic extractions of *Q. robur* L. leaves, the antimicrobial activity against the *P. aeruginosa* strain in a four-fold dilution, as well as against microorganisms *S. aureus, E. coli,* and *C. albicans* in an eight-fold dilution, was observed (Table 1). When comparing 40% water-ethanolic extractions with the "negative" standard (a minimum inhibitory concentration for 40% water-ethanol), no differences in the antimicrobial activity between the test sample and the reference sample were observed (Table 2). This indicates that there is no contribution of the complex of biologically active compounds available in the extract, to the pharmacological effect at the given extraction concentration.

For 70% water-ethanolic extractions from *Q. robur* L. leaves, the antimicrobial activity was expressed against *P. aeruginosa* in an eight-fold dilution; against *S. aureus* – when diluted 32 times; for the *C. albicans* strain – in an eight-fold dilution. Growth inhibition of the *E. coli* strain was observed in a two-fold dilution (Table 1).

An antimicrobial activity of 80% water-ethanolic extractions of *Q. robur* L. leaves was expressed against *E. coli* when diluted 64 times; against *S. aureus, C. albicans* and *P. aeruginosa* – in a 16-fold dilution (Table 1). For water-ethanolic extractions from leaves with 80% ethanol, the maximum growth retardation of microorganisms is observed. When compared with the "negative" standard of ethanol at the concentration of 80%, a significant growth inhibition of microorganisms is observed (Table 1; Table 2).

An antimicrobial activity of 96% water-ethanolic extractions of *Q. robur* L. leaves was expressed against all strains in an eight-fold dilution; for *S. aureus*, *C. albicans* and *E. coli* in a 16-fold dilution; with respect to *E. coli*, the highest activity was observed in a 32- fold dilution (Table 1). The indices of the "negative" control for 96% ethanol concentration, were significantly lower compared to the test sample (Table 2).

The analysis of 40% water-ethanolic extractions from *Q. robur* L. buds gives less antimicrobial effect in comparison with 40% water-ethanolic extractions from *Quercus robur* L. leaves (Table 1; Table 2). In particular, the extraction from the buds at the given concentration of ethanol is expressed in relation to strains of *P. aeruginosa, S. aureus, E. coli* and *C. albicans* in an eight-fold dilution; along with other strains, in 32-fold dilution, a more pronounced activity was observed against the *C. albicans* strain (Table 1).

During testing of 70% water-ethanolic extractions from *Q. robur* L. buds, a pronounced antimicrobial activity was noted against the strains of such microorganisms as *P. aeruginosa, S. aureus, E. coli* and *C. albicans* in eight-fold dilution. With respect to *P. aeruginosa* strains, an antimicrobial activity was observed in 16-fold dilutions. The most pronounced antimicrobial effect was recorded against the *C. albicans* strain in 32 fold dilutions (Table 1).

An antimicrobial activity of 80% water-ethanolic extractions of *Q. robur* L. buds was observed against the *E. coli* strain when diluted 32 times, as well as against the *S.aureus, C. albicans* and *P. aeruginosa* strains when diluted 16, 16, and 32 times, respectively (Table 1). When compared with the "negative" standard of ethyl alcohol at the concentration of 80%, a significant growth inhibition of microorganisms is observed (Table 1; Table 2).

The study of the antimicrobial activity of 96% wa-

[4] Determination of the sensitivity of microorganisms to antibacterial drugs. Guidelines. 4.2.1890-04. Clinical microbiology and antimicrobial chemotherapy. 2004; 6 (4): 306–359. The guidelines were approved and put into effect by the Chief State Sanitary Doctor of the Russian Federation – First Deputy Minister of Health of the Russian Federation G.G. Onishchenko, March 4, 2004.

[5] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. 13th ed. CLSI standard M02. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2018.

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ter-ethanolic extractions from *Q. robur* L. buds gave the following results: a distinct antimicrobial activity against all strains in a 16-fold dilution; against *P. aeruginosa* and *C. albicans*, the activity was also observed in 32 – fold dilutions (Table 1).

During the screening analysis of water-ethanolic extractions of *Q. robur* L. leaves and buds, the conditions for obtaining the dosage form of the tincture were determined. 70% ethanol was chosen as an extractant for the manufacture of tincture from *Q. robur* L. leaves and tincture from *Q. robur* L. buds, since this concentration is the optimal extractant for raw materials containing a complex of biologically active substances of the flavonoid group that provide an antimicrobial effect of the drug [15, 20].

The tested tincture from *Q. robur* L. leaves in 70% ethanol showed the following results. The antimicrobial effect was observed for all of these strains. In particular, in a sixteen-fold dilution, the antimicrobial activity is observed against the *P. aeruginosa* strain and the *S. aureus* strain; when diluted 32 times – against *E. coli,* and when diluted 64 times – against *C. albicans* (Table 1).

In the course of testing the preparation of *Q. robur* L. bud tincture in 70% ethanol, the following results were obtained: the antimicrobial effect was observed against the *P. aeruginosa* strain when diluted 64 times; at sixteen-fold dilution, is observed against the *S. aureus* strain in a sixteen-fold dilution; the antimicrobial activity against *E. coli* and *C. albicans* took place when diluted 32 times (Table 1).

Thus, a screening study was carried out to research the antimicrobial activity of water-ethanolic extractions from *Q. robur* L. leaves and buds, as a result of which an antimicrobial effect on a number of pathogenic microorganisms strains (*P. aeruginosa; S. aureus; E. coli; C. albicans)* was found out *in vitro*:.

According to the results of the work performed, it established that all the studied samples of water-ethanolic extractions from *Q. robur* L. leaves and buds, give a stable antimicrobial effect against *S. aureus* and *C. albicans* strains.

The water-ethanolic 80% extract from *Q. robur* L. leaves and buds in the raw material:extractant ratio of 1:50 and preparations of tincture ~~of~~ from *Q. robur* L. leaves and buds in 70% alcohol in the raw material:extractant ratio of 1: 5, are the most effective objects, and give the maximum growth retardation of the microorganisms when diluted 32 and 64 times.

A sophisticated action with the maximum antimicrobial effect on *C. albicans* is provided by the preparation of a tincture from *Q. robur* L. leaves in the raw material:extractant ratio of 1: 5; for *S. aureus* strains – 70% water-ethanolic extractions in the raw material:extractant ratio of 1: 50 (Fig. 1). Water-ethanolic extractions from *Q. robur* L. buds have a similar stable maximum antimicrobial effect against *C. albicans*. For *P. aeruginosa, S. aureus* and *E. coli* strains, the maximum antimicrobial effect is noted in the extractant concentration of 96% ethyl alcohol.

The minimum antimicrobial activity is noted for 70% water-ethanolic extractions from the leaves of *Q. robur* L. in the raw material:extractant ratio of 1: 50 for *E. coli* strains, as well as for 40% water-ethanolic extractions from the *Q. robur* L buds in the raw material:extractant ratio of 1: 50 for *P. aeruginosa strains* (Fig. 1).

The choice in favor of 70% alcohol concentration as an extractant for obtaining tincture from the oak leaves and buds was made on the basis that for dosage forms with these extraction parameters, the greatest antimicrobial effect is observed against the studied strains of microorganisms (Fig. 1). Also, extracts at a given alcohol concentration have a better penetrating ability into the deep layers of the epidermis in comparison with higher and lower alcohol concentrations[6]. It should be noted that the leaves have a large phytomass in comparison with the buds, their collection can be carried out in a longer period of time than for the buds, the collection time of which, as a rule, falls in the winter-spring period [7].

## CONCLUSION

All the results obtained in the course of the study, can serve for the creation of new antibacterial drugs based on *Q. robur* L. leaves and buds, as well as for the introduction of tincture preparations from *Q. robur* L. leaves and buds in 70% alcohol into medical and pharmaceutical practice.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest related to the publication of this article.

[6] State Register of Medicines. Available from: https://www.rlsnet.ru/tn_index_id_6283.htm.

[7] State Register of Medicines. Available from: https://www.rlsnet.ru/tn_index_id_6283.htm.

## AUTHORS' CONTRIBUTION

Nikolay A. Ryabov – data collecting, experiment conducting, analyzing and interpreting the data obtained, preparing a draft manuscript, analyzing the literature, writing a manuscript and finally approving of it for publication; Vitaly M. Ryzhov – planning of the study, participation in the development of the concept and design of the study, collection of plant material for analysis; Vladimir A. Kurkin – final approval of the manuscript for publication, processing the results obtained, verification of critical intellectual content; Svetlana D. Kolpakova – participation in research, literature analysis; Alexander V. Zhestkov – participation in the description and analysis of the results obtained, participation in manuscript writing and its final approval for publication; Artem V. Lyamin – participation in the writing of the manuscript, critical analysis of the research.

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# CHARACTERISATION AND STUDY OF 1- [2- (2-BENZOYLPHENOXY) ETHYL] -6-METHYLURACIL MECHANISM OF ACTION

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**The aim** of the study is to identify 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil using various methods of analysis, as well as to study its action mechanism against wild-type and mutant forms of HIV-1 reverse transcriptase (RT).

**Materials and methods.** To characterize the structure of the test substance, a few kinds of analysis (X-ray diffraction, elemental, thermal) as well as a few kinds of spectroscopy (UV, IR, and NMR) have been used. The study of the action mechanism of the compound as a potential drug was carried out by evaluating the inhibitory activity against HIV-1 RT wild-type and its mutant forms corresponding to drug-resistant viral strains.

**Results.** The studies have been carried out to confirm the structure of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil. The UV spectrum has a pronounced absorption maximum when measuring a solution of the substance in tetrahydrofuran at the concentration of 0.10 mg/ml. In the IR spectrum, there are specific bands in the range of 4000-370 $cm^{-1}$. These factors make it possible to use UV and IR spectra to identify the test compound in the substance. It has also been established that the number and mutual arrangement of functional groups, the integrated intensity of signals in the 1H-NMR spectrum, as well as the structure of the carbon skeleton, correspond to the structure of 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil. The results of studying the action mechanism showed that the test compound is an effective inhibitor of wild-type HIV-1 RT with an inhibition constant of 0.2 μM, as well as an enzyme inhibitor (mutation G190A) with an inhibition constant of 8 μM; enzyme (mutation Y181C) with an inhibition constant of 10 μM, as well as a reverse transcriptase (RT) inhibitor (mutation L100I, K103N, V106A) and a double mutant K103N / Y181C with an inhibition constant of more than 20 μM.

**Conclusion.** As a result of the performed X-ray structural, elemental, $^{1}$H-NMR and $^{13}$C-NMR analyzes, the structure of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil has been confirmed. The possibility of using UV, IR and NMR spectroscopy, as well as thermal analyzes to confirm the authenticity during the verification of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil, has been shown. The developed methods can be used in the quality control and included in the draft of practice guidelines for the investigated substance. The studies of the action mechanism of the compound of HIV-1 RT reverse transcriptase have shown that this compound belongs to the group of non-nucleoside reverse transcriptase inhibitors (NNRTIs) of HIV-1.

**Keywords:** 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil; identification; X-ray structural analysis; thermal analysis, elemental analysis; UV spectroscopy; IR spectroscopy; NMR spectroscopy; action mechanism; HIV-1 reverse transcriptase

**Abbreviations:** TGA – thermal gravimetric analysis; DSC – differential scanning calorimetry; IR spectroscopy – infrared spectroscopy; NMR spectroscopy – nuclear magnetic resonance spectroscopy; HIV – human immunodeficiency virus; RT – reverse transcriptase; NNRTIs – non-nucleoside reverse transcriptase inhibitors

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# ХАРАКТЕРИЗАЦИЯ И ИССЛЕДОВАНИЕ МЕХАНИЗМА ДЕЙСТВИЯ 1-[2-(2-БЕНЗОИЛФЕНОКСИ) ЭТИЛ]-6-МЕТИЛУРАЦИЛА

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**Цель** – идентификация 1-[2-(2-бензоилфенокси)этил]-6-метилурацила с использованием различных методов анализа, а также исследование его механизма действия в отношении дикого типа и мутантных форм обратной транскриптазы (ОТ) ВИЧ-1.
**Материалы и методы.** Для характеризации структуры исследуемого вещества использовали рентгеноструктурный анализ, элементный анализ, термический анализ, а также УФ-, ИК- и ЯМР- спектроскопии. Изучение механизма действия соединения, как потенциального лекарственного средства, проводили путем оценки ингибирующей активности в отношении ОТ ВИЧ-1 дикого типа и ее мутантных форм, соответствующих лекарственно-устойчивым штаммам вируса.
**Результаты.** Проведены исследования, подтверждающие структуру 1-[2-(2-бензоилфенокси)этил]-6-метилурацила. УФ-спектр имеет выраженный максимум поглощения при измерении раствора субстанции в тетрагидрофуране в концентрации 0,10 мг/мл, в ИК спектре наблюдаются специфичные полосы в области 4000–370 $см^{-1}$, что позволяет использовать УФ и ИК спектры для идентификации исследуемого вещества в субстанции. Также было установлено, что количество и взаимное расположение функциональных групп, интегральная интенсивность сигналов в спектре $^{1}$H-ЯМР, а также строение углеродного скелет, соответствуют структуре 1-[2-(2-бензоилфенокси)этил]-6-метилурацила. Результаты изучения механизма действия показали, что исследуемое соединение является эффективным ингибитором ОТ ВИЧ-1 дикого типа с константой ингибирования 0,2 μM, а также ингибитором фермента (мутация G190A) с константой ингибирования 8 μM; фермента (мутация Y181C) с константой ингибирования 10 μM, а также ингибитором ОТ (мутация L100I, K103N, V106A) и двойном мутанте K103N/Y181C с константой ингибирования более 20 μM.
**Заключение.** В результате проведенных рентгеноструктурного, элементного, $^{1}$H-ЯМР и $^{13}$C-ЯМР анализов была подтверждена структура 1-[2-(2-бензоилфенокси)этил]-6-метилурацила. Показана возможность применения УФ-, ИК- и ЯМР-спектроскопии, а также термических анализов для подтверждения подлинности при входном контроле качества 1-[2-(2-бензоилфенокси)этил]-6-метилурацила. Разработанные методы могут быть использованы в контроле качества и включены в проект НД на исследуемую субстанцию. Исследования механизма действия соединения в отношении ОТ ВИЧ-1 показали, что данное соединение относится к группе ненуклеозидных ингибиторов обратной транскриптазы (ННИОТ) ВИЧ-1.
**Ключевые слова:** 1-[2-(2-бензоилфенокси)этил]-6-метилурацил; идентификация; рентгеноструктурный анализ; термический анализ, элементный анализ; УФ-спектроскопия; ИК-спектроскопия; ЯМР-спектроскопия; механизм действия; обратная транскриптаза ВИЧ-1
**Сокращения:** ТГА – термогравиметрический анализ; ДСК – дифференциальная сканирующая калориметрия; ИК-спектроскопия – инфракрасная спектроскопия; ЯМР-спектроскопия – спектроскопия ядерного магнитного резонанса; ВИЧ – вирус иммунодефицита человека; ОТ – обратная транскриптаза; ННИОТ – ненуклеозидный ингибитор обратной транскриптазы

## INTRODUCTION

The HIV pandemia is the most urgent problem and still an open challenge in the world public health. Despite the fact that, according to Rospotrebnadzor, it was possible to reduce the growth rate of new HIV infections from 13.4% in 2012 to 0.9% in 2017, the epidemiological situation remains severe.[1] Thus, according to the preliminary data, in 2019, 94,668 new cases of HIV infec-

[1] Resistance to HIV and other socially dangerous diseases: some indicators for 6 years, Government of the Russian Federation, 11.04.2018, Available from: http://government.ru/info/32200/. Russian

tion[2] were detected in the Russian Federation, and the number of people living with HIV in the world, reached approximately 38.0 million.[3]

Nevertheless, the HIV infection continues to be an incurable disease. Its danger is explained by the unique effect of the virus on the human body: the reproduction of the virus in the cells of the immune system does not only make the virus less vulnerable to the action of the latter, but also contributes to the development of other infectious diseases [1]. Consequentially, bacterial and viral diseases caused by opportunistic infections – pneumonia, herpesvirus, as well as cancer, damage to the cardiovascular, gastrointestinal and nervous systems – are often developed in HIV-infected people. It is these phenomena that are the main causes of death in the HIV-infected [2]. A modern approach to antiretroviral therapy is aimed at prolonging and improving the quality of patients' lives [3]. Currently, the best method of treating the HIV infection is highly active antiretroviral therapy (HAART), which involves the use of several active substances with different mechanisms at the same time. These are: at least one drug from the group of HIV nucleoside reverse transcriptase inhibitors (NRTIs) in combination with a HIV non-nucleoside reverse transcriptase inhibitor (NNRTI) and / or inhibitors of other classes [4]. HAART drugs are constantly improving, and the development of new pharmacological units is gaining strength due to the variety of side effects and toxicity, as well as the development of drug resistance in strains [5, 6].

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a promising group of antiretroviral drugs, which are organic compounds of various classes with a significant proportion of aromatic hydrophobic radicals [7]. These are noncompetitive enzyme inhibitors interacting with the allosteric center of reverse transcriptase, affecting the mobility and flexibility of the polymerization center, which ultimately leads to a decrease in the enzyme efficiency [8-10]. The inhibitory effect of drugs manifests itself in several ways, for example, the binding of nevirapine causes the translation of the hydrophobic residues position, and, as a result, the tertiary structure of the reverse transcriptase protein expands [11]. It also manifests itself due to the influence on the dynamic processes of RT with a nucleic acid matrix [12–14]. There are two generations of NNRTI drugs. The first class includes nevirapine, efavirenz and delaverdine, and the second one includes etravirine and rilpivirine [15, 16]. However, despite a slow development of resistance to the second generation NNRTIs, the HIV mutant strains allowing the virus to resist the action of these drugs, are already encountered in practice [17].

Currently, the approaches to the development of new NNRTIs include the following factors: increasing positional adaptability and conformational flexibility in the drug binding pocket [18]; targeting conserved residues in the binding pocket [19, 20]; improving physicochemical properties with the help of prodrugs, or introducing solubilizing groups [21].

As a result of the multi-year research carried out at the Department of Pharmaceutical and Toxicological Chemistry, as well as at the Research Institute of Pharmacology of Volgograd State Medical University (the Ministry of Health of Russia) in collaboration with scientists from the Institute of Molecular Biology n. a. V.A. Engelhardt (Federal Agency for Scientific Organizations), virologists from the USA and Western Europe (a pharmaceutical company of ImQuestBioSciences Inc., USA; Rega Institute for Medical Research, Belgium), a new class of highly active non-nucleoside inhibitors of viral reproduction has been discovered [22, 23].

Among other compounds, 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil (Fig. 1) showed a high ability to suppress the reproduction of HIV-1 *in vitro.* The compound also suppressed the reproduction of mutant HIV-1 strains and had a resistance profile close to that of efavirenz [24, 25].

The obtained results of preclinical studies make it possible to consider the proposed compound as a promising drug candidate for the treatment of HIV-1 infection.

In the course of the pharmacy development, high standards are being imposed on the quality and safety of medicines. In this regard, it becomes necessary to use research methods in the pharmaceutical analysis that allow achieving maximum specificity and reliability of the results [26]. Thus, the requirements for the use of UV, IR, NMR spectroscopy and a thermal analysis, are increasingly being introduced into the drafts of regulatory documents for pharmaceutical substances.

**THE AIM** of the study is to identify 1-[2- (2-benzoylphenoxy) ethyl] -6-methyluracil using X-ray, thermal and elemental analyzes, UV, IR, NMR spectroscopy, as well as to study its action mechanism against wild-type and mutant forms of HIV-1 reverse transcriptase (RT).

## MATERIALS AND METHODS

The objects of the study were the samples of the pharmaceutical substance 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil, provided by Volgograd State Medical University of the Ministry of Health of Russia. The studied substance is a crystalline powder, practically insoluble in water and organic solvents.

[2] Help HIV infection in the Russian Federation in 2019 (prepared at the Federal Scientific and Methodological Center for the Prevention and Control of AIDS, Central Research Institute of Epidemiology, Rospotrebnadzor). Available from: http: //www.hivrussia.info/wp-content/uploads/2020/02/VICH-infektsiya-v-Rossijskoj-Federatsii-na-31.12.2019.pdf. Russian

[3] WHO HIV / AIDS Fact Sheet, Available from: https: //www.who.int/ru/news-room/fact-sheets/detail/hiv-aids. Russian

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### X-ray diffraction study

An X-ray diffraction study of the compound was carried out on a Bruker APEX II diffractometer (Bruker, Germany). The structures were solved by the direct method and refined by the geometric least squares mean (LSM) in the anisotropic full-matrix approximation in terms of the structure factor ($F^2_{hkl}$). Hydrogen atoms were calculated geometrically and refined with restrictions imposed on the C-H bond lengths and their isotropic displacement parameters. All calculations were performed using ShelXl, SHELXT, and Olex-2 programs.[4,5,6]

### X-ray phase study

An X-ray phase study was performed to identify possible polymorphs. The composition investigations of the sample of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil by a powder X-ray diffraction, were performed on a Bruker D8 Advance diffractometer (Bruker, Germany) equipped with a nickel β-filter and a system of controlled slits for monochromatization (l[CuKα] = 1.5418 Å), and a position-sensitive detector LynxEye, in the angular range of 4-60° with a step of 0.02° anglewise**.** A certain amount of the substance was ground in a mortar and applied to a flint plate as suspension in heptane, and then the resulting sample was dried. After obtaining the diffraction data on the results of a single crystal study, the theoretical diffraction pattern was calculated and compared with the experiment. The dependence of the background on the 2q angle was modeled using a series of Chebyshev polynomials up to the fifth order. To take into account the features of the device, the method of fundamental parameters determined in advance using a sample of lanthanum boride LaB6, was used. All calculations were performed using the TOPAS program[7].

### Thermogravimetric analysis and differential scanning calorimetry

To determine the thermal properties of the substance, the thermal analysis methods were used – a thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The TGA of the test compound was carried out on a Derivatograph-C device (MOM, Hungary) at the heating rate of 10° C / min. The data obtained were graphically recorded in the form of curves: thermogravimetric (TG), differential thermogravimetric (DTG), and differential thermal (DTA). On the derivatogram, the TG curve shows the change in the sample mass during the study period, and the DTG curve shows the decomposition rate and is useful for accurately assessing the decomposition steps. The DTA reflects the differentiation of thermal effects, contains information on endo- and exothermic maxima, and is used for a qualitative assessment of the derivatogram. The experimental TGA data were processed using the Winder C program. The DSC studies were performed on a DSC-822e device (Mettler-Toledo, Switzerland) in the temperature range from –145 to +260°C, at the heating rate of 10°C/min. All calculations were performed using the STAR$^e$ program.

### Elemental analysis

Elemental analysis was performed on an automatic CHN analyzer VarioMicrocube (Elementar, Germany). Acetanilide (71.098% C; 6.71% H, 10.36% N) was used as a standard sample. The carrier gas was helium, the oxidizing agent was high purity oxygen. The oxidizing column was filled with copper oxide, and the reducing column was filled with wire copper. The combustion of standard weighed samples and 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil substance, pre-selected in tin capsules and weighed on an XP6 ultramicrobalance (MettlerToledo, Switzerland) with an accuracy of 0.001 mg, was carried out at the temperature of 950° C. The reduction of the combustion products on the wire copper was carried out at 550° C. The gaseous destruction products were separated on a chromatographic column and detected in a katharometer. The calculation of the determination results was carried out automatically according to the program supplied with the device.

### Ultraviolet and visible Spectrophotometry

In the UV-visible range, the spectra were recorded on a Cary 4000 spectrometer (Varian, USA) by measuring the absorption of radiation in a cuvette with a substance solution at the concentration of 0.1 mg / ml. The sample was dissolved in a volumetric flask in tetrahydrofuran (THF, spectroscopic grades, “Component-reagent”), as well as in dimethyl sulfoxide (DMSO, UV-IR-HPLC-GPC grades, Panreac). The measurements of the obtained solutions were carried out in a quartz cuvette with an optical path length of 1.00 mm (Hellma). The data were processed in the software of the WinUV spectrometer (Varian).

### Infrared spectrometry

The experimental work was carried out on a Vertex 70 FT-IR spectrometer (BrukerOptik GmbH, Germany). The samples for recording the spectra were prepared by direct pressing with optically pure potassium bromide; the spectra were measured in the systematic scanning mode in the range of 4000–370 $cm^{-1}$. A diffuse reflection attachment (Shimadzu, Japan) was used to record the absorption spectrum in the near IR area. All data were processed using the OPUS spectrometer control software (Bruker, Germany).

[4] Dolomanov O.V., Bourhis L.J., Gildea R.J., Howard J.A.K., PuschmannH. OLEX2: a complete structure solution, refinement and analysis program. J. Appl. Cryst. 2009;42: 339–341. DOI: 10.1107/S002188980804272

[5] Sheldrick G.M. SHELXT – Integrated space-group and crystal-structure determination. Acta Cryst. 2015; A71: 3–8. DOI: 10.1107/S2053273314026370

[6] Sheldrick G.M. Crystal structure refinement with SHELXL // ActaCryst. – 2015. – V. C71. – P. 3–8. DOI: 10.1107/S2053229614024218.

[7] Bruker AXS: TOPAS V4: General profile and structure analysis software for powder diffraction data. – User’s Manual, Bruker AXS, Karlsruhe, Germany, 2009: 72 p.

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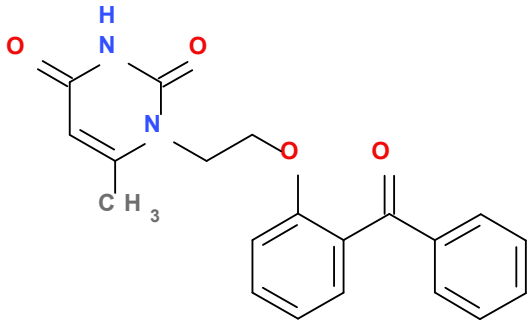


**Figure 1 – Structural formula of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil**

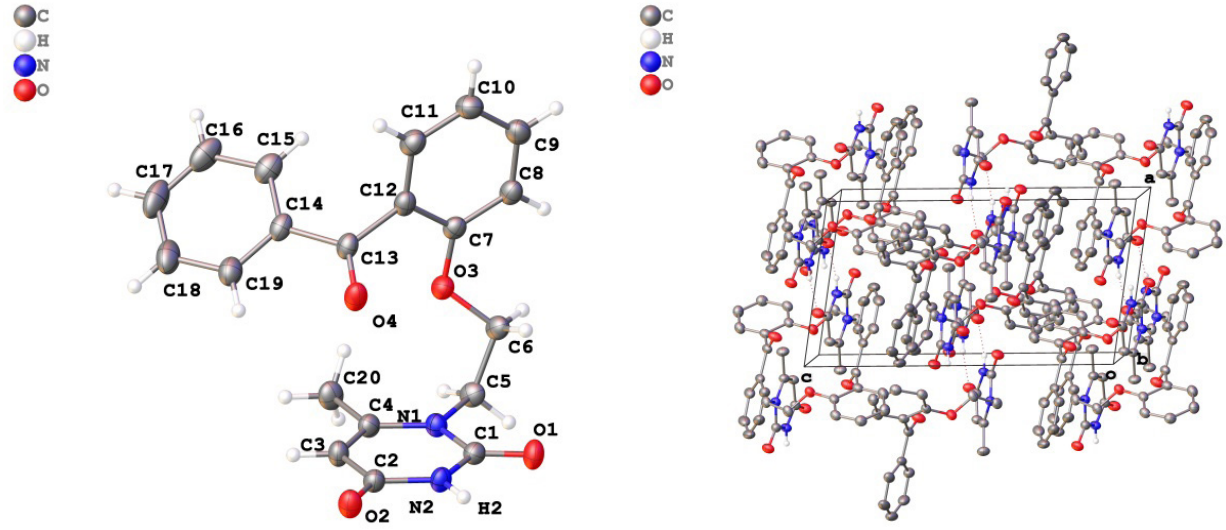


**Figure 2 – Visualization of the 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil molecule**

Note: (A) – General view of the molecule and (B) – Crystal packing

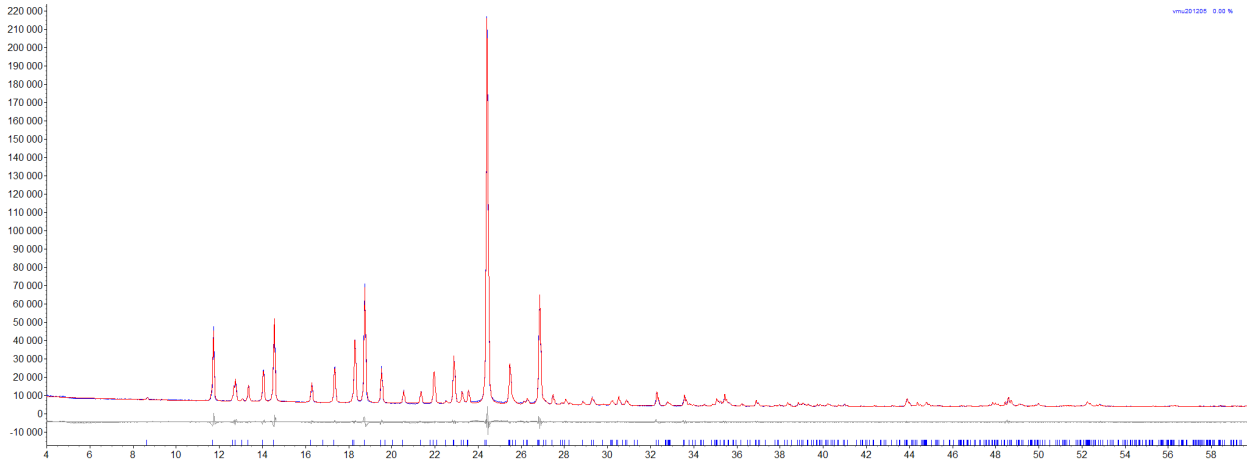


**Figure 3 – General view of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil diffractogram (a blue line – experiment, a red line – calculation, a gray line – a difference curve)**

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**Table 1 – Basic crystallographic data and parameters of the structure refinement for 1-[2-(2-benzoylphenoxy) ethyl] -6-methyluracil**

| | |
|---|---|
| Gross formula | $C_{20}H_{18}N_2O_4$ |
| Molecular mass | 350.36 |
| Temperature, K | 120 |
| Space group, Z | $P2_1/n$, 4 |
| Cell parameters: | 4 |
| a, Å | 8.1352(7) |
| b, Å | 13.7868(11) |
| c, Å | 15.0957(12) |
| a, ° | 90 |
| b, ° | 98.443(2) |
| g, ° | 90 |
| Cell volume, V, $Å^3$ | 1674.8(2) |
| Density, $d_{calc}$, g $cm^{-3}$ | 1.390 |
| Absorption coefficient, m, $cm^{-1}$ | 0.98 |
| Structure factor F (000) | 736 |
| Crystals size, mm | 0.25 × 0.17 × 0.14 |
| Crystalline form, color | Призмы, коричневый |
| $2q_{max}$, | 61.36 |
| Number of measured reflections | 22276 |
| Number of independent reflections | 5160 |
| Number of reflections with I>2s (I) | 3182 |
| Number of refined parameters [I>2s (I)]: | 286 |
| $R_1$ | 0.0511 |
| $wR_2$ | 0.1258 |
| GOF | 1.000 |
| Residual electron density, e·$Å^{-3}$($r_{min}$/$r_{max}$) | 0.344/-0.212 |

**Table 2 – Diffraction maxima of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil sample**

| Angle 2q, ° | D-space (d), Å | Relative intensity | Angle 2q, ° | D-space (d), Å | Relative intensity |
|---|---|---|---|---|---|
| 4.706 | 18.7609 | 0.30% | 35.156 | 2.55063 | 1.30% |
| 8.676 | 10.18426 | 0.50% | 35.439 | 2.53089 | 2.90% |
| 11.735 | 7.53531 | 19.00% | 36.223 | 2.47787 | 0.70% |
| 12.765 | 6.92951 | 5.40% | 36.892 | 2.43449 | 1.40% |
| 13.074 | 6.76618 | 0.50% | 37.367 | 2.4046 | 0.30% |
| 13.358 | 6.62292 | 4.00% | 37.799 | 2.37813 | 0.30% |
| 14.048 | 6.29941 | 8.10% | 37.967 | 2.368 | 0.50% |
| 14.555 | 6.08082 | 21.20% | 38.364 | 2.34439 | 1.00% |
| 16.287 | 5.43779 | 5.20% | 38.853 | 2.31603 | 1.00% |
| 17.352 | 5.10653 | 9.30% | 39.073 | 2.30348 | 0.90% |
| 18.292 | 4.84624 | 16.30% | 39.292 | 2.29113 | 0.50% |
| 18.746 | 4.7297 | 30.70% | 39.86 | 2.25981 | 0.40% |
| 19.518 | 4.54444 | 9.50% | 40.209 | 2.24098 | 0.70% |
| 20.544 | 4.31971 | 3.60% | 40.775 | 2.21116 | 0.20% |
| 21.352 | 4.15813 | 3.10% | 40.996 | 2.19974 | 0.60% |
| 21.957 | 4.0448 | 8.10% | 41.818 | 2.15838 | 0.10% |
| 22.549 | 3.93992 | 0.50% | 41.767 | 2.1609 | 0.10% |
| 22.882 | 3.88337 | 12.00% | 42.364 | 2.13184 | 0.30% |
| 23.253 | 3.82218 | 3.00% | 43.254 | 2.09001 | 0.10% |
| 23.552 | 3.77445 | 3.10% | 43.892 | 2.06109 | 1.90% |
| 24.415 | 3.64283 | 100.00% | 44.381 | 2.03952 | 1.00% |
| 25.463 | 3.49533 | 10.20% | 44.793 | 2.0217 | 1.10% |
| 26.287 | 3.38752 | 1.30% | 45.324 | 1.99923 | 0.20% |
| 26.854 | 3.31735 | 27.00% | 46.412 | 1.95488 | 0.30% |

| Angle 2q, ° | D-space (d), Å | Relative intensity | Angle 2q, ° | D-space (d), Å | Relative intensity |
|---|---|---|---|---|---|
| 27.469 | 3.24438 | 2.30% | 46.735 | 1.94213 | 0.10% |
| 28.068 | 3.1765 | 1.30% | 47.417 | 1.91577 | 0.10% |
| 28.864 | 3.09073 | 1.00% | 47.931 | 1.89643 | 0.40% |
| 29.282 | 3.04751 | 2.10% | 47.989 | 1.89426 | 0.80% |
| 29.804 | 2.99533 | 0.20% | 48.597 | 1.87199 | 2.10% |
| 30.239 | 2.95321 | 1.20% | 49.131 | 1.85285 | 0.50% |
| 30.520 | 2.92669 | 2.40% | 49.999 | 1.82271 | 0.70% |
| 30.865 | 2.89478 | 1.50% | 51.332 | 1.77848 | 0.30% |
| 31.227 | 2.86196 | 0.10% | 52.285 | 1.74826 | 0.90% |
| 32.285 | 2.77059 | 3.60% | 52.831 | 1.73149 | 0.50% |
| 32.802 | 2.72812 | 1.10% | 56.284 | 1.63318 | 0.30% |
| 33.576 | 2.66693 | 2.60% | 57.546 | 1.60032 | 0.10% |
| 33.998 | 2.63483 | 0.40% | 58.432 | 1.57815 | 0.10% |
| 34.508 | 2.59705 | 0.50% | 59.064 | 1.56277 | 0.20% |
| 34.869 | 2.57093 | 0.40% | | | |

**Table 3 – Results of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil elemental analysis**

| Pharmaceutical substance | C,%(M±σ), n=2 | H,%(M±σ), n=2 | N,%(M±σ), n=2 |
|---|---|---|---|
| 1-[2-(2-benzoylphenoxy)ethyl]-6 methyluracil | 68.22±0.08 | 5.24±0.03 | 7.81±0.04 |
| Theoretical calculation | 68.60 | 5.11 | 8.00 |

**Table 4 – Characteristic maxima of absorption bands of a sample substance (in $cm^{-1}$) in the near and middle IR range of 8000–370 $cm^{-1}$**

| Pharmaceutical substance | Absorption maxima, $cm^{-1}$ |
|---|---|
| 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil | 4002.5; 3940.0; 3914.1; 3896.3; 3861.3; 3847.4; 3831.5; 3811.5; 3788.7; 3775.7; 3741.0; 3707.2; 3697.4; 3682.9; 3653.6; 3640.3; 3623.9; 3318.4; 3186.1; 3157.8; 3080.0; 3060.6; 3036.4; 3014.2; 2967.7; 2933.3; 2900.5; 2881.0; 2864.3; 2792.1; 2587.1; 2552.3; 2470.9; 2443.9; 2427.6; 2386.4; 2354.8; 2341.2; 2303.6; 2240.7; 2189.0; 2105.5; 2090.1; 2075.2; 2038.8; 2016.0; 1987.2; 1951.2; 1924.4; 1888.1; 1860.0; 1823.9; 1701.3; 1665.7; 1613.6; 1596.2; 1578.6; 1531.9; 1483.0; 1473.8; 1462.9; 1449.1; 1442.1; 1427.0; 1409.3; 1393.1; 1358.1; 1313.7; 1292.2; 1270.8; 1242.8; 1179.2; 1151.7; 1121.2; 1107.8; 1075.2; 1062.4; 1043.3; 1025.0; 996.0; 982.0; 971.9; 959.6; 944.6; 929.9; 894.2; 869.8; 852.2; 833.3; 807.7; 775.5; 766.4; 755.3; 731.4; 717.1; 704.2; 689.1; 634.1; 609.9; 567.6; 533.5; 509.0; 461.9; 435.9; 418.2; 376.7 |

**Table 5 – Results of $^{1}H$, $^{13}C$ NMR spectra analysis of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil**

| Pharmaceutical substance | Position | Group | Chemical shift, δ $^{1}H$, ppm | Chemical shift, δ $^{13}C$, ppm |
|---|---|---|---|---|
| 1-[2-(2-benzoylphenoxy) ethyl] -6-methyluracil | 1 | Ar, α-CH | 7.21 d | 113.16 s |
| | 2 | Ar, β-CH | 7.10 t | 121.61 s |
| | 3 | Ar, β-CH | 7.50 t | 132.23 s |
| | 4 | Ar, α-CH | 7,29 d | 128.94 s |
| | 5 | Ar, ipso-C | – | 155.71 s |
| | 6 | Ar, ipso-C | – | 129.13 s |
| | 7 | C=O | – | 196.05 s |
| | 9 | Ar, ipso-C | – | 136.92 s |
| | 10.14 | Ar, α-CH | 7.67 d | 129.71 s |
| | 11.13 | Ar, β-CH | 7.46 t | 129.08 s |
| | 12 | Ar, γ-CH | 7.60 t | 134.07 s |
| | 16 | O-$CH_2$ | 4.18 t | 66.25 s |
| | 17 | N-$CH_2$ | 3.85 t | 43.47 s |
| | 19 | C=O | – | 151.82 s |
| | 20 | NH | 11.10 s | – |
| | 21 | C=O | – | 162.76 s |
| | 22 | =CH | 5.12 s | 101.55 s |
| | 23 | =C | – | 154.49 s |
| | 26 | $CH_3$ | 1.82 s | 19.88 s |

Note: t – triplet, s – singlet, d – doublet, k – quartet

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### Nuclear magnetic resonance spectroscopy

The experimental work to determine the NMR spectra of the test substance was carried out on a BrukerAvance-IIIHD 500 NMR spectrometer (Bruker, Germany). A portion of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil, 20 mg in weight, was dissolved in 600 µL of deuterated dimethyl sulfoxide (DMSO-d6). The resulting solution without further processing by "asis" was placed in an NMR spectrometer for recording $^{1}H$, $^{13}C$, HC-HMQC and HC-HMBC spectra. The $^{1}H$-NMR spectra were recorded at the operating frequency of 500.13 MHz, $^{13}C$ – at the operating frequency of 125.76 MHz. To confirm the structure of the carbon skeleton of the potential product, $^{13}C$ spectra were recorded in the phase-sensitive JMOD version ($CH_3$, CH – signals with negative phases, $CH_2$, C – signals with positive phases), as well as inverse heteronuclear correlations HC – HMQC (direct interactions C – H), HC – HMBC (long range interactions C – H). Assignment in the $^{13}C$-JMOD spectrum was performed based on the analysis of two-dimensional inverse correlations HC-HMQC and HC-HMBC. To confirm the structure of the carbon skeleton, $^{13}C$ NMR spectra were recorded in the phase-sensitive version of JMOD (C, $CH_2$ signals were directed upward; CH, CH3 signals were directed downward), HC-HMQC, HCHMBC.

### Investigation of the action mechanism *in vitro*

The study of the action mechanism of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil was carried out by evaluating the inhibitory activity against wild-type HIV-1 RT and its mutant forms corresponding to drug-resistant viral strains using radioactively labeled nucleotides. To express the wild-type heterodimer of HIV-1 reverse transcriptase, the cells of *E. coli* strain M15 [pRep4] (Qiagen, Germany) transformed with the p6HRT-PROT plasmid, were used. To obtain mutant forms of HIV-1 reverse transcriptase, the cells of *E. coli* strain Rosetta DE3 (Qiagen, Germany) transformed with the target plasmids, were used.

During the study of the inhibitory activity of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil against mutant forms of HIV-1 RT, a panel of HIV-1 RT mutant forms with amino acid substitutions L100I, K103N, V106A, Y181C, G190A was used: they are prevalent in patients with resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), lead to resistance through a variety of mechanisms and are accepted in defining the resistance profile of new anti-HIV drugs. Additionally, the inhibitory activity of 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil against a double mutant with the two most common substitutions K103N and Y181C was investigated. The substance of the drug efavirenz was used as a positive control of the HIV-1 RT inhibitor. The inhibition constant for the substance was determined by the Dixon method, i. e., based on the dependence of the inverse rate of the enzymatic reaction in the presence of an inhibitor on its concentration.

## RESULTS AND DISCUSSION

A general view of the molecule and its crystal packing are shown in Fig. 2. The main crystallographic data and refinement parameters are presented in Table 1.

The general view of the diffraction pattern is shown in Fig. 3, the main characteristics of the diffraction maxima are shown in Table 2.

Refinement of the divergence between the experimental and theoretical data showed that the sample corresponds to one crystalline phase of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil. No other crystalline phases have been found out.

The obtained data of the thermal analysis of the substance 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil are shown in Fig. 4–6.

The process of weight loss begins at the temperature of 25-50° C. The decrease in weight in this temperature range is about 1%, which is associated with the removal of residual solvents or moisture from the sample. In the temperature range of 184–227° C, the weight loss is about 3%, which is associated with the destruction of the crystalline hydrate. After 300° C, the thermal changes begin to occur with the substance, ending in thermo-oxidative destruction. On the DTG curve, the peak with a top at 203° C corresponds to the temperature at which crystalline hydrate water is removed. On the DTA curve, the endothermic peak with a top at 230° C corresponds to the sample melting point.

It has been established that crystallization of 1-[2-(2-benzoylphenoxy) ethyl] -6-methyluracil is observed at the temperature of 191° C (Fig. 5). The melting point and the fusing heat upon repeated heating are somewhat lower than on the first one, which is associated with different conditions for the formation of the crystalline phase.

An endothermic peak with a minimum at 195° C was recorded on the thermogram. It is associated with the removal of crystallization water. The melting point of the crystalline phase was 227° C (Fig. 6).

The elemental analysis results are presented in Table 3.

Based on the results obtained, shown in Table 3, it can be concluded that the content of the analytes of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil corresponds to the theoretical content of the analytes calculated on the basis of the gross formula $C_{20}H_{18}N_2O_4$.

The spectra of 1-[2-(2-benzoylphenoxy)ethyl] -6-methyluracil in the UV-visible range are shown in Fig.7, 8.

The spectra show an absorption maximum at 251.0 nm for the solution in THF and 254.5 nm for the solution in DMSO. In this case, the optical density of DMSO increases much faster than of THF; therefore, in the case of THF, the measurement from 210 nm is possible. To obtain a pronounced maximum absorption of the substance in the UV-visible range, the following measurement conditions are recommended: a solution in tetrahydrofuran of spectral purity, the concentration of 0.10 mg/ml, measurements in a quartz cuvette with an optical path length of 1.00 mm, a registration of the spectrum in the range of 210–900 nm, the background should be pure THF.

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**Table 6 – Inhibitory activity of NNRTIs against RT of wild (WT) and mutant strains of HIV-1 *in vitro***

| Back transcriptase | Inhibition constant (Ki, μM) | |
|---|---|---|
| | 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil | Efavirenz |
| Wild type | 0,23 ± 0,04 | 0,011± 0,002 |
| L100I | >20[a] | 0,14± 0,01 |
| K103N | >20[a] | 0,52± 0,1 |
| V106A | >20[a] | 0,11± 0,01 |
| Y181C | 12 ± 2,4 | 0,053± 0,006 |
| G190A | 8,3 ± 0,4 | 0,091± 0,008 |
| K103N/Y181C | >20[a] | 0,52± 0,08 |

Note: [a]The test substance, 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil, inhibits mutant forms of HIV-1 RT L100I, K103N, V106A and the double mutant K103N / Y181C at the concentration of 20 μM with the efficiency of 38%, 33%, 22% and 35%, respectively. No increase in inhibition of these mutant forms of HIV-1 RT at higher concentrations of 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil was observed, which is most likely associated with the achievement of the solubility limit of the compound in the reaction mixture containing 10% DMSO

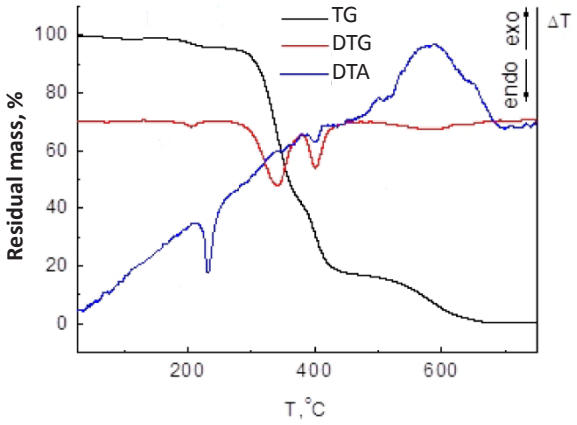


**Figure 4 – Derivatogram of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil**

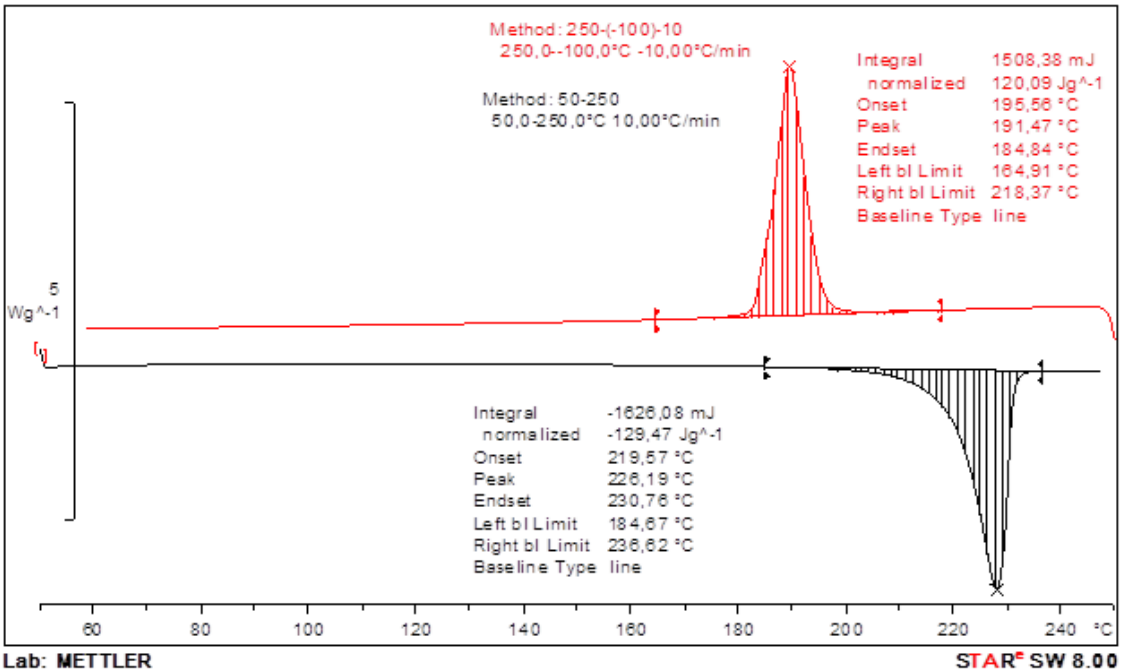


**Figure 5 – DSC thermogram obtained by scanning 1- [2- (2-benzoylphenoxy) ethyl] -6-methyluracil**

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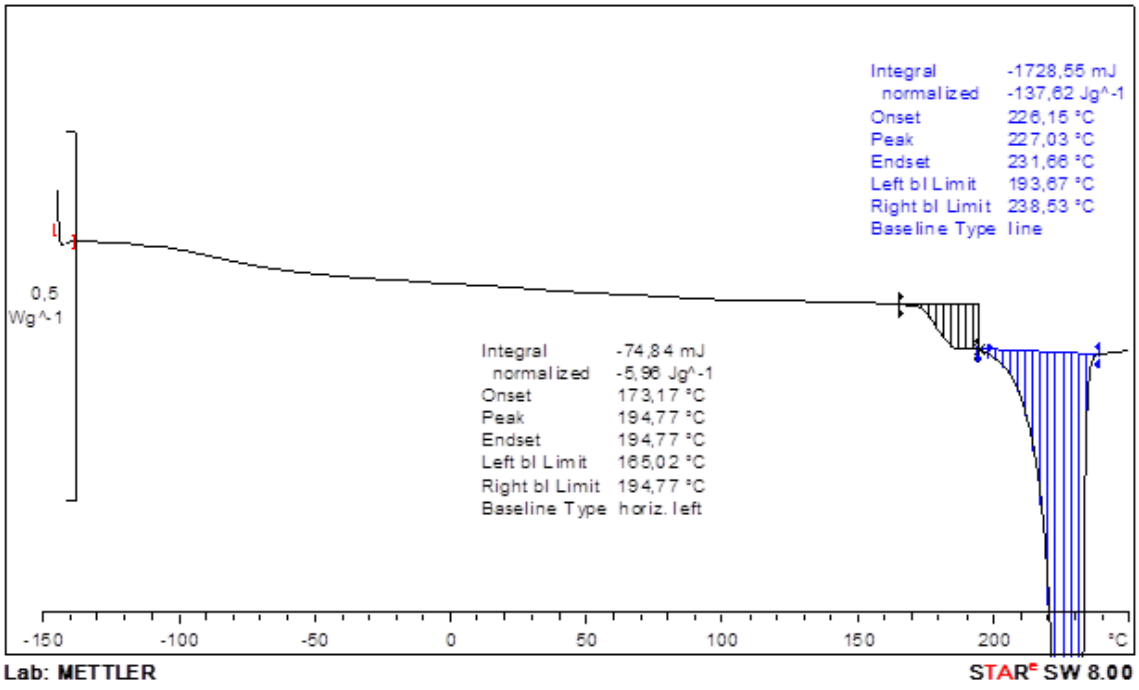


**Figure 6 – DSC thermogram obtained by scanning 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil**

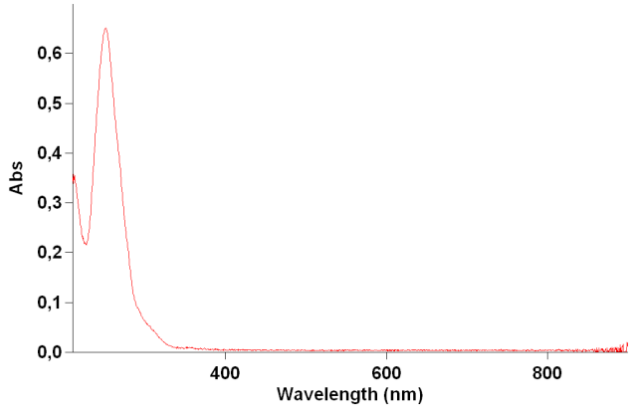


**Figure 7 – Absorption spectrum of 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil tetrahydrofuran at the concentration of 0.1 mg/ml in the range of 210–900 nm in a cuvette with an optical path length of 1.00 mm**

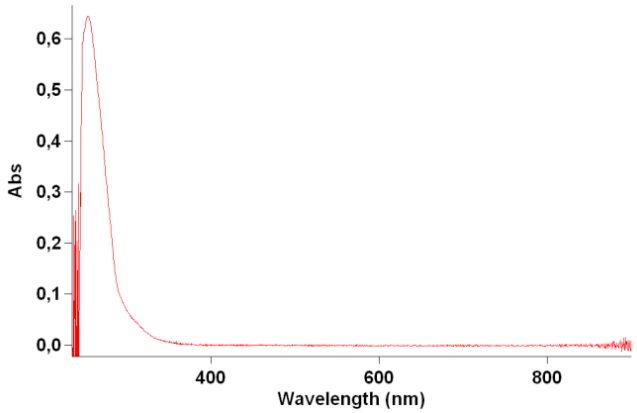


**Figure 8 – Absorption spectrum of 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil dimethyl sulfoxide at the concentration of 0.1 mg/ml in the range of 235–900 nm in a cuvette with an optical path length of 1.00 mm**

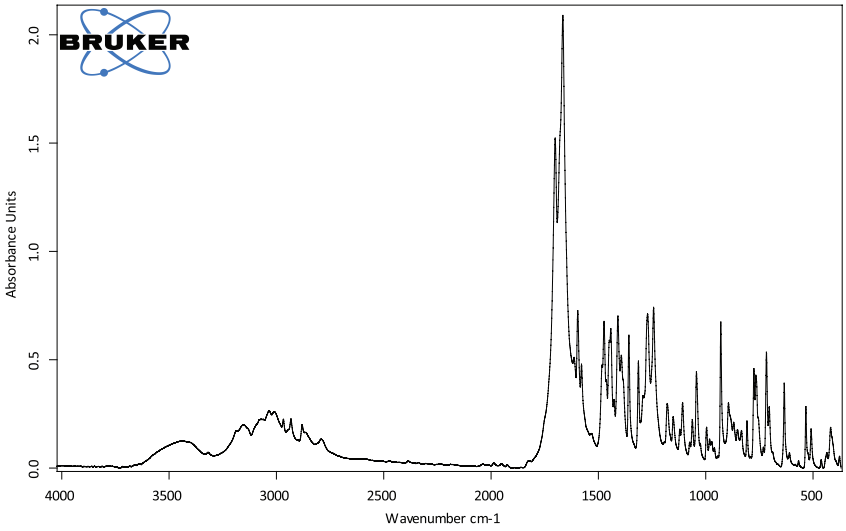


**Figure 9 – IR absorption spectrum of 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracilav in the range of 4000-370 cm$^{-1}$ in potassium bromide tablets**

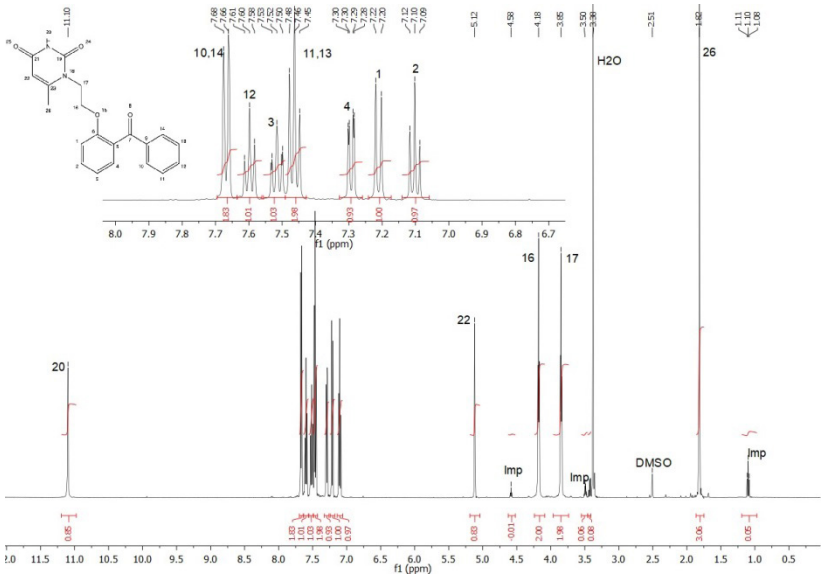


**Figure 10 – 1H NMR spectrum of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil**

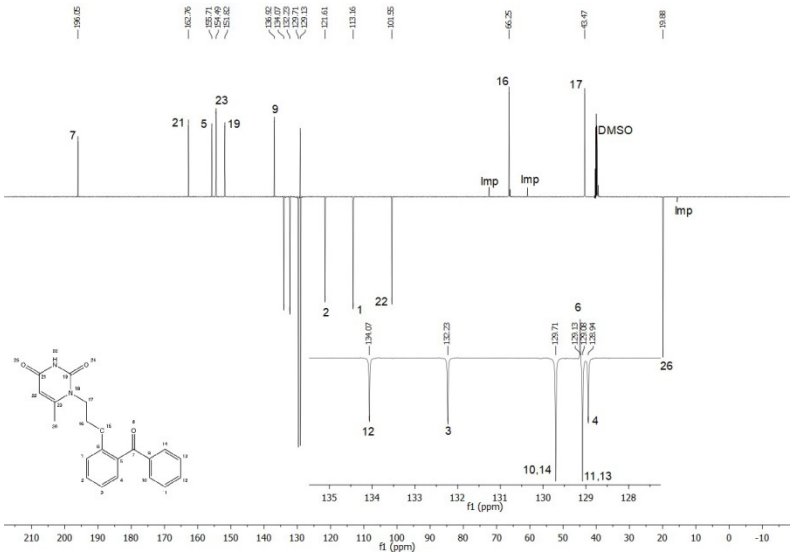


**Figure 11 – $^{13}$C NMR JMOD spectrum of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil**

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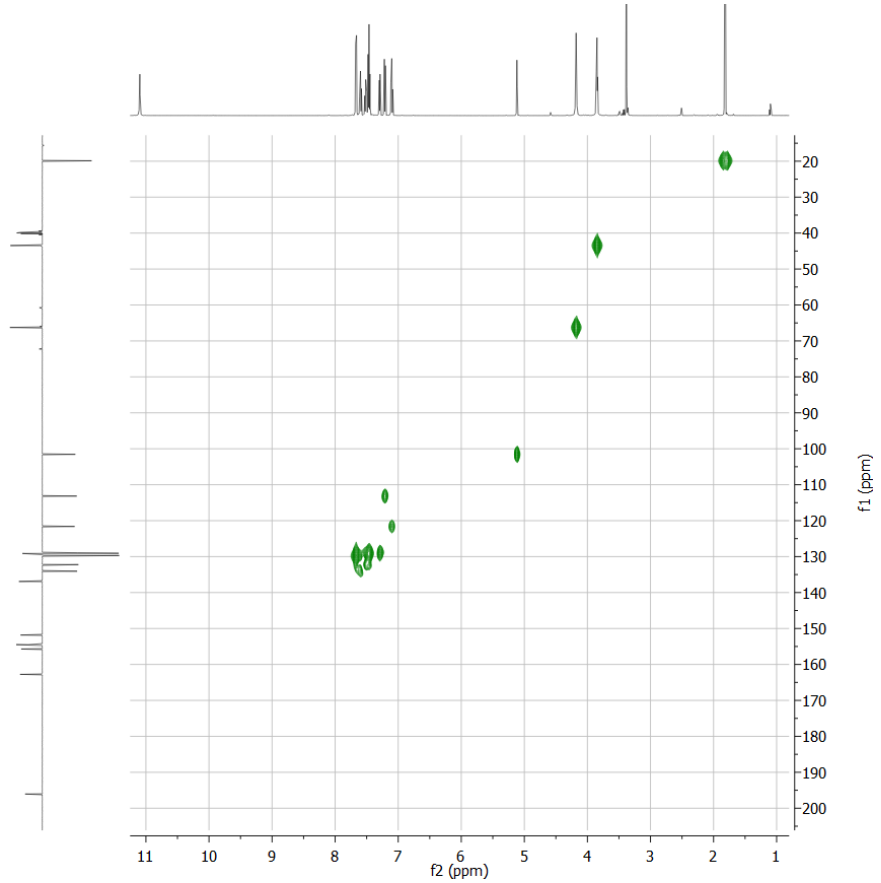


**Figure 12 – NMR HC-HMQC spectrum of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil**

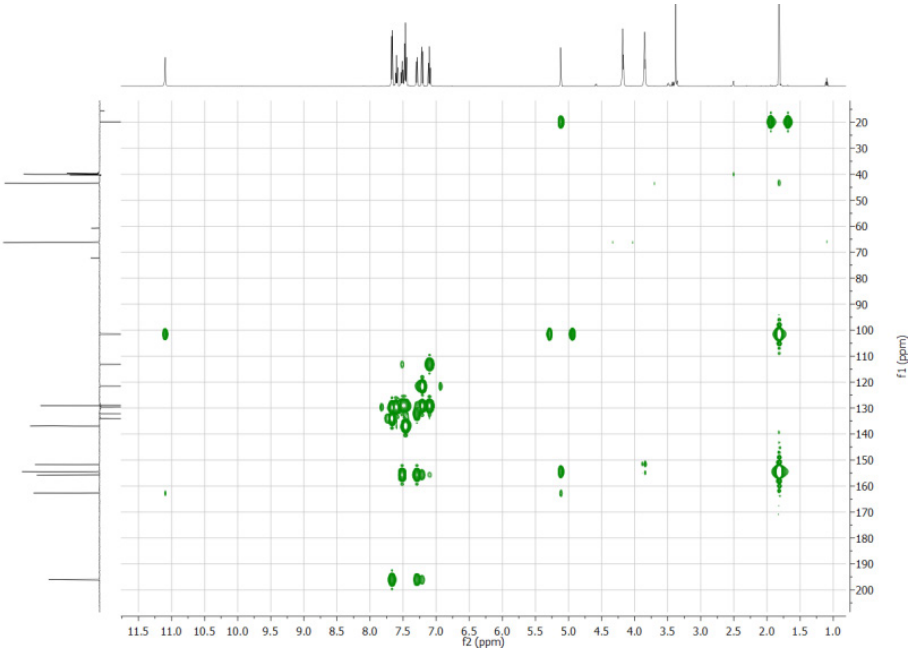


**Figure 13 – HC-HMBC NMR spectrum of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil**

Fig. 9 shows a general view of the IR absorption spectrum of the sample in potassium bromide tablets.

In the spectra of the sample in the IR range, there are many narrow and characteristic bands, which are summarized in Table 4. In addition, the spectrum of the sample in KBr in the area of 3400 cm – 1 contains a broad band corresponding to the moisture absorbed by potassium bromide; therefore, it was excluded from consideration.

Due to the complexity of the molecule, it is not possible to identify and unambiguously assign all signals to specific functional groups. Therefore, IR spectroscopy for a given molecule can be used as an additional one, along with NMR spectroscopy.

The obtained $^{1}$H NMR spectrum of the analyte is shown in Fig. 10.

The most important characteristics of the NMR spectrum is its chemical shift (δ), which depends on the structure of the molecule. The electron density of protons in molecules is determined by the nature of the chemical bond and the induction effects of the surrounding groups, as a result of which the screening of protons becomes different and their signals appear in different areas of the spectrum.

The signals were assigned in the $^{1}$H spectrum (Fig. 10). The spectrum shows that the sample contains signals related to both aliphatic fragments and aromatic rings. The signal of the NH group is also visible. In general, the range of the main product does not contradict the proposed structure. In the aromatic region, the spectrum of $^{1}$H also contains low-intensity signals of impurities in the trace amounts, which, due to their low content, cannot be identified.

To confirm the structure of the carbon skeleton, $^{13}$C NMR spectra were recorded in the phase-sensitive version of JMOD (C, $CH_2$ – signals upward, CH, $CH_3$ – signals downward), HC-HMQC, HCHMBC. These spectra are shown in Fig. 11–13.

The signals were assigned in the $^{13}$C spectrum (Fig. 11). The spectrum shows that the sample contains signals related to both aliphatic fragments and positions in the aromatic rings of carbonyl groups. In general, the spectrum of 1- [2- (2-benzoylphenoxy) ethyl] -6-methyluracil does not contradict the proposed structure. The signals of minor impurities are also visible. Their content in the sample is below the detection limit of $^{13}$C NMR spectroscopy.

Based on the interpretation results of the inverse two-dimensional correlation HC-HMQC and HC-HMBC spectra (Fig. 12,13), a complete assignment of signals in the $^{1}$H and $^{13}$C spectra was made, and the structure of the carbon skeleton product was confirmed. Fig. 12,13 also show that the distribution of two-dimensional correlation signals does not contradict the suggested structure of the proposed product. The results of assigning the bands to the functional groups of the molecules of the test substance are presented in Table 5.

It was found out that the number and a mutual arrangement of functional groups, the integral intensity of the signals in the $^{1}$H spectrum corresponds to the structure of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil. The structure of the carbon skeleton corresponds to the structure of the test compound.

Modern instrumental methods of analysis make it possible to fully characterize a pharmaceutical substance, which is extremely necessary for its standardization, development of quality control methods and their subsequent inclusion in draft regulatory documents [26]. In this work, the results of X-ray, elemental, thermogravimetric, DSC analyzes, UV, IR and NMR spectroscopy of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil – the compound developed as a drug candidate for the HIV infection treatment – have been demonstrated.

The data obtained confirm the expected structure of the molecule: visualization of the molecule by an X-ray diffraction study, the gross formula $C_{20}H_{18}N_2O_4$ calculated by an elemental analysis, the mutual arrangement of functional groups and the structure of the carbon skeleton in the NMR spectrum correspond to the expected structure of the compound. The revealed characteristic peaks in the UV and IR spectra indicate the presence of basic functional groups characteristic of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil. The results of the X-ray phase study indicate the presence of one crystalline phase of the sample, which is consistent with the data of the thermal analysis methods, which have established the absence of substance polymorphs. During the NMR analysis, low-intensity signals of impurities in the trace amounts were detected, but their content was extremely low, which indicates the proper synthesis and a high degree of the substance purification.

The most important stage of the research was the study of the action mechanism of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil in relation to HIV-1 RT.

It was shown that 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil is an effective inhibitor of RT as a wild strain of HIV-1, and a number of its mutant forms (Table 6). The activity of the test object depends on mutations in the binding site of non-nucleoside RT inhibitors, acting by a non-competitive mechanism.

The results presented in Table 6 confirm that the object of the study belongs to the group of HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs). It should be noted that efavirenz inhibited an RT polymerase activity at lower concentrations than 1-[2-(2-benzoylphenoxy)ethyl] -6-methyluracil, with an equal antiviral activity *in vitro*.

A high variability of HIV leads to numerous substitutions of amino acids in RT, which form the resistance of the virus to NNRTIs [27]. According to the literature data [28], most of the identified mutations are localized in the hydrophobic pocket, the binding site of HIV NNRTIs, near the catalytic site of the enzyme. Among patients with resistance to NNRTIs, the most common mutations

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are K103N (56.98%) and Y181C (24.95%). Mutations G190A, L100I, V106A are less common (8.16%, 6.92% and 2.37%, respectively), but they also strongly affect the success of antiretroviral therapy, as they lead to the loss of the inhibitory activity of nevirapine by more than 2 orders of magnitude [29]. Mutations L100I, K103N, and Y181C are also characteristic of the patients with resistance to efavirenz and delaverdine [30]. Mutations Y181C and V106A are known to cause a drop in the activity of capravirin, a potential second-generation NNRTI withdrawn from clinical trials [31]. Lersivirin is an NNRTI based on capravirin. Having passed the clinical phase IIb, it has succeeded in achieving resistance to these mutations, but there was a decrease in its activity notified in the presence of the L100I mutation [32]. A moderate activity loss against the V106A mutation was characteristic of compound GW69564 [33], the structural modification of which made it possible to create another molecule (GW695634), which reached the III phase of clinical trials. Several mechanisms of RT resistance to the action of NNRTIs have been proposed: mutations L100I and G190A sterically prevent the placement of NNRTIs in the hydrophobic pocket [34], the Y181C mutation leads to the loss of interactions with amino acid residues inside the pocket [35], the K103N mutation makes it difficult for NNRTIs to enter the hydrophobic pocket [36, 37].

The work demonstrated the ability of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil to inhibit not only wild-type HIV-1 RT, but also its most common mutant forms with amino acid substitutions L100I, K103N, V106A, Y181C, G190A, widely present in NNRTI-resistant patients and leading to the resistance through a variety of mechanisms. These mutations are often studied when defining the resistance profile of new antiretroviral drugs. Additionally, the inhibitory activity of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil was shown against a double mutant with the two most common substitutions K103N and Y181C, which is extremely important for the development of a new antiretroviral drug aimed at overcoming the resistance of the virus. To confirm the activity of the compound against various strains and clinical isolates of infection, *in vitro* studies on the cell cultures infected with HIV, are required.

## CONCLUSION

Thus, the results of the studies performed indicate that the UV spectrum of the compound has a pronounced absorption maximum when measuring a solution of the substance in tetrahydrofuran at the concentration of 0.10 mg / ml. In the IR spectrum there are specific bands in the range of 4000–370 $cm^{-1}$, which make it possible to use UV and IR spectra for the identification of the test compound in the substance. As a result of the performed X-ray structural, elemental, $^{1}$H-NMR and $^{13}$C-NMR analyzes, the structure of 1-[2-(2-benzoylphenoxy)ethyl]-6-methyluracil has been confirmed.

The possibility of using UV, IR and NMR spectroscopy, as well as thermal analyzes to confirm the authenticity of the substance during its verification, has been shown. The developed methods can be used in the quality control and are included in the draft regulatory document for the substance 1-[2-(2-benzoylphenoxy) ethyl]-6-methyluracil.

The studies of the action mechanism of the test substance against HIV-1 RT showed that the compound belongs to the HIV-1 NNRTI group.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHORS' CONTRIBUTION

Ekaterina A. Jain (Korsakova) – research methodology, interpretation of results, text writing;
Dmitry V. Demchenko – statistical processing of results, text writing;
Alexander A. Ozerov, Vadim Yu. Balabanyan – working out the research concept and design;
Marina N. Makarova, Valery G. Makarov – interpretation and visualization of results

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# ESTIMATION OF SOCIO-ECONOMIC BURDEN OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE FOR A 5-YEAR PERIOD: A REGIONAL ASPECT

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**The aim** of the study was to estimate the economic damage by COPD, including direct medical and non-medical costs and indirect costs associated with premature deaths of working-age individuals.
**Materials and methods.** First, estimation of the economic COPD burden in Astrakhan region (AR) was carried out using the clinical and economic analysis of the "cost of illness" (COI). Direct medical costs of inpatient, outpatient, ambulance and emergency medical care, as well as direct non-medical costs associated with the disability benefits payments, were taken into account. Indirect costs were defined as economic losses from undelivered products due to premature deaths of working-age individuals.
**Results.** From 2015 to 2019, the economic COPD burden in AR amounted to 757.11 million rubles in total, which is equivalent to 0.03% of the gross regional product covering a five-year period of the study. Direct medical and non-medical costs totaled 178.02 million rubles. In the structure of direct medical expenses, expenses for inpatient, as well as ambulance and emergency medical care during the study period, increased by 92.5% and 45.5%, respectively. While the costs for the outpatient care decreased by 31.9%, the increase in direct non-medical costs associated with the disability benefits payments, increased by 5.1% (2019). Indirect losses amounted to 579.09 million rubles.
**Conclusion.** The structure of the main damage is dominated by indirect losses in the economy associated with premature deaths of working-age individuals. In the structure of direct medical costs, inpatient care costs prevailed. These studies indicate the need to continue an advanced analysis of the economic burden of COPD, as well as to optimize the treatment and prevention of the exacerbations development of this disease.
**Keywords:** Chronic Obstructive Pulmonary Disease; economic burden; direct medical costs; direct non-medical costs; indirect costs; Astrakhan region
**List of abbreviations:** AR – Astrakhan region; RDs – respiratory diseases; COPD – Chronic Obstructive Pulmonary Disease; EU – European Union; TD – temporary disability; TCMIF – Territorial Compulsory Medical Insurance Fund; A – Ambulance; EMC – emergency medical care; DRG – Diagnosis-related group; GRP – gross regional product; YPLL – years of potential life lost

# ОЦЕНКА СОЦИАЛЬНО-ЭКОНОМИЧЕСКОГО БРЕМЕНИ ХРОНИЧЕСКОЙ ОБСТРУКТИВНОЙ БОЛЕЗНИ ЛЕГКИХ ЗА 5-ЛЕТНИЙ ПЕРИОД – РЕГИОНАЛЬНЫЙ АСПЕКТ

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**Цель.** Оценка экономического ущерба от ХОБЛ, включая прямые медицинские и немедицинские затраты и непрямые затраты, связанные с преждевременной смертью лиц трудоспособного возраста.
**Материалы и методы.** Впервые проведена оценка экономического бремени ХОБЛ в Астраханской области (АО) с использованием клинико-экономического анализа «стоимость болезни». В исследовании учитывались прямые медицинские затраты на стационарное, амбулаторное лечение, скорую и неотложную медицинскую помощь, а также прямые немедицинские затраты, связанные с выплатами пособий по инвалидности. Непрямые затраты определялись как экономические потери от не произведенной продукции вследствие преждевременной смерти в экономически активном возрасте.
**Результаты.** Экономическое бремя ХОБЛ в АО за период с 2015 по 2019 гг. суммарно составило 757,11 млн рублей, что эквивалентно 0,03% валового регионального продукта за пятилетний период исследования. Прямые медицинские и немедицинские затраты суммарно составили 178,02 млн. рублей. В структуре прямых медицинских затрат расходы на стационарную, а также скорую и неотложную медицинскую помощь за период исследования увеличились на 92,5% и 45,5% соответственно. В то время как затраты на амбулаторную помощь уменьшились на 31,9%, прирост прямых немедицинских затрат, связанных с выплатами пособий по инвалидности, вырос на 5,1% к 2019 г. Непрямые потери составили 579,09 млн рублей.
**Заключение.** В структуре основного ущерба преобладают непрямые потери в экономике, связанные с преждевременной смертью населения экономически активного возраста. В структуре прямых медицинских затрат превалировали затраты на стационарную помощь. Данные исследования свидетельствуют о необходимости продолжения более углубленного анализа экономического бремени ХОБЛ, а также оптимизации лечения и профилактики развития обострений данного заболевания.
**Ключевые слова:** хроническая обструктивная болезнь легких; экономическое бремя; прямые медицинские затраты; прямые немедицинские затраты; непрямые затраты; Астраханская область
**Список сокращений:** АО – Астраханская область; БОД – болезни органов дыхания; ХОБЛ – хроническая обструктивная болезнь легких; ЕС – Европейский союз; ВН – временная нетрудоспособность; ТФОМС – Территориальный фонд обязательного медицинского страхования; СМП – скорая медицинская помощь, НМП – неотложная медицинская помощь; КСГ – клинико-статистические группы; ВРП – валовый региональный продукт; ПГПЖ – потерянные годы потенциальной жизни.

## INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is the most common chronic respiratory disease (RD). Currently, more than 250 million people suffering from this pathology, are registered worldwide [1]. In recent decades, there has been an increase in morbidity and mortality from COPD [2]. If in Western Europe the prevalence of COPD is 10–27% [3], in Russia it is 21.8% [4]. COPD is the third leading cause of death in the world [5]. The high prevalence, combined with disability and mortality, represents COPD as a global medical, social and economic problem [6, 7].

The study of the economic damage caused by COPD is carried out all over the world. In the United States of America alone, direct costs for hospital admissions in cases of COPD exacerbations, is $18 billion [8]. In the European Union (EU) countries, respiratory diseases burden health budgets by € 47.3 billion. With a total health expenditure of € 800 billion (9% of gross domestic product), direct costs for respiratory diseases account for approximately 6% of the total health budget of these countries [9]. Similar studies are being carried out in the Russian Federation. So, in the research by I.S. Krysanov [10], direct costs were studied, and they amounted to 54.6 billion rubles in 2007. By 2012, these costs had increased to 61.6 billion rubles. According to the results of the study by the "Quality of Life" (QOL) social fund, the COPD economic burden in the Russian Federation (RF) in 2013 was estimated at more than 24 billion rubles, including medical costs, the costs associated with the disability benefits payments (DBPs), and presenteeism[1]. In 2016, a group of Russian researchers headed by A.V. Kontsevaya, calculated the economic damage from respiratory diseases (RRs) and COPD in the Russian Federation for 2016. It amounted to 903.9 billion rubles for respiratory diseases (RDs) and 170.3 billion rubles for COPD (18.8% of RDs and 0.2% of GRP) [12].

As evidenced by statistical regional data, in Astrakhan region (AR), just as in the Russian Federation as a whole, there are dismal projections of an increase in hospital admissions, disabilities and mortality due to COPD[2]. The regional economic burden of COPD has not been calculated previously, which makes this study relevant.

**THE AIM** of the study was to estimate the economic damage from COPD, including direct medical and non-medical costs and indirect costs associated with premature deaths of working-age individuals.

## MATERIALS AND METHODS

For the first time, an assessment of the economic COPD burden was made in Astrakhan region (AR) for the period of 2015–2019. The research included a step-by-step calculation of direct costs of the AR health care sys-

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tem in the study period, as well as indirect costs in the form of economic losses. The research included statistics on COPD (the ICD code is J44).

In order to assess the impact of COPD on the regional budget, a type of clinical and economic analysis was used – the cost of illness (COI) analysis, which includes both direct and indirect government costs [10]. The analysis was performed according to the following formula:

$$COI = DC + IC,$$

where COI – Cost of Illness; DC – Direct Costs; IC – Indirect Costs.

To assess the economic burden of COPD, the following resources and databases were analyzed.

1. The data on the incidence of COPD and RDs in the population presented by Federal State Budgetary Institution "The Central Research Institute for Organization and Information Technologies" (the Ministry of Health of Russia) for the study period, including the absolute number of total and new identified cases of COPD[3].

2. The data on the annual forms of statistical reporting of the Territorial Compulsory Medical Insurance Fund of Astrakhan Region: "Information on the activities of the subdivisions of a medical organization providing medical care in inpatient conditions" (a number of hospital admissions, a number of ambulance calls; emergency medical care (EMC) (Form N14)), "Information on the number of diseases registered in patients living in the service areas of medical organizations" (Form N12), "Information on the activities of day hospitals of medical organizations" (Form N141)[4].

3. Regional economic parameters of the gross regional product (GRP), as well as statistical data on the number of deaths due to COPD by five-year age groups for each study year, were provided by the Federal State Statistics Service for Astrakhan region and the Republic of Kalmykia[5]. The statistical data were requested from the Federal State Institution "Main Office of Medical and Social Assessment of Astrakhan Region" (the Ministry of Labor of Russia) on the number of people with disabilities due to COPD by disability groups for each year of the study period.

When calculating direct medical costs, such as costs of visits to outpatient patients, emergency medical services and treatment in inpatient conditions, the cost indicators according to the tariffs of the Territorial Compulsory Medical Insurance Fund (TCMIF) in AR for the study period, were used. The economic analysis of direct medical costs of the state for the provision of COPD medical care covering a five-year period in the Astrakhan region was based on the aggregate of the above-listed costs.

Direct non-medical costs of disability benefits payments (DBPs) were calculated taking into account the number of the COPD disabled in each group and the estimated value of the disability benefits for each group and for each study year. The parameters used to calculate the direct non-medical costs associated with disability due to COPD, are presented in Table 1.

Indirect costs associated with losses in the production of GRP due to the premature deaths of working-age individuals, were calculated taking into account the employment rate of the population. The calculation procedure was corresponded to the methodology approved in accordance with the Order dated 10 April 2012 No. 192 / 323n / 45n / 113 of the Ministry of Economic Development of the Russian Federation "On approval of the Methodology for calculating economic losses from mortality, morbidity and disablement of the population"[6].

The main formula for calculating the loss from mortality of the population was:

$$LPMY_{x,s,d} = ND_{x,s,d} \frac{NE_{x,s}}{PS_{x,s}} \times \frac{GRP}{NE} \times 0{,}5,$$

where: $LPMY_{x,s,d}$ – lost profits in the GRP production (volume of underproduced GRP) as a result of mortality of persons in the reporting year at the age (x) of sex (s) due to deaths (d) in the Russian Federation in the reporting year; $ND_{x,s,d}$ – number of deaths at the age (x) of sex (s) due to deaths (d) in Astrakhan region; $NE_{x,s}$ – number of employed at the age (x) of sex (s) in Astrakhan region;$PS_{x,s}$ – population size at the age (x) of sex (s) in Astrakhan region; GRP – Gross regional product of Astrakhan region; NE – number of employed in the region; 0.5 – coefficient, taking into account the distribution of the time of deaths during the year.

The years of potential life lost (YPLL) were calculated according to the formula:

$$YPLL = (72 - Age_{gr.}) \times NDAge_{gr.},$$

where YPLL – years of potential life lost; 72 – extreme age limit for economic activity; $Age_{gr.}$ – Age group; $NDAge_{gr.}$ – number of deaths in the studied age group.

The computational part of the study was carried out using descriptive statistics methods in the MS Excel 10.0 program (Microsoft, USA).

[3] Federal Research Institute for Health Organization and Informatics of Ministry of Health of the Russian Federation. Available from: https://mednet.ru/miac/meditsinskaya-statistika/.Russian

[4] Territorial fund of compulsory health insurance of the Astrakhan region. Tariffs. Available from: https://astfond.ru/oms/tarify/. Russian

[5] Office of the Federal State Statistics Service for the Astrakhan Region and the Republic of Kalmykia. Available from: https://www.astrastat.gks.ru.

[6] Order of the Ministry of Economic Development of the Russian Federation, the Ministry of Health and Social Development of the Russian Federation, the Ministry of Finance of the Russian Federation and the Federal State Statistics Service of April 10, 2012 N 192 / 323-n / 45-n / 113 "On approval of the Methodology for calculating economic losses from mortality, morbidity and disability of the population". Available from: https://base.garant.ru/70170542/. Russian

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**Table 1 – Cost indicators used in the analysis**

| Indicator | Value | | | | |
|---|---|---|---|---|---|
| Coefficient of relative input intensity in DRG for COPD | 0.91 | | | | |
| Costs: | Costs, rubles | | | | |
| | 2015 | 2016 | 2017 | 2018 | 2019 |
| 1 hospital admission on the policy of compulsory medical insurance | 14933.72 | 15141.61 | 13524.03 | 15016.73 | 17725.39 |
| 1 prophylactic treatment | 302.8 | 160.0 | 97.6 | 120.3 | 137.0 |
| 1 treatment for a disease | 803.20 | 620.298 | 617.30 | 830.01 | 849.93 |
| 1 emergency call taking into account the DRG coefficient | 453.2 | 490 | 443.8 | 568.5 | 592.9 |
| 1 ambulance call taking into account the DRG coefficient | 1724.5 | 1747.7 | 1819.5 | 2122.7 | 2248.92 |
| 1 case of day hospital treatment taking into account the DRG coefficient | 10056.18 | 8538.3 | 7 622.19 | 8253.27 | 11 627.83 |
| Indicator | | | | | |
| Gross regional product (GRP), mln. rubles | 2015 | 2016 | 2017 | 2018 | 2019 |
| | 322303.0 | 346779.4 | 420961.1 | 553395.7 | 561695.9 |
| Gross regional product (GRP) per caput, mln. rubles | 315996.9 | 340398.3 | 413440.6 | 544793.4 | 552965.3 |
| Average monthly salary, rubles | 25499 | 27423.1 | 29599.2 | 33630.1 | 35791.5 |
| Calculated value of the disability benefits payments (DBPs) taking into account the indexation coefficient | Group | 2015 | 2016 | 2017 | 2018 |
| | I | 16285.6 | 17934.3 | 24466.6 | 20270.7 |
| | II | 13193.2 | 14613.3 | 20915.9 | 16062.6 |
| | III | 10777.1 | 12070.8 | 18318.3 | 13489.6 |

**Table 2 – COPD and RDs incidence for 5 years in AR (2015–2019)**

| Indicator | 2015 | 2016 | 2017 | 2018 | 2019 |
|---|---|---|---|---|---|
| Number of persons with RDs | 289876 | 316565 | 300636 | 313889 | 310706 |
| Number of persons with COPD | 2869 | 2788 | 2721 | 2913 | 3180 |
| COPD incidence, incident cases | 445 | 226 | 266 | 310 | 496 |
| Share of COPD in RDs,% | 0,99 | 0,88 | 0,91 | 0,93 | 1,02 |

**Table 3 – Direct medical costs in AR associated with COPD covering the period of 2015–2019**

| Inpatient care for COPD patients within 5 years (2015–2019) | | | | | |
|---|---|---|---|---|---|
| Indicator | 2015 | 2016 | 2017 | 2018 | 2019 |
| Number of hospital admissions | 1121 | 1365 | 1699 | 1671 | 1818 |
| Number of bed-days, in total | 10089 | 12285 | 16141 | 15039 | 16362 |
| Average duration of treatment, bed-days | 9,0 | 9.0 | 9.5 | 9.0 | 9.0 |
| Costs for hospital admissions, mln. rubles | 16.74 | 20.67 | 22.97 | 25.09 | 32.22 |
| Outpatient care for COPD patients covering 5 years (2015–2019) | | | | | |
| Number of visits for preventive and other purposes | 2656 | 2419 | 2554 | 2596 | 3333 |
| Number of treatments for diseases | 2737 | 2445 | 2163 | 1965 | 1595 |
| Number of day hospital admissions. | 165 | 185 | 174 | 144 | 117 |
| Outpatient care costs, mln rubles | 4.66 | 3.48 | 2.91 | 3.13 | 3.17 |
| Emergency medical care (ambulance+ emergency) for COPD patients covering 5 years (2015–2019) | | | | | |
| Number of ambulance calls | 1844 | 2013 | 1830 | 1994 | 2031 |
| Number of emergency medical care cases | 554 | 951 | 868 | 844 | 722 |
| Costs for ambulance and emergency care, mln rubles | 3.43 | 3.98 | 3.71 | 4.71 | 4.99 |
| Summary total of direct medical costs | | | | | |
| Direct medical costs for COPD, mln rubles | 24.83 | 28,14 | 29.60 | 32.94 | 40.39 |
| Share of costs for inpatient care,% | 67.4 | 73.5 | 77.6 | 76.2 | 79.8 |
| Share of costs for outpatient care,% | 18.8 | 12.4 | 9.8 | 9.5 | 7.8 |
| Share of costs for ambulance and emergency care, mln rubles | 13.8 | 14.1 | 12.5 | 14.3 | 12.4 |

**Table 4 – Number of people with disabilities due to COPD and direct non-medical costs in AR for 5 years (2015–2019)**

| Disablement group | Number of COPD disabled persons | | | | |
|---|---|---|---|---|---|
| | 2015 | 2016 | 2017 | 2018 | 2019 |
| I | 0 | 0 | 0 | 2 | 0 |
| II | 5 | 5 | 5 | 11 | 10 |
| III | 27 | 15 | 19 | 14 | 14 |
| In total | 32 | 20 | 24 | 27 | 24 |
| Number of RDa disabled persons | 201 | 153 | 142 | 130 | 142 |
| Share of persons disabled due to COPD in RDs, % | 15,9 | 13 | 16,9 | 20.7 | 16.9 |
| Costs of disability benefits, mln rubles | 4.28 | 3.05 | 5.43 | 4.87 | 4.48 |

**Table 5 – Structure of mortality due to RDs and COPD in AR within 2015–2019**

| Years | | 2015 | 2016 | 2017 | 2018 | 2019 |
|---|---|---|---|---|---|---|
| Deaths due to all diseases | Male | 6418 | 6340 | 5838 | 5893 | 5780 |
| | Female | 6119 | 5889 | 5782 | 5841 | 5646 |
| In total | | 12537 | 12229 | 11620 | 11734 | 11426 |
| Deaths due to RDs | Male | 291 | 271 | 253 | 273 | 291 |
| | Female | 139 | 114 | 116 | 144 | 123 |
| In total | | 430 | 385 | 369 | 417 | 414 |
| Deaths due to COPD | Male | 48 | 46 | 43 | 52 | 58 |
| | Female | 10 | 10 | 11 | 11 | 15 |
| In total | | 58 | 56 | 54 | 63 | 73 |
| Share of deaths due to COPD in RDs | | 13.5 | 14.5 | 14.6 | 15.1 | 17.6 |
| Relative mortality rate among COPD patients,% | | 2.0 | 2.0 | 2.0 | 2.2 | 2.3 |

**Table 6 – Age structure of mortality from COPD, and YPLL in AR for 2015–2019**

| Years / Age | Up to 69 years old | | Older than 70 years | | Years of potential life lost (YPLL) |
|---|---|---|---|---|---|
| | n | % | n | % | |
| 2015 | 35 | 60% | 23 | 40% | 500 |
| 2016 | 42 | 75% | 14 | 25% | 556 |
| 2017 | 24 | 44% | 30 | 56% | 274 |
| 2018 | 32 | 51% | 31 | 49% | 321 |
| 2019 | 37 | 51% | 36 | 49% | 396 |
| Average value | 34.0 ±6.7 | 56%±12% | 26.8±8.5 | 44%±12% | 409.4±118.4 |

**Table 7 – Economic burden of COPD in RDs for the period of 2015–2019**

| Cost type | 2015 | 2016 | 2017 | 2018 | 2019 | For 5 years |
|---|---|---|---|---|---|---|
| Direct costs for COPD, mln rubles | 29.12 | 31.19 | 35.03 | 37.81 | 44.87 | 178.02 |
| Indirect costs due to premature COPD deaths, mln rubles | 102.63 | 124.94 | 82.63 | 118.65 | 150.24 | 579.09 |
| Summary total of losses and costs due to COPD, mln rubles | 131.75 | 156.12 | 117.66 | 156.46 | 195.11 | 757.11 |
| Share in GRP, % | 0.04 | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 |

## RESULTS

The analysis showed an increase in the incidence of RDs from 2015 to 2019 by 7.2%. The number of COPD patients in the study period also increased by 10.8%. The largest share of COPD in RDs was 1.02% in 2019 (Table 2).

Covering a five-year period, there was an increase in hospital admissions of patients with COPD by 62.2%, with its highest value of 1818 cases in 2019. The number of bed-days spent by patients with COPD in a round-the-clock hospital during the analyzed period, with a relatively equal average duration of treatment for each patient, also increased by 62.2%. The actual costs of in-patient care for COPD patients during the study period increased by 92.5% (Table 3).

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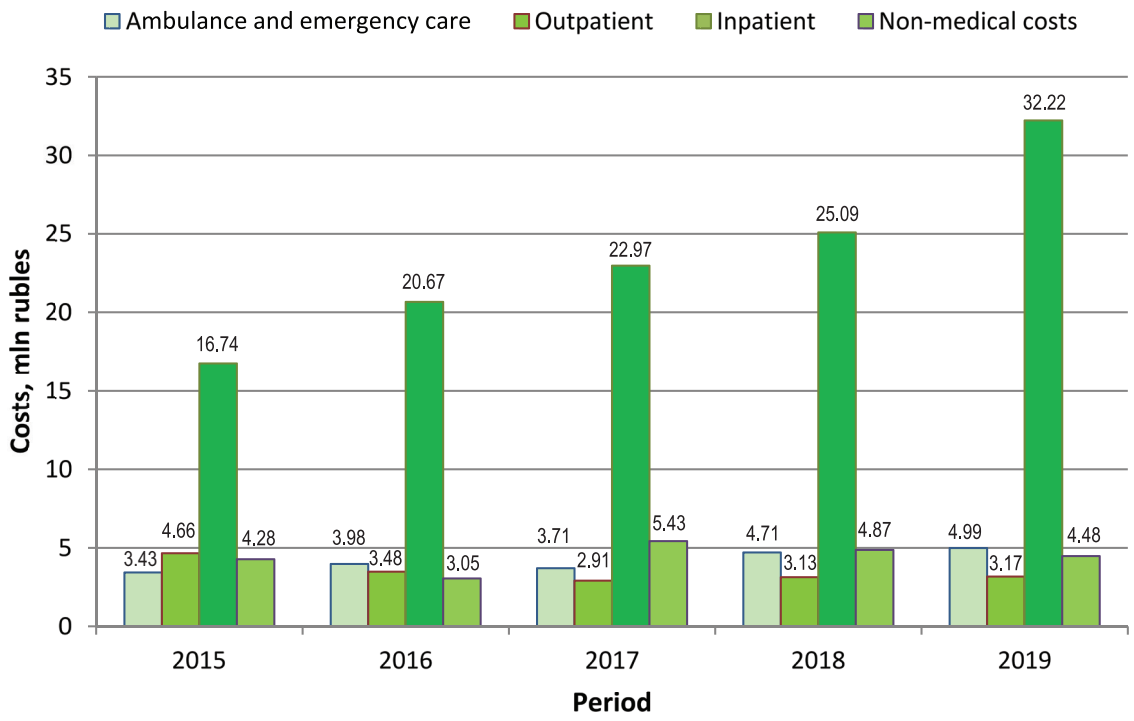


**Figure 1 – Structure of direct costs for COPD in Astrakhan region within a five-yearperiod (2015–2019)**

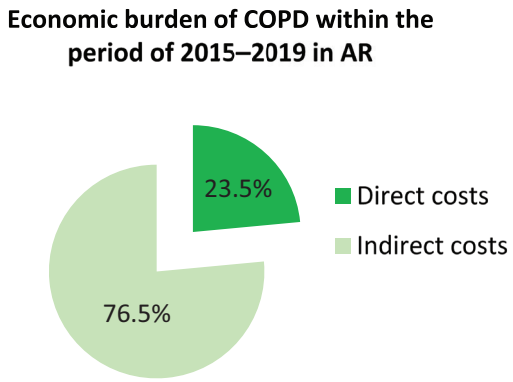


**Figure 2 – Structure of COPD economic burden in Astrakhan region within the period of 2015–2019**

In calculating the direct medical costs of the RF for the outpatient care of COPD patients, the following indicators were taken into account: the number of visits to patients for preventive and other purposes; the number of treatments for diseases; number of day hospital admissions. During the study period, the indicator "a number of visits for preventive purposes" increased by 25.5%, while "treatments for diseases" and "the number of day hospital admissions" decreased by 41.7% and 29%, respectively. In this regard, direct medical costs for outpatient COPD care during the study period, decreased by 31.9%, amounting to 3.17 million rubles in 2019 (Table 3).

During the study period, there was an increase in the number of ambulance calls and the provision of emergency medical services to COPD patients by 10.1% and 30.3%, respectively. This contributed to an increase in the emergency medical care costs for the period 2015–2019 by 45.5%, and in monetary terms, it amounted to 4.99 million rubles in 2019 (Table 3).

Direct medical costs for treating COPD patients increased by 62.7% covering the five-year period, and amounted to 40.39 million rubles in 2019. The analysis of the structure of direct medical costs associated with COPD in RDs for the study period (Table 3) shows, that the largest share of costs for inpatient COPD patients coincided with 2019 (79.8% of direct medical costs), the share of costs for outpatient care prevailed in 2015 (18.8% of direct medical costs), and the share of expenses for emergency medical services and emergency medical care was the largest in 2018 (14.3% of direct medical costs).

In the calculation of direct non-medical costs, the cost of disability benefits for each surveyed year was taken into account. Table 4 shows the number of people with disabilities due to COPD and RDs according to disability evaluation groups. In the period of 2015-2019, the number of persons with disabilities due to COPD decreased from 32 to 24, the number of persons with disabilities due to RDs also decreased from 201 to 142. However, the proportion of persons with disabilities due to COPD in RDs during this period increased by 1%. The analysis of direct non-medical costs associated with disability benefits payments shows an increase by 5.1% in 2019, at the same time, the largest cost indicator coin-

cided with 2017, which, in monetary terms, corresponds to 5.43 million rubles.

The structure of direct costs for COPD in AR within the study period is shown in Fig. 1. The main share of direct costs was the cost of inpatient care.

Indirect losses in the economy were analyzed on the basis of premature deaths of working-age individuals. As Table 5 shows, a total of 304 people (217 men and 87 women) died due to COPD within the period of 2015–2019. There was an increase in the absolute number of deaths due to COPD, and it amounted to 25.9% for a five-year observation period. An increase in the share of deaths due to COPD in relation to the number of deaths due to RDs, which increased from 13.5% to 17.6% for a five-year period, is also worth notifying. At the same time, a relative mortality rate among COPD patients remained relatively stable, due to the increase in the number of COPD cases, and averaged to 2.1±0.13%.

Due to COPD, within the entire study period, 2047 years of potential life were lost on the basis of premature deaths of working-age individuals. In particular, the largest number of years (500) of YPLL took place in 2015, and the smallest (274 years) – in 2017. This is due to the fact that in 2015, 60% of patients died at premature deaths of working-age individuals (up to 69 years old); in 2017 their number decreased to 44%, and COPD patients began to die at a later age (Table 6).

The total amount of losses and costs for the five-year observation period reached 757.1 million rubles, which is equivalent to 0.03% of the GRP of Astrakhan region. In the structure of the main damage by COPD, indirect losses associated with premature deaths of working-age individuals, prevail in economy. In economic terms it means that within a five-year observation period, all losses and costs amounted to 579.09 million rubles (76.5%). The total amount of direct costs for the study period reached 178.02 million rubles (23.5% of all losses and costs) (Table 7; Fig. 2).

## DISCUSSION

COPD is the cause of colossal costs in the health care system of all the countries around the world. According to the studies from various countries, the prevailing share of costs for COPD associated with hospital admissions of patients during exacerbations [12–15], is represented by direct government costs. These costs increase with the severity of COPD exacerbations requiring emergency care, longer hospital stays or intensive care units [16]. Recent studies of economic costs in Asian countries have confirmed that these results are universal. However, the burden of chronic diseases, such as COPD, is a particular problem in low-income countries, where health resources have traditionally been focused on episodic management of acute diseases, especially infectious ones, and are not adapted to the treatment of chronic diseases [14]. The tendency to increase costs depending on the severity of COPD was noted in the studies of scientists from Italy [17] and Great Britain [18]. The economic research on determining the burden of COPD is regularly conducted in the United States. In a 2010 study, direct costs for COPD were $ 32 billion and indirect costs were $ 20.4 billion [19]. Another study, which included an estimation of the total costs for COPD in 2010 and projected medical costs up to 2020, found out that the economic damage by COPD was $ 36 billion per year. Of these, the direct medical costs associated with COPD and its consequences, were estimated at $ 32.1 billion, and the costs associated with the loss of 16.4 million workdays, was $ 3.9 billion [20]. In the UK, the damage by COPD was 1.9 billion pounds [21]. However, in a 2016 study conducted in 12 countries (USA, UK, Germany, Italy, etc.), it was shown that indirect costs were several times higher than direct ones, which reflects the hidden nature of the economic burden associated with a decrease in labor productivity [22]. Losses in labor productivity due to the unexcused absence and premature retirement are key drivers of higher indirect costs [23]. Determining the indirect costs associated with COPD, can be challenging [24], but it is clear that accounting for them will help to identify the true socioeconomic burden of COPD.

The study by A.V. Kontsevaya et al. [11] also showed the prevalence of indirect losses associated with premature COPD deaths of working-age individuals. In this study, similar results of the COPD burden in Astrakhan region within a 5-year period, were obtained. Indirect costs significantly exceeded direct ones in them (Fig. 2).

In the structure of direct medical costs, there is an increase in costs for inpatient care, emergency care and emergency medical services, with a simultaneous decrease in outpatient care costs associated with a decrease in visits for illness and hospital admissions in a day hospital. In the structure of direct medical costs, there is an increase in costs for inpatient care, ambulance care and emergency medical services, with a simultaneous decrease in outpatient care costs associated with a decrease in treatments for diseases and day hospital admissions. This indicates an increase in the frequency and severity of COPD exacerbations, which indirectly leads to high costs for the treatment of these exacerbations in hospital settings. Reducing the cost of outpatient care requires its optimization, since economically, it is less expensive than inpatient care and, therefore, a possible increase in investment of health care resources in the treatment and prevention of this disease is justified.

### Limitations of the study

The study did not include the cost of medical care at the outpatient stage of treatment. When calculating indirect costs, the costs associated with temporary disability (TD) and primary disability output due to COPD, were not taken into account. According to the methodology for calculating economic losses due to premature deaths, the calculation was carried out

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taking into account the number of deaths by age (one-year groups) and gender. However, in connection with the provision of statistics for five-year age groups, it was decided to calculate economic losses at the upper limit of the five-year age group (Example: the group of 45–49 – 49 years were used), which probably underestimates the result of total economic losses.

Due to the availability of information on the number of the working-age population by age groups and gender only for 2018 and 2019, the average values of 2018 and 2019 were used when calculating indirect costs for previous years.

## CONCLUSION

This paper was the first to assess the impact of the economic COPD burden on the regional budget of Astrakhan region for the period of 2015-2019. Economically, the damage by COPD in AR for this period amounted to 757.11 million rubles, which is equivalent to 0.03% of the GRP for the study period. The structure of damage is dominated by the losses associated with premature deaths of working-age individuals, amounting to 579.09 million rubles. Direct costs (medical and non-medical ones) totaled 178.02 million rubles. In the structure of direct medical costs, inpatient care costs prevail.

Taking into account the fact that there are unaccounted costs associated with temporary disability, primary disability, and other factors that limit this study, it seems relevant to conduct a further analysis of the economic COPD burden, which will contribute to the most complete assessment of the costs of this disease. For the leaders of the regional health care system, it is also very important to pay attention to the development of measures aimed at preventing the contraction of the disease and reducing COPD exacerbations. To do this, it is necessary to increase investments in the treatment, improve the possibilities of the outpatient link. At the outpatient stage, sustaining COPD drug therapy will make it possible to "control" the course of the disease, reducing the number of hospital admissions – the most expensive treatment option. In addition, it is necessary to stimulate the coverage of pneumococcal vaccination in this category of chronic patients, which will help to reduce exacerbations and significantly reduce hospitalization costs.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

Ekaterina A. Orlova – material collecting, text writing, editing; Adelya R. Umerova – text writing and editing; Inna P. Dorfman – material collecting, editing; Mikhail A. Orlov – text writing and editing; Musalitdin A. Abdullaev – text writing and compiling a bibliographic list.
All authors made a significant contribution to the search and analytical work and preparation of the article; read and approved of the final version before publication.

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# ISOLYQUIRITIGENIN AFFECTS PHAGOCYTES FUNCTIONS AND INCREASES MICE SURVIVAL RATE IN STAPHYLOCOCCAL INFECTION

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The results of studying the effect of isoliquiritigenin on animal survival in the model of staphylococcal infection and the function of human and animal phagocytes are presented in this article.

**The aim** of the investigation was to study the effect of an isoliquiritigenin preliminary administration on the survival of animals against the background of staphylococcal infection, as well as on the function of phagocytes in mice and humans.

**Materials and methods.** To assess the survival of Balb/C mice, a model of infection caused by *Staphylococcus aureus J49 ATCC 25923* with the construction of Kaplan-Meier curves, was used. The effect on the phagocytes functions was studied by assessing the peptone-induced migration of phagocytes into the abdominal cavity of Balb/C mice, the absorption activity of phagocytes (neutrophils and monocytes) of human blood, as well as their production of reactive oxygen intermediates (ROIs) using a flow cytometry.

**Results.** It was found out that a preliminary triple intraperitoneal administration of isoliquiritigenin (30 mg/kg) increases the survival rate of Balb/C mice in staphylococcal infection caused by *Staphylococcus aureus J49 ATCC 25923.* At the same time, isoliquiritigenin dose-dependently activates the production of reactive oxygen intermediates by human neutrophils and monocytes without statistically significantly suppressing a phagocytic activity of monocytes and neutrophils against fluoresceinisothiocyanate-labeled *Staphylococcus aureus J49 ATCC 25923,* as well as peptone-induced migration of phagocytes into the abdominal cavity of mice.

**Conclusion.** Thus, a preliminary administration of isoliquiritigenin increases the survival rate of mice with staphylococcal infection and increases the production of reactive oxygen intermediates by phagocytes. The data obtained, can become the basis for further research of antibacterial and immunotropic effects of isoliquiritigenin in order to find new drugs for the treatment of staphylococcal infection.

**Keywords:** isoliquiritigenin; *Staphylococcus aureus;* innate immunity; phagocytosis; oxidative burst; phagocyte migration

**List of abbreviations:** ROI(s) – reactive oxygen intermediate(s)**;** DHR 123 – dihydrorhodamine 123**;** DMSO – dimethylsulfoxide**;** ISL – isoliquiritigenin; CFUs – colony-forming units**;** ConA – concanavalin A**;** NADP-oxidase – nicotinamide adenine dinucleotide phosphate oxidas**;** NTs – neutrophil traps**;** FITC – fluorescein isothiocyanate; PMA – phorbolmyristate acetate; phorbol-12 myristate-13-acetate; MTT – 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide; OD – optical density

# ИЗОЛИКВИРИТИГЕНИН ВЛИЯЕТ НА ФУНКЦИИ ФАГОЦИТОВ И ПОВЫШАЕТ ВЫЖИВАЕМОСТЬ МЫШЕЙ ПРИ СТАФИЛОКОККОВОЙ ИНФЕКЦИИ

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*RESEARCH ARTICLE*

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В данной статье представлены результаты изучения влияния изоликвиритигенина на выживаемость животных в модели стафилококковой инфекции и функции фагоцитов человека и животных.

**Цель.** Изучить влияние предварительного введения изоликвиритигенина на выживаемость животных на фоне стафилококковой инфекции, а также на функции фагоцитов мышей и человека.

**Материалы и методы.** Для оценки выживаемости мышей линии Balb/C использовали модель инфекции, вызванной *Staphylococcus aureus J49 ATCC 25923,* с построением кривых Каплан-Мейера. Влияние на функции фагоцитов изучали, оценивая пептон-индуцированную миграцию фагоцитов в брюшную полость мышей Balb/C, поглотительную активность фагоцитов (нейтрофилов и моноцитов) крови человека, а также продукцию ими активных форм кислорода с помощью проточной цитометрии.

**Результаты.** Установлено, что предварительное трехкратное внутрибрюшинное введение изоликвиритигенина (30 мг/кг) увеличивает выживаемость мышей Balb/C при стафилококковой инфекции, вызванной *Staphylococcus aureus J49 ATCC 25923*. При этом изоликвиритигенин дозозависимо активирует продукцию активных форм кислорода нейтрофилами и моноцитами крови человека, статистически значимо не подавляя фагоцитарную активность моноцитов и нейтрофилов в отношении флюоресцеинизотиоцианат-меченого *Staphylococcus aureus J49 ATCC 25923*, а также пептон-индуцированную миграцию фагоцитов в брюшную полость мышей.

**Заключение.** Таким образом, предварительное введение изоликвиритигенина повышает выживаемость мышей при стафилококковой инфекции и увеличивает продукцию активных форм кислорода фагоцитами. Полученные данные могут стать основой для дальнейшего исследования антибактериальных и иммунотропных эффектов изоликвиритигенина с целью изыскания новых лекарственных средств для лечения стафилококковой инфекции.

**Ключевые слова:** изоликвиритигенин; *Staphylococcus aureus*; врожденный иммунитет; фагоцитоз; кислородный взрыв; миграция фагоцитов

**Сокращения:** АФК – активные формы кислорода; ДГР 123 – дигидродамин 123; ДМСО – диметилсульфоксид; ИЛГ – изоликвиритигенин; КОЕ – колониеобразующие единицы; КонА – конканавалин А; НАДФН-оксидаза – никотинамидадениндинуклеотидфосфатоксидаза; НЛ – нейтрофильные ловушки; ФИТЦ – флюоресцеинизотиоцианат; ФМА – форбол-12-миристат-13-ацетат; МТТ – 3-(4,5-диметилтиазол-2-ил)-2,5-дифенил-тетразолиум бромид; OD – оптическая плотность.

## INTRODUCTION

Recognition and elimination of microbial pathogens by a macroorganism occurs due to the activation of innate and adaptive immunity. Innate immunity prevents the introduction of microbes into tissues and is able to remove them before the mechanisms of the acquired immunity are activated. Innate immunological responses to the pathogen are almost instantaneous and are mainly based on the reactions of inflammation and phagocytosis, while the adaptive immunological response turns on only after a few days (optimally 7–14 days), since it requires proliferation and differentiation of lymphocytes.

A significant bacterial load in the model of acute bacterial infection causes death in laboratory animals during the first days of observation. This model of infection makes it possible to assess not only the antibacterial effects of the studied compounds, but also its influence on the functions of the effectors of innate immunity [1], among which phagocytes play an important role.

Polyphenolic compounds of higher plants (flavonoids) have a wide spectrum of biological activity, including antimicrobial and immunomodulatory effects [2]. For example, a parenteral administration of licorice root flavonoids increases the resistance of mice to acute staphylococcal infection at the doses that did not significantly affect the functions of innate immunity effectors [3], but prevented the activation and proliferative response of lymphocytes [4]. Dozens of different flavonoids have been isolated from licorice roots, one of the main of them being isoliquiritigenin (ISL). At various concentrations, ISL exhibits antibacterial properties, influences the proliferation of lymphocytes and their secretion of cytokines at the early stages of the immune response during staphylococcal infection in mice [2].

**THE AIM** of the investigation was to study the effect of isoliquiritigenin on the phagocytes functions in mice and humans as well as its effect against the background of staphylococcal infection in mice.

## MATERIALS AND METHODS

### Test agent

ISL (98% purity, Xi'An YiyangBio-Tech Co., China) was used as a test substance. In the experiments, a solution of ISL in dimethyl sulfoxide (DMSO, Panreac, Spain) was used so that *in vitro* the final concentration of the solvent in the test samples did not exceed 1%. Considering that the MIC of ISL against *S. aureus J49 ATCC 25923* is 64 μg/ml [5], ISL in the concentration range of 16–128 μg/ml was used for *in vitro* experiments. In the series of *in vivo* experiments, the ISL matrix solution in DMSO was diluted in phosphate-buffered saline (pH=7.4, PanEcoLLC, Russia), injected intraperitoneally in the volume of 0.5 ml as a true solution with a DMSO concentration of no more than 5%. Given the low toxicity and bioavailability of ISL [2], in *in vivo* experiments, ISL was injected intraperitoneally at the total dose of 30 mg/kg. The control samples / groups were injected with appropriate volumes / concentrations of solvent instead of ISL.

### Bacterial strain and conditions for its cultivation

The cultivation of the *S. aureus J49 ATCC 25923* strain (Federal State Budgetary Institution Scientific

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Center for Expertise of Medicinal Products of the Ministry of Health of Russia (Moscow, Russia)), was carried out in Mueller-Hinton broth (Medicaplus LLS, Russia) at 37°C in glass aerated vials. For *in vitro* and *in vivo* experiments, dilutions for the cultivation and intraperitoneal administration to animals were prepared from an overnight bacterial culture in the middle log phase. To count colony forming units (CFUs), the optical density (OD) of the bacterial suspension was measured at 630 nm in a microplate photometer (ImmunoChem 2100, USA) using the McFarland standard, based on the following ratio: 1 optical unit OD630 = 8.5×108 CFUs/ml.

### Experimental animals

Balb/C mice (males, 20–22 g, 6–8 weeks old) were obtained from the Research and Production Enterprise "Nursery of Laboratory Animals" of the Institute of Biology of the Russian Academy of Sciences (Pushchino, Russia). The animals were cared for and treated in accordance with the ARRIVE principles [6]. The animals were kept with free access to food and water. For the experiments, mice were randomly assigned to groups of 8. Withdrawal from the experiment was carried out without anesthesia by decapitation or cervical dislocation. When performing the experiments, the provisions of the Declaration of Helsinki (Brazil, 2013) were observed, the protocol of these experiments was approved by the ethical committee of Chuvash State University n. a. I.N. Ulyanov "(Protocol No. 20-04 dated April 17, 2020).

### Obtaining blood from healthy volunteers

To determine the absorption activity of human blood phagocytes and the production of reactive oxygen intermediates by them, on the day of the experiment, the blood was taken into heparinized test tubes from healthy volunteers (60 people) aged 18–25 years after receiving voluntary informed consent. The protocol of these experiments had been approved by the ethics committee of the FSBEI HE "Chuvash State University n. a. I.N. Ulyanov" (Protocol No. 20–04 dated April 17, 2020).

### Preparation of fluorescein isothiocyanate-labeled *S. aureus*

To inactivate S. aureus J49 ATCC 25923, an overnight bacterial culture was exposed in the water bath at 95ºC for 30-40 minutes, then it was centrifuged (1000 g, 25 min.) [7]. The killed precipitated bacteria were scoured once with a carbonate-bicarbonate buffer (0.1 M, pH 9.5), and a cell suspension was prepared with a bacterial concentration of 2×108 microbial bodies per ml. Fluorescein isothiocyanate (FITC) dissolved in DMSO, was added to the inactivated bacterial suspension at the rate of 0.05 mg per 108 bacteria, followed by incubation for 1 h at the room temperature without access to light. Then, the inactivated bacterial cells were scoured three times with phosphate-buffered saline by centrifugation (1000 g, 10 min), and a suspension of FITC-labeled bacteria was diluted to a concentration of 5×108 microbial bodies per ml. Aliquots were stored at –70°C.

### Model of *S. aureus* infection in mice

A suspension of S. aureus in a phosphate-salt buffer was administered intraperitoneally $10^9$ CFUs/per mouse. The day of infection was considered the zero day of the experiment. The survival of mice was evaluated every 6 hours on the first day and daily from the second day and for the next 20 days of the experiment. The experimental animals were injected with ISL before the infection (a total dose of 30 mg/kg, three times after 4 hours, intraperitoneally). As a reference, the animals of the control group were injected with the appropriate volumes and concentrations of the solvent.

### Assessment of phagocyte chemotaxis

To assess the migration of phagocytes into the abdominal cavity, the authors were guided by the method proposed by Miyazaki [8]. The experimental animals were divided into 4 groups, which received the following: group 1 (negative control) – a sterile phosphate-salt buffer three times (0.5 ml, intraperitoneally); group 2 – a sterile peptone solution in a phosphate-salt buffer (3% – 3 ml, intraperitoneally); group 3 – a solvent three times (5% – 0.5 ml, intraperitoneally), then – a sterile peptone solution in a phosphate-salt buffer (3% – 3 ml, intraperitoneally); group 4-ISL (three times, intraperitoneally), then a sterile solution of peptone in a phosphate-salt buffer (3% – 3 ml, intraperitoneally). After 24 hours and 72 hours, the animals were withdrawn from the experiment and 20 ml of phosphate-salt buffer was injected intraperitoneally. After palpatory massaging of the abdomen, the rinsewaters were taken into plastic tubes for the subsequent centrifugation. The deposited cells were counted in Gorjaev's chamber. After that, the stimulation index was calculated as the ratio of the number of cells in the groups receiving peptone, to the number of cells in the negative control group.

### Assessment of phagocyte activity absorption

The phagocyte activity absorption was assessed using a flow cytometry [7]. For this purpose, ISL was added to the samples of heparinized human blood at the concentrations of 16–128 μg/ml. The experimental samples were cultured for 30 minutes (t=37°C, ϕ=100%, $CO_2$=5%). Then, FITC-labeled *S. aureus* was added to the samples, and the incubation was continued for 30 minutes (t=37°C, ϕ=100%, $CO_2$=5%). After the incubation time for lysis of erythrocytes, the lysis solution (Backman Coulter, USA) was added and

incubated for 10 minutes. The samples were analyzed on a Cytomics FC500 flow cytometer (Backman Coulter, USA) and the phagocytic index (the number of phagocytes absorbing FITC-labeled bacteria to the total number of phagocytes) and the fluorescence intensity were calculated.

### Assessment of producing reactive oxygen intermediates by phagocytes

The producing reactive oxygen intermediates by phagocytes was assessed using a flow cytometry [7]. To perform the test, PMA (0.1 μg/ml, samples with activated phagocytes) or 0.2% EDTA (control, non-stimulated samples of phagocytes <1% DMSO), phorbol-12-myristate-13-acetate (PMA)-induced "oxidative burst" were added to the samples of heparinized human blood, pre-incubated with ISL at concentrations of 16-128 μg/ml for 30 minutes (incubation conditions: t=37ºC; ϕ=100%; $CO_2$=5%), and incubated for 10 minutes. After the incubation, the fluorogenic substrate dihydrorhodamine 123 (GDR 123) was added to the samples and incubated for another 10 minutes. The lysed blood samples were analyzed on a Cytomics FC500 flow cytometer (Backman Coulter, USA) to determine the percentage of activated neutrophils and monocytes, as well as indicators of spontaneous and stimulated fluorescence intensity.

### Statistical analysis

All experiments were performed in at least three repetitions. The data obtained were statistically processed using GraphPadPrism 8.4.0 Software. To assess the dynamics of the death of mice, Kaplan-Meier curves were constructed. The results obtained followed the law of normal distribution, were processed by the methods of variation statistics and were presented as the arithmetic mean (M) ± standard error of the mean (SEM). The significance of the differences between the groups in the experiments, was determined by the Student's test, conducting a pairwise comparison. The differences were considered significant at p <0.05, where p is the level of significance.

## RESULTS

### Effect of ISL on the survival rate of Balb/C mice infected with *S. Aureus J49 ATCC25923*

It was established that on the second day of the experiment, when infected with 109 CFU/per mouse, the beginning of death of the animals in both experimental groups was noted. In the control group, mortality increased more dynamically, and by the 4th day, the survival rate was only 17.0±7.6% (Fig. 1). In the group that received preliminary injections of ISL at the dose of 30 mg/kg, the survival rate was significantly higher, and on the 7$^{th}$ day of the experiment it was 67.0±16.5% (p <0.05).

### Effect of ISL on peptone-induced migration of phagocytes into the abdominal cavity of mice

The migration of phagocytes into the abdominal cavity was assessed by calculating the stimulation index – the number of cells stimulated by intraperitoneal injection of peptone, relative to phosphate-buffered saline. The stimulation indices in mice treated with ISL and in control animals stimulated with peptone, did not differ significantly (Fig. 2). So, after 24 hours, the stimulation index in the control group was 2.4±0.1 vs 2.0±0.1 in the ISL-treated group; after 72 hours, the stimulation index values were characterized by values of 1.6±0.1 (control group) compared with 1.8±0.1 (the group receiving ISL).

### Influence of ISL on the absorptive activity of human blood phagocytes

The study of the ISL influence on the absorption activity of phagocytes was carried out by the cytometric method. It was shown that the *in vitro* pretreatment of phagocytes with ISL does not lead to a significant change in the percentage of phagocytic neutrophils and monocytes compared to the control (Table 1).

In the control samples, the phagocytic index in neutrophils was 93.9±6.0%, and in monocytes it was 73.9±14.1%. At the ISL concentration of 128 μg/ml, an insignificant tendency towards a decrease in the phagocytic index of neutrophils (92.5±5.5%) and monocytes (64.1±13.7%) was observed. However, the assessment of the fluorescence intensity showed that, in comparison with the control, in the samples with the addition of ISL, there was an increase in the proportion of fluorescent neutrophils (184.8±44.8 vs 145.5±41.1) and monocytes (58.5±17.2 vs 64.1±18.1).

### Effect of ISL on ROIs production by human blood phagocytes

The effect of ISL on reactive oxygen intermediates (ROIs) production by phagocytes was assessed without their activation by PMA (Table 2). Herewith, in the absence of ISL, the proportion of fluorescent neutrophils (3.9±1.8%) and monocytes (3.0±2.0%) was very low. Compared with the control values, the addition of ISL dose-dependently increased the proportion of fluorescent neutrophils at the concentrations of 128 μg/ml (100.0±0.1%; p<0.05), 64 μg/ml (99.6±0.5%; p <0.05), 16 μg/ml (34.7±8.9%; p<0.05), and at the concentrations of 128 μg/ml (86.2±11.7%; p<0.05), 64 μg/ml (47.2±18.7%; p <0.05) is the percentage of fluorescent monocytes (Fig. 3). At the same time, the neutrophil fluorescence intensity significantly differed from the control parameters (2.5±0.3 U) in the presence of 128 μg/ml ISL (6.6±1.6 U; p <0.05), 64 μg/ml (3.8±0.2 U; p<0.05), and monocytes – at 128 μg/ml (3.0±0.15 U; p <0.05).

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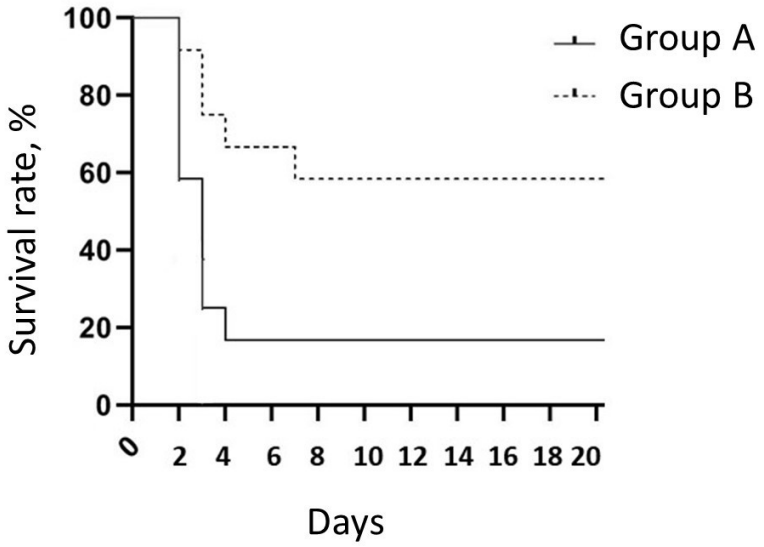


**Figure 1 – Survival rate of Balb/C mice infected with *S. aureus J49 ATCC 25923***
Note: group A – control, $10^9$ CFUs/per mouse; group B – preliminary injection of ISL (30 mg/kg), $10^9$ CFUs/per mouse

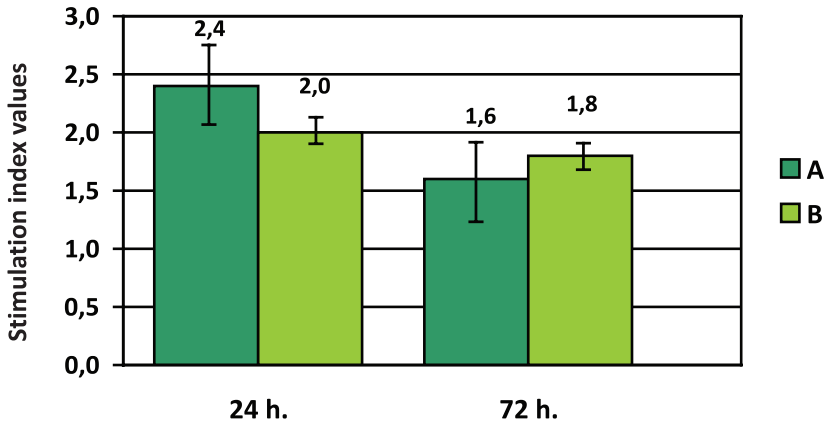


**Figure 2 – Effect of ISL on the migration of phagocytes into the abdominal cavity of Balb/C mice**
Note: A – control; B – ISL

**Table 1 – Influence of ISL on the parameters of the phagocytic index (%) and fluorescence intensity (U) of neutrophils and monocytes of human blood**

| Phagocytes | | Control | 128 mkg/ml | 64 mkg/ml | 16 mkg/ml |
|---|---|---|---|---|---|
| Neutrophils | % | 93,9±6,0 | 92,5±5,5 | 92,1±7,6 | 95,6±2,4 |
| | U. | 145,5±41,1 | 184,8±44,8 * | 129,1±55,6 | 118,1±40,5 |
| **Monocytes** | % | 73,9±14,1 | 64,1±13,7 | 71,4±11,2 | 71,4±11,4 |
| | U. | 64,1±18,1 | 58,5±17,2 * | 51,4±21,9 | 45,4±15,4 |

Note: * – reliable changes at p <0.05

**Table 2 – Effect of ISL on production of ROIs by neutrophils and macrophages/monocytes of human blood**

| | | | Control | ISL | | |
|---|---|---|---|---|---|---|
| | | | | 128 mkg/ml | 64 mkg/ml | 16 mkg/ml |
| Neutrophis | PMA activation | % | 93.3±14.9 | 100.0±0.1 | 99.9±0.1 | 99.5±0.4 |
| | | Ед | 11.3±3,7 | 15.8±3.0 * | 14.8±4.2 * | 14.1±5.2 |
| | Without PMA activation | % | 3.9±1.8 | 100.0±0.1 * | 99.6±0.5 * | 34.7±8.9 * |
| | | Ед | 2.5±0.3 | 6.6±1.6 * | 3.8±0.2 * | 2.1±0.2 |
| Monocytes | PMA activation | % | 44.5±27.2 | 97.8±1.8 * | 88.1±7.8 * | 62.7±17.7 |
| | | Ед | 3.4±0.7 | 6.0±1.3 * | 4.6±1.1* | 4.0±1.0 |
| | Without PMA activation | % | 3.0±2,0 | 86.2±11,7 * | 47.2±18.7 * | 4.6±2.4 |
| | | Ед | 2.3±0.5 | 3.0±0.15 * | 2.3±0.2 | 2.4±0.7 |

Note: Unit (U) – units of fluorescence intensity (UFI); * – reliable changes at p <0.05

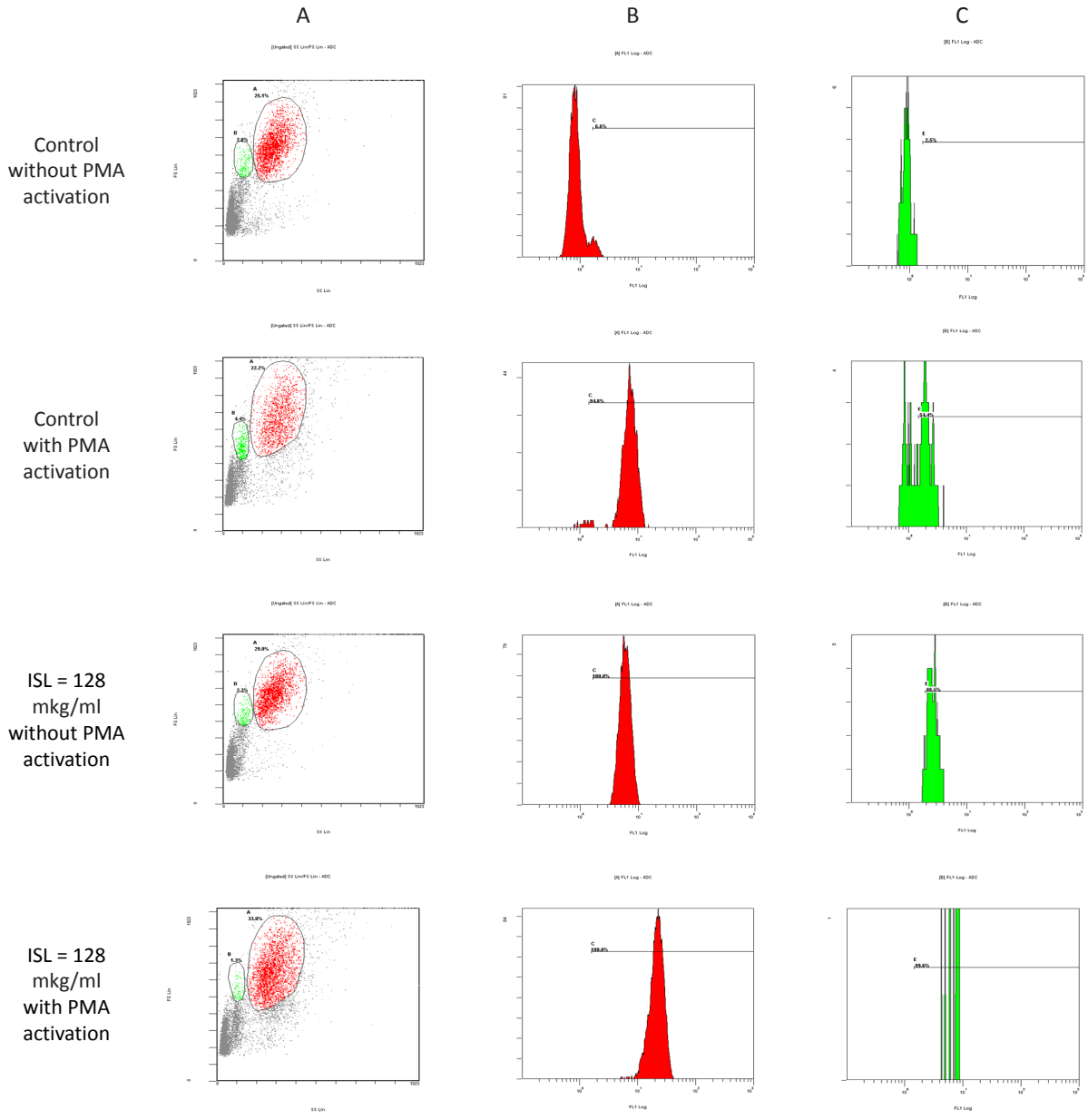


**Figure 3 – Distribution of blood cells by lateral light scattering (A) and fluorescence histogram corresponding to the gates of neutrophils (red) and macrophages/monocytes (green) (B, C)**

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The effect of ISL on the ability of human phagocytes to produce ROIs in response to the protein kinase C activator – PMA – has also been studied. In the control samples, the number of neutrophils producing ROIs was 93.3±14.9%, monocytes – 44.5±27.2% (Table 2). In the presence of ISL at the concentrations of 128 μg/ml, 64 μg/ml, and 16 μg/ml, an insignificant tendency towards a dose-dependent increase in the proportion of fluorescent neutrophils was observed: 100.0±0.1% (p= 0.07), 99.9±0, 1% (p = 0.07) and 99.5±0.4% (p=0.08), respectively (Fig. 2). Compared with the control samples (11.3±3.7 U) in the presence of ISL at the concentrations of 128 μg/ml (15.8±3.0 U, p <0.05) and 64 μg/ml (14.8±4.2 U, p<0.05), a significant increase in fluorescence intensity was noted, and at the concentration of 16 μg/ml, there was just a tendency to that (14.1±5.2 U, p = 0.09). When assessing the functions of monocytes in the presence of ISL at the concentrations of 128 μg/ml (97.8±1.8%, p<0.05), 64 μg/ml (88.1±7.8%, p <0.05), there was a dose-dependent significant increase observed in the number of fluorescent cells compared to the control values (44.5±27.2 U). The data on the increase in the proportion of fluorescent monocytes upon the exposure to ISL at the concentrations of 64–128 μg/ml were comparable with a dose-dependent increase in the degree of their fluorescence (Table 2).

## DISCUSSION

The present research is devoted to the study of the ISL influence on some mechanisms of innate immunity (a phagocyte function) as possible factors affecting the survival in the early stages of bacterial infection. A parenteral administration of ISL before infecting the mice, increased the survival rate of the experimental animals in the model of staphylococcal sepsis and septic shock, when the animals died in the first 24–48 hours against the background of a pronounced bacterial load of $1.5\times10^9$ CFUs/per mouse [5]. In the present experiment, at the infectious dose of $10^9$ CFUs/per mouse, the dynamics of the death rate was different (the animals died later), but the preliminary intraperitoneal injection of ISL was also effective and increased the lifespan of Balb/C mice.

The experiments have shown that ISL has an antibacterial activity [5]. However, the antibacterial activity is manifested at sufficiently high concentrations (64 μg/ml), the probability of reaching and maintaining of which in biological fluids/foci of infection *in vivo,* is low, especially since ISL has a rather short half-life [9, 10]. Taking into account these features, in the present work the emphasis was made on the study of the ISL influence on the functions of phagocytes.

Phagocytes are effector cells of innate immunity that provide the first line of defense against invasion of infectious pathogens. The main stages of phagocytic reactions are chemotaxis, absorption, killing and digestion of an infectious pathogen. The effect on chemotaxis was studied in a peptone-induced migration of phagocytes into the abdominal cavity of mice [8]. It was found that ISL has no suppressive effect on the migration of phagocytes both after 24 hours (chemotaxis of predominantly neutrophils) and after 72 hours (chemotaxis of predominantly macrophages).

The absorption of an infectious agent by phagocytes is realized through such mechanisms as: convergence of a phagocyte and a pathogen; establishing contact; preparation for dipping; circumfluence of the pathogen; membrane closure; absorption of an object. These stages were summarized using the cytometric method and FITC-labeled bacteria. The collection strain *S. aureus J49 ATCC 25923* was used as a bacterial agent for phagocytosis. In the studied concentration range (16–128 μg/ml) ISL did not reduce the proportion of neutrophils and monocytes able of absorbing bacterial cells. However, the fluorescence intensity of monocytes when exposed to certain concentrations of ISL, tended to decrease. Anyhow, this fact is not significant, since in high concentrations, ISL is able of realizing a direct antibacterial activity.

Thus, an intraperitoneal administration of ISL at the total dose of 30 mg/kg does not significantly affect the chemotaxis of neutrophils and macrophages in response to the standard migration activator peptone in Balb/C mice. It does not inhibit the absorption function of neutrophils and human blood monocytes against *S. aureus J49 ATCC 25923* in the concentration range of 16–128 μg/ml, either. It should be noted that at the indicated concentrations, ISL strongly inhibits the proliferation of mitogen-activated T-lymphocytes [5], which indicates that ISL exhibits selective suppressive properties in relation to adaptive immunity effectors, without affecting the main innate immune responses of phagocytes.

Killing and digestion of bacterial pathogens by phagocytes occurs due to the oxygen (ROIs production) and nitrogen metabolism. ROIs is a group of highly reactive oxygen-containing chemicals associated not only with pathological (chronic inflammation, pathological cell proliferation) [11], but also with physiological processes (survival, growth, cell proliferation and differentiation, an immune response) [12]. In particular, an important link in the implementation of innate immune responses is the launch of a massive ROI production ("oxidative burst") in phagocytes. It initiates the onset of the "oxidative burst" of nicotinamide adenine dinucleotide phosphate oxidase (NADP oxidase) (in phagocytes, the main isoform is type 2 NADP oxidase) [13], which can be activated in a signaling cascade associated with protein kinase C, phorbol esters, e.g., PMA [14]. In this regard,

the effect of ISL on the digestive ability of phagocytes was assessed by the production of ROIs by neutrophils and monocytes using the fluorogenic substrate of DHR 123, which interacts with ROIs to form fluorochrome rhodamine 123. The intensity of the "oxidative burst" was assessed using a flow cytometry. During the experiments, it was found that the addition of ISL (16–128 μg/ml) even to non-activated PMA cells dose-dependently increased the proportion of fluorescent neutrophils and monocytes, as well as the fluorescence intensity compared to the control samples, which indicates the accumulation of ROIs in phagocytes. The addition of ISL to PMA-activated phagocytes also increased ROIs production compared to the PMA-activation without ISL exposure. In this case, summation effects were observed in terms of fluorescence intensity. Thus, the sum of the fluorescence intensity of PMA-activated neutrophils (11.3±3.7 U) and neutrophils not activated by PMA, but incubated with ISL (128 μg/ml) (6.6±1.6 U), is comparable to the values of fluorescence intensity of PMA-activated neutrophils incubated with ISL at the dose of 128 μg/ml (15.8±3.0 U). A similar summation of the effect was found in monocyte samples.

On the other hand, flavonoids are well known as antioxidants that can reduce ROIs production by phagocytes. For example, resveratrol inhibits the activity of NADP oxidase, myeloperoxidase and, as a consequence, the formation of hypochlorous acid [15]. ROIs are involved in the implementation of one of the mechanisms of the innate immune response – the formation of "neutrophil traps" (NL) [16]. Neutrophils activated by bacteria *(S. aureus* and *E. coli)* or chemical substances (PMA) produce ROIs and form neutrophilic extracellular conglomerates, which are aimed at curbing bacterial dissemination from the focus of infection [17]. It has been demonstrated that flavonoids (epicatechin, catechin hydrate and rutin trihydrate, as well as luteolin, kaempferol) suppress the formation of ROI-dependent NTs [20, 21], and resveratrol improves lungs functions during acute respiratory tract infections or chronic inflammatory lungs diseases [22].

In some biological systems, as well as at high concentrations, natural polyphenols can demonstrate prooxidant properties [23–26]. Thus, ISL increases the production of ROIs in various tumor cells [27], which is considered as one of its antitumor mechanisms. Apparently, similar mechanisms of ROIs production with the participation of flavonoids, are possible in neutrophils and monocytes, as demonstrated in the present study in the samples with ISL. It is possible that this is important in realizing the antistaphylococcal effects of flavonoid compounds. However, one should also take into account the possibility of an irreversible damage to mitochondria, death of macrophages caused by an increase in ROI production and dissemination of absorbed bacteria during incomplete phagocytosis, which has been demonstrated in the study of resveratrol against mycobacteria [28].

## CONCLUSION

It has been established that a preliminary intraperitoneal administration of ISL at the dose of 30 mg/kg to male Balb/C mice significantly increases the survival rate of animals in the model of the infectious process caused by *S. aureus J 49 ATCC 25923,* does not inhibit the chemotaxis of neutrophils and macrophages in Balb/C mice. It has also been revealed that ISL at the concentrations of 16–128 μg/ml does not statistically significantly affect the absorption of human peripheral blood by monocytes and neutrophils of *S. aureus J49 ATCC 25923*, but dose-dependently increases the number of phagocytes producing ROIs, as well as the intensity of the "oxidative burst" of activated neutrophils and human peripheral blood monocytes. Thus, these effects together can be considered as one of the possible mechanisms of the mice survival at the early stages of the development of staphylococcal infection in mice.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## AUTHORS' CONTRIBUTION

E.A. Solenova – execution of experimental work, statistical processing of results, processing of research results, writing the text of an article; S.I. Pavlova – development of the concept and design of the study, management of experimental work, processing of research results, editing the text of the article.

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*ОРИГИНАЛЬНАЯ СТАТЬЯ*

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# PHARMACOKINETIC PROPERTIES OF A NEW KAPPA-OPIOID ANALGESIC RU-1205 COMPOUND AT A SINGLE PERORAL ADMINISTRATION

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**The aim** of the study is the investigation of the pharmacokinetic properties of the RU-1205 compound, with previously identified kappa-agonistic and analgesic effects, at a single oral administration, as well as comparison of the relationship between its pharmacokinetic and analgesic properties.

**Materials and methods.** Pharmacokinetic parameters of RU-1205 after the oral administration at the dose of 50 mg/kg were investigated using the method of High Performance Liquid Chromatography with determination of the concentration of the compound according to the previously constructed calibration schedule. The indices of the area under the pharmacokinetic curve, clearance, half-life, residence time of the drug molecule in the body, a total (apparent) volume of distribution, as well as the indicator of absolute bioavailability, were calculated. The tissue distribution and excretion of RU-1205 were studied. Potential metabolites of RU-1205 were predicted using the PALLAS 3.00 program. The study of the analgesic activity was carried out on a model of central somatogenic pain with electricalstimulation, with the dynamics assessment of the voltage amplitude of the corresponding reaction of the «tail-flick» reflex.

Results. The compound under study is rapidly adsorbed from the gastrointestinal tract, reaching a maximum concentration by the end of the first hour of the study, and is determined in plasma within 12 hours. Its half-life is 17.7 hours. The absolute oral bioavailability is 37.3%. It was found out that the compound is withdrawn within 3–4 days. The main route of excretion is extrarenal. Biotransformation of a substance probably proceeds mainly with the formation of oxidized forms of the initial molecule by reactions of the first phase of metabolic transformation. The analgesic effect is long-lasting: it starts after 15 minutes and lasts for 12 hours with flattening of the curve by the 8th hour.

**Conclusion.** When administered orally, the test substance undergoes a long process of elimination, has the greatest tropism for the elimination organs and undergoes active biotransformation processes in the body of animals. As a result of it, active metabolic products with an analgesic activity are, possibly, formed.

**Keywords:** kappa agonists; opioid analgesics; benzimidazoles; pharmacokinetics; peroral route; bioavailability; excretion; tissue distribution

**List of abbreviations:** HPLC – High Performance Liquid Chromatography; RCF – relative centrifugal field; AUC – area under the curve; Kel – elimination constant; Cl – clearance; T1/2 – half-life of a drug; MRT – mean residence time; Vd – Volume of distribution; AVD – apparent volume of distribution; TA – tissue availability; ACN – acetonitrile; GIT – gastrointestinal tract; МАРК – mitogen-activated protein kinase

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# ФАРМАКОКИНЕТИЧЕСКИЕ СВОЙСТВА НОВОГО КАППА-ОПИОИДНОГО АНАЛЬГЕТИКА – СОЕДИНЕНИЯ RU-1205 ПРИ ОДНОКРАТНОМ ПЕРОРАЛЬНОМ ВВЕДЕНИИ

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**Цель**. Исследование фармакокинетических свойств соединения RU-1205, с ранее выявленным каппа-агонистическим и анальгетическим действием, при однократном пероральном введении, а также сопоставление взаимосвязи между его фармакокинетическими и обезболивающими свойствами.
**Материалы и методы.** Фармакокинетические параметры RU-1205 после перорального введения в дозе 50 мг/кг исследовали с помощью метода высокоэффективной жидкостной хроматографии с определением концентрации соединения по предварительно построенному калибровочному графику. Рассчитывали показатели площади под фармакокинетической кривой, клиренса, периода полувыведения, время пребывания в организме молекулы препарата, общего (кажущегося) объём распределения, а также показатель абсолютной биодоступности. Изучали тканевое распределение и экскрецию RU-1205. Осуществляли прогноз возможных метаболитов соединения RU-1205 с помощью программы «PALLAS 3.00». Исследование анальгетической активности проводили на модели центральной соматогенной боли при электрическом раздражении с оценкой динамики амплитуды напряжения соответствующей реакции «отдергивания хвоста».
**Результаты.** Изучаемое соединение быстро адсорбируется из желудочно-кишечного тракта с достижением максимальной концентрации к концу первого часа исследования и определяется в плазме в течение 12 часов. Период полувыведения составляет 17,7 ч. Абсолютная биодоступность при пероральном пути – 37,3%. Установлено, что соединение выводится за 3–4 дня. Основной путь выведения внепочечный. Биотрансформация вещества вероятно протекает в основном с образованием окисленных форм исходной молекулы по реакциям первой фазы метаболической трансформации. Анальгетическое действие продолжительное: начинается через 15 минут и сохраняется в течении 12 часов с выходом на плато к 8 часу.
**Заключение.** Исследуемое вещество подвергается длительному процессу элиминации при пероральном введении, имеет наибольшую тропность к органам элиминации и подвергается активным процессам биотрансформации в организме животных, в результате которых, возможно, образуются активные продукты метаболизма с анальгетической активностью.
**Ключевые слова**: каппа-агонисты; опиоидные анальгетики; бензимидазолы; фармакокинетика; пероральный путь; биодоступность; экскреция; тканевое распределение
**Список сокращений:** ВЭЖХ – высокоэффективная жидкостная хроматография; AUC – площадь под фармакокинетической кривой; Kel – константа элиминации; Cl – клиренс; $T_{1/2}$ – продолжительность периода полувыведения; MRT – среднее время пребывания в организме молекулы; Vd – общий (кажущийся) объём распределения; fT – тканевая доступность; ACN – ацетонитрил; ЖКТ – желудочно-кишечный тракт; МАРК – митоген-активируемая протеинкиназа.

## INTRODUCTION

For many decades, opioid analgesics continue to be the mainstay of the pharmacotherapy of severe pain syndromes. However, the global opioid crisis requires a change in the established generally accepted practice of opioids clinical use with the need to replace some of the traditionally used narcotic analgesics with a narrow therapeutic index, a pronounced narcogenic potential, and non-optimal pharmacokinetics [1, 2]. Nonselective agonists of opioid receptors (for example, morphine), especially in injections, have a rather high risk of non-medical use due to their pronounced narcogenic potential (due to the activation of predominantly μ-opioid receptors). This significantly complicates their regulatory accessibility, complicates the work of medical personnel due to complex accounting rules for narcotic analgesics, in-

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creasing the degree of control in accordance with the Decree of the Government of the Russian Federation No. 681 dated June 30, 1998 (as amended on 03.12.2020), and thereby significantly limits them clinical use, not fully meeting the patients' need for strong analgesics approved for use [3].

To solve the problem of narcogenicity of opioid analgesics, several directions have been proposed. One of them is the search and development of new opioid analgesics with a safer profile of a receptor activity due to the selectivity of the drug's action on various subtypes of opioid receptors, which will ensure an increase in the effectiveness of the analgesia performed together with minimization of side effects. [4.5]. Partial agonists and mixed agonists-antagonists of various subpopulations of opioid receptors (buprenorphine, butorphanol, nalbuphine), developed to increase the selectivity of the receptor profile, have found only a limited use in clinical practice, since they had retained a number of negative properties of traditional opiates, in particular narcogenicity [6].

The study of the analgesic properties of RU-1205 in experimental nociceptive models showed that in the analgesic activity, the studied compound is superior to morphine and butorphanol [7]. Unlike the reference drugs, it does not cause respiratory depression and does not possess pharmacological properties that can be regarded as specific predictors of substance capacity to form physical dependence, cause aversion or addiction [8,9]. According to the modern concept of therapy for a severe chronic pain, non-invasive dosage forms of opioid analgesics are recognized as the most effective and safe drugs that provide the best life quality for patients (except the final stages of the disease). One of the important advantages of the test substance is its effectiveness in various administration modes, including an oral administration. This opens up prospects for the creation of an effective oral tablet dosage form on its basis, in contrast to butorphanol, which undergoes intensive metabolism in the liver and is used in medical practice only in the form of an injection solution [10].

At the stage of creating new original drugs, one of the necessary stages of preclinical studies is the research of the pharmacokinetic parameters of the compound being developed. These parameters will make it possible to solve the whole range of the applied issues, such as studying the degree and the rate of adsorption, biotransformation, permeability of drugs through tissue barriers and distribution between blood and peripheral tissues, excretion of the studied drugs, as well as assessing bioavailability during an extravascular administration of a drug [11].

**THE AIM** of the study is the investigation of the pharmacokinetic properties of the RU-1205 compound at a single oral administration, as well as comparison of the relationship between its pharmacokinetic and analgesic properties.

## MATERIALS AND METHODS

### Experimental animals

The studies were carried out on sexually mature male rats weighing 200-230 g, in the amount of 74 individuals, obtained from the Rappolovo laboratory animal nursery of the Russian Academy of Medical Sciences (St. Petersburg, Russia). Before the start of the experiment, the animals were subjected to the adaptation quarantine for 14 days in the vivarium of the Department of Pharmacology and Bioinformatics of Volgograd State Medical University (the Ministry of Health of Russia). The rats had a round-the-clock access to feeding troughs and drinkers *ad libitum*, and were kept in standardized vivarium conditions (Decree of August 29, 2014 No. 51 "On the approval of SP 2.2.1.3218-14" Sanitary and epidemiological requirements for the device, equipment and maintenance of experimental biological clinics (vivariums) "; Directive of the European Parliament and the Council of the European Union 2010/63 / EU of September 22, 2010" On the protection of animals used for scientific purposes"). The animals were kept in groups of 5 individuals in a controlled combined light regime (12/12 h) and the temperature of 20-22°C. 12 hours before the experiment, the animals were deprived of food, but there was a free access to water. The experiments were approved by the Regional Research Ethics Committee of Volgograd Region (registration number IRB 00005839 IORG 0004900 (OHRP), protocol No. 2077-2018 dated October 30, 2018).

### Test substances

Compound RU-1205 is (9-(2-morpholinoethyl)-2-(4-fluorophenyl)imidazo[1,2-a]-benzimidazole dihydrochloride) (Fig. 1) in the form of a substance synthesized at the Scientific Research Institute of Physical and Organic Chemistry of Rostov-on-Don Southern Federal University (Pat. RF No. 2 413 512 C1 dated July 29, 2009). The purity of the compound was at least 99.46%.

### Study design

At the first stage of the investigation, the pharmacokinetic properties of RU-1205 were studied at the dose of 50 μg/kg at a single intragastric administration by method of High Performance Liquid Chromatography. The animals were randomly divided into 7 groups – a control group that received a 0.9% sodium chloride solution in the volume of 100 μl per 100 g of animal weight, and 6 groups that received the RU-1205 compound at the dose of 50 μg/kg (n=6). For the intragastric administration, atraumatic probe No. 14 (Kent Scientific, USA) was used. In order to determine the background peaks before the experiment, the blood was taken from all 42 animals, followed by obtaining plasma samples in the volume of 1.5–2 ml. After the administration of the RU-1205 compound in 30 minutes, 1, 2, 4, 8, 12 hours,

the animals were decapitated using a guillotine (OpenScience, Russia), followed by blood and organ sampling. Decapitation and material sampling from the control group animals were carried out 20 minutes after the injection of the solvent. To obtain plasma, the blood samples were stabilized with a 5% aqueous sodium citrate solution (Polisintez, Russia) (pH 6.0), followed by centrifugation in a 15 min. mode at 3000 rpm, RCF = 604 g (ELMI, Latvia). For precipitation of plasma proteins and extraction of the test substance, acetonitrile was added to the blood samples in a 1:1 ratio. The organs under study (brain, liver, kidneys, heart, lungs, omentum, and muscle tissue) were subjected to grinding and homogenization (Silent Crusher, Germany) to obtain a 20% aqueous homogenate.

The study of tissue bioavailability is an integral part of pharmacokinetic research of new compounds. After obtaining the results, it is possible to judge the intensity of the substances distribution between the peripheral tissues and the target organ (for the RU-1205 compound, the target organ is the brain). When studying the tissue availability of RU-1205, the concentration of the compound was investigated in the following organs: the brain, the elimination organs (liver, kidneys), heart, lungs in especially vascularized tissues, moderately vascularized tissues (muscles, the quadriceps femoris muscle was selected for the experiment), omentum (in weakly vascularized tissues) [12].

The quantitative determination of RU-1205 in blood plasma and organ homogenates was studied according to the previously developed method [13] using High Performance Liquid Chromatography (HPLC) with a diode array detection on a liquid chromatograph (Shimadzu, Japan). The determination was carried out with High Performance Liquid Chromatography System consisting of a SUPELCOSIL LC-18 chromatographic column (5 µm 100×4.6 mm) with a diode-matrix ultraviolet detector (thermostatting temperature of 50°C). To prepare the mobile phase, acetonitrile (UF210) (Russia) was used in a 1:1 ratio with a buffer system (monosubstituted potassium phosphate 50 mM, pH = 5.0). The detection wavelength was 205 nm, and the flow rate of the mobile phase was 1 ml/min. The concentration of the RU-1205 compound in the samples was determined according to the previously constructed calibration graph. For this, the dependence of standard concentrations (0.5; 1; 5; 10; 25 µg/ml) on the areas of chromatographic peaks was plotted using the method of absolute standards. The regression coefficient ($R^2$) was 0.998.

To assess the pharmacokinetic properties of RU-1205 using Microsoft Office Excel 2007 software (USA), the following pharmacokinetic parameters were calculated:

1. AUC (Area Under the Curve) – the area under the pharmacokinetic curve "concentration – time" (by a model-independent method of statistical moments [14]);
2. Kel – elimination constant;
3. Cl – clearance;
4. T1/2 – the duration of the half-life;
5. MRT is the mean residence time of the RU-1205 molecule in the body;
6. Vd – total (apparent) volume of distribution.

The evaluation of the penetration intensity of the RU-1205 compound into various organs and tissues was carried out using the tissue availability parameter (fT), which is the ratio of the organ / tissue AUC value to the blood plasma AUC value.

At the second stage of the study, the excretion of the RU-1205 compound was studied by HPLC in urine and feces samples at a single intragastric administration. The experiments were carried out on 20 sexually mature male rats, randomly divided into control and experimental groups (n=10). The experimental group received a single injection of the RU-1205 compound at the dose of 50 mg/kg intragastrically with an atraumatic probe, the animals from the control group received a solvent – a 0.9% sodium chloride solution. After 24, 48, 72 and 96 hours, the samples were taken in the Thermoplast metabolic chambers (Italy). The samples were subjected to the sample preparation and analysis by HPLC as described above.

At the third stage, the dynamics of the analgesic activity was studied depending on the concentration of RU-1205 in the blood plasma after a single intragastric administration in the electric stimulation test of the tail head. The studies were carried out on 12 sexually mature male rats (n=6). The animals of the control group were injected once intragastrically with the RU-1205 compound at the dose of 5 mg/kg with an atraumatic probe (0.9% sodium chloride solution). For 12 hours, the analgesic activity was studied in the electric stimulation test of the tail head when stimulation was applied through subcutaneous electrodes (rectangular pulses with a frequency of 100 Hz, the duration of 10 ms, the stimulation duration of 1 sec) (Laboratory electro-stimulator ESL-2, Russia) with gradual sequential increase in voltage [15, 16]. The value of the pain threshold (before the manifestation of the spinal "*tail-flick*" reflex) was assessed as an indicator of tension, expressed in volts. The presence of analgesic properties of the RU-1205 compound was judged on the basis of the voltage amplitude changes compared with the values of the control animals.

The PALLAS 3.00 program (CompuDrug Chemistry Ltd.) was used to predict possible metabolites of RU-1205.

### Statistical processing of results

Statistical processing of the obtained data was carried out using Microsoft Office Excel 2007 Software (USA).

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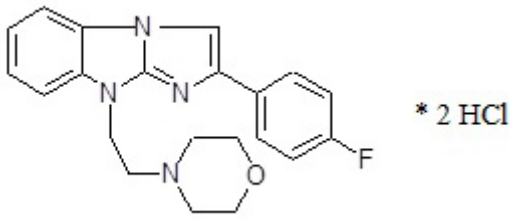
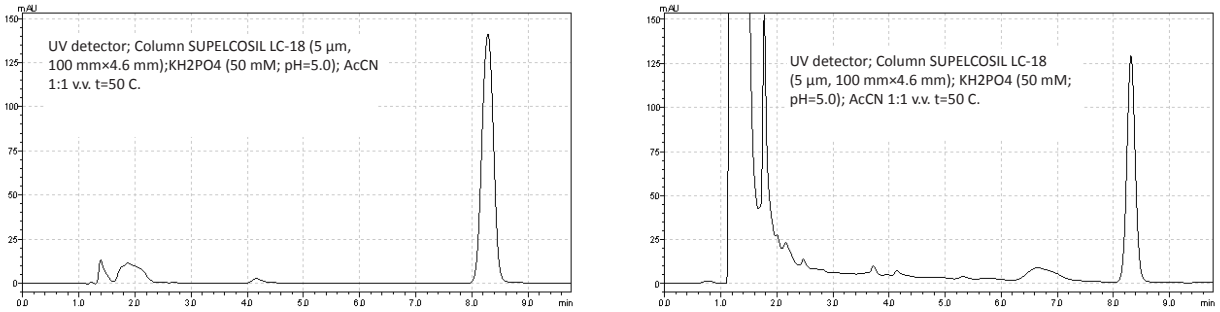



**Figure 1 – Structural formula of RU-1205 compound**




**A** **B**

**Figure 2 – Chromatogram of substance of RU-1205 compound at concentration of 5 µg/ml in aqueous solution (A) and biological material (B). Retention time – 8.00–8.83 min**

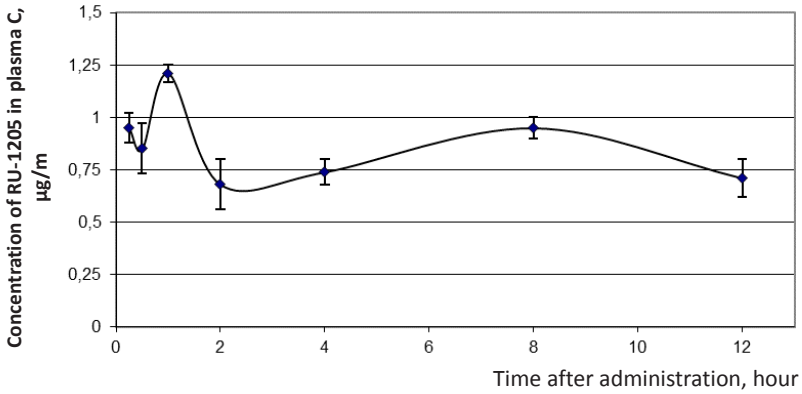
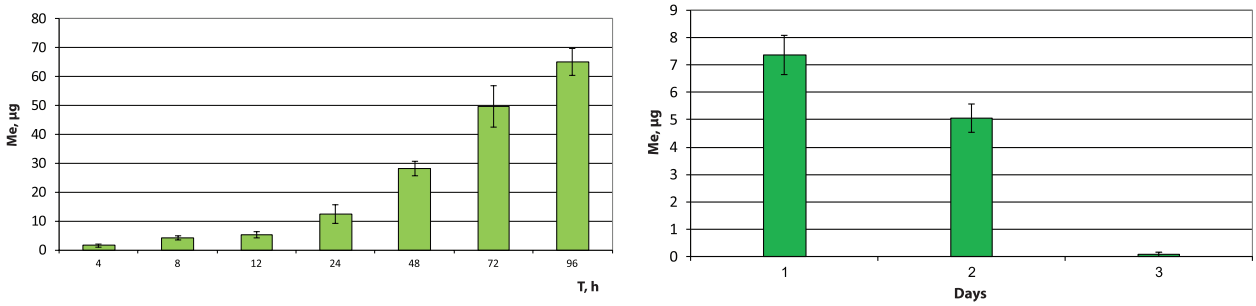



**Figure 3 – Kinetic curve of RU-1205 compound in rat blood plasma**
Note: administration – oral, dose – 50 mg/kg




**A** **B**

**Figure 4 – Cumulative excretion of compound RU-1205 through the kidneys (A) and gastrointestinal tract (B) in intragastric administration**
Note: on the abscissa – time, h; on the ordinate – amount of RU-1205, µg

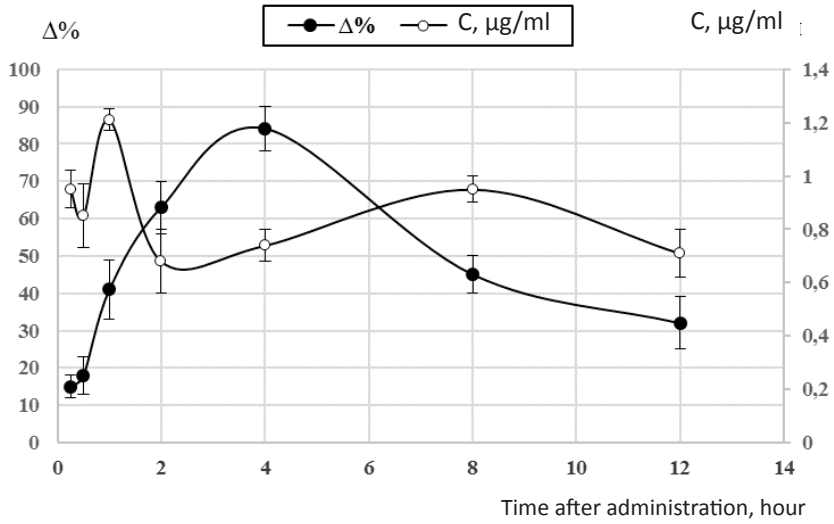
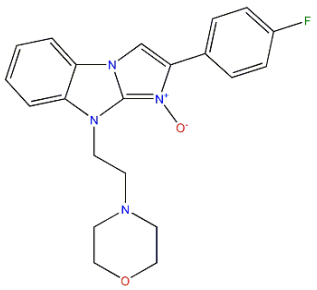
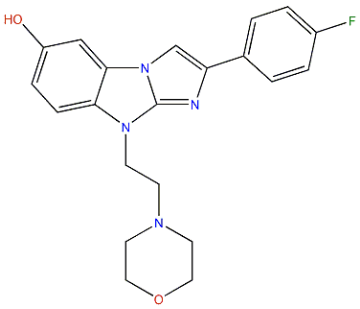
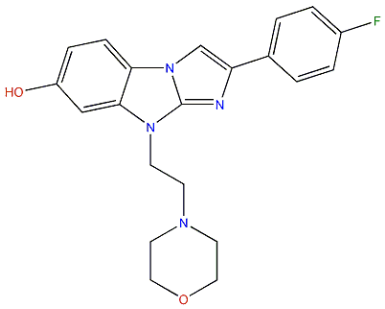
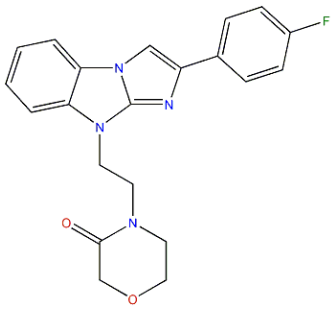
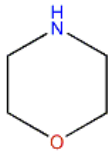
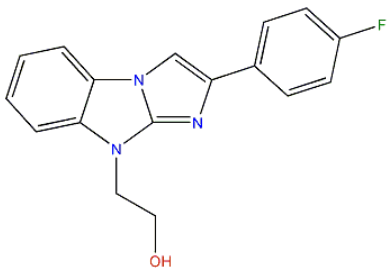
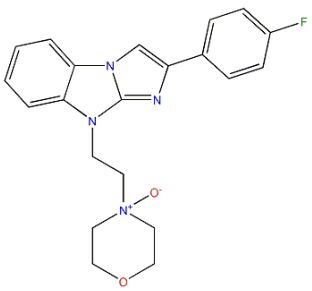
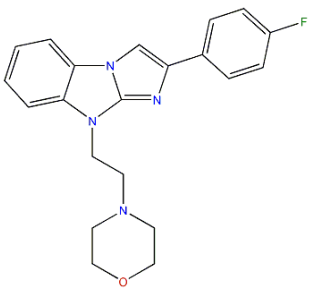



**Figure 5 – Dependence of the dynamics of RU-1205 antinociceptive activity on the concentration of the compound in blood plasma and time at intragastric administration**

Note: on the abscissa – time (hours); on the ordinate: on the left – pain threshold shift (Δ% in relation to the control), on the right – compound concentration in blood plasma (μg/ml)

**Table 1 – Projection of possible metabolites of RU-1205 compound *in silico* (program "PALLAS 3.00")**

| A | B | C |
|---|---|---|
|  |  |  |
| **D** | **E** | **F** |
|  |  |  |
| **G** | **Structure of RU-1205 compound** | |
|  |  | |

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## RESULTS

Using the developed method of quantitative determination, chromatograms of standard aqueous and plasma solutions of RU-1205 were obtained (Fig. 2).

The pharmacokinetic curve of the concentration changes in the substance of the RU-1205 compound in the blood plasma after the oral administration is shown in Fig. 3. The figure shows that the kinetic curve has a two-phase nature of the dynamics of concentration changes. The first phase of increasing the concentration characterizes the process of the substance absorption. The rapid stage of concentration increasing begins from the 30-th minute, and the maximum concentration of the compound in the plasma ($C_{max}$ = 1.05 μg/ml) is already reached by the 60-th minute of the study, which indicates the rapid adsorption of the compound RU-1205 from the gastrointestinal tract (Fig. 3). The second part of the pharmacokinetic curve characterizes the elimination of the substance of the compound from the blood plasma, initially with a sharp decrease in the concentration during the second hour of the study and a subsequent gradual decrease within 12 hours, which indicates a long elimination process. The area under the pharmacokinetic curve was 27.56 μg*hour/ml. The long-term nature of the elimination of the RU-1205 compound is also confirmed by high values of such indicators as $T_{1/2}$ and MRT, which are 17.7 h and 7.85 h, respectively, as well as a low Cl indicator (1.81 l/h/kg).

To characterize the distribution of the medicinal substance, the index of the apparent volume of distribution (AVD) was calculated. This makes it possible to determine one of the distribution options for the compound: being in the blood plasma without leaving the vascular bed (with the indicator less than the plasma volume); the distribution in the extracellular and intracellular fluid (with comparable indicators with the total amount of fluid in the body) or being mainly in the tissues (with the values higher than the total volume of the body fluid). The obtained value – 43.88 l/kg – exceeds the real volume of fluid in the body of rats (0.67 l/kg) by more than 65 times, which may indicate intensive penetration of the compound into the organs and tissues of the body and deposition in peripheral tissues [17].

The absolute bioavailability was determined by a comparative study of the concentrations dynamics of the test substance in the blood plasma after oral and intravenous administrations. This indicator was calculated as the ratio of the area under the curve for the oral administration of the drug substance to the area under the curve for the intravenous route. Based on the previous studies, the AUC index for the intravenous administration of RU-1205 is 14.76 μg*hour/ml [18], based on which the absolute bioavailability in the oral route corresponds to 37.3%.

The main result of the distribution processes of the drug in the body is its further transport to the zone of a potential action, where it interacts with specific targets, which are both the whole organ and individual cells or specific molecular structures that determine the pharmacological effect of the drug. The penetration intensity of the studied substance into peripheral tissues is characterized by tissue accessibility. The RU-1205 compound is intensively distributed in the tissues of the studied organs, herewith, it was determined that there is a significant heterogeneity in the distribution of the drug among the organs. The analysis of the absolute values of tissue availability (fT) of RU-1205 showed that it has the least tropism to the heart, lungs, spleen, and muscles. In the liver and kidneys, there is a significant content of the studied substance, with AUC values of 17.65 and 82.94 μg*hour/ml, respectively. The tissue distribution parameter for renal tissue was 3.01 and 0.64 for liver tissue, respectively. The substance under study is determined in these target organs for 12 hours in the liver and 8 hours in the kidneys. The highest content was noted in the kidney tissues, which may indicate the predominant renal excretion of RU-1205. In high concentrations, the compound is also determined in the adipose tissue, in the omentum, which, apparently, is due to its lipophilicity. In the brain, after the oral administration, the content of the studied substance is below the detection threshold.

In the study of the RU-1205 excretion after the oral administration, it was determined that the excretion of the compound occurs via renal excretion and through the intestine. Therefore, the test compound is excreted in the urine for four days and in the feces for two days. The intensity of the excretion process during the next three days of the study after the oral administration of the compound is presented by the data of cumulative urinary and intestinal kinds of excretion (Fig. 4). The cumulative urinary excretion is 65.12 μg, which corresponds to about 0.65% of the administered dose. It was also found that more of the studied substance is detected in urine than in feces (Fig. 4 A.). At the same time, it was revealed that the extrarenal (metabolic) clearance of the RU-1205 compound significantly predominates over the renal, which, according to the literature data, indicates an active metabolism of the studied substance in the liver [19, 20].

The study of the analgesic properties of the compound showed that a significant analgesic effect was observed already 15 minutes after the oral administration of RU-1205. In this case, the latent period of the nociceptive reaction increases by 16%. The antinociceptive effect gradually increases and reaches its highest values by the 4th hour after the administration, then gradually decreases, reaching about 50% of the maximum efficiency indicators by 8 hours of observation (Fig. 5). The observed analgesic effect is long lasting, keeping for 12 hours.

A computer projection of the studied substance metabolites *in silico* using the PALLAS 3.00 program, made it possible to determine seven possible metabolites. These metabolites are mainly products of oxidation reactions,

in particular, 3 predicted metabolites under the codes "a", "d" and "g", possibly formed as results of the hydroxylation reaction (Table 1).

## DISCUSSION

In recent years, one of the promising groups of opioid analgesics with a selective mechanism of action without any risk of developing respiratory depression and drug dependence (characteristic of μ-agonists), has been considered selective kappa-opioid agonists. In contrast to μ- or δ-agonists, along with a high analgesic activity, they do not stimulate the dopaminergic "reward" system [6, 21-23]. Although kappa-opioid receptor agonists have been long recognized as analgesics with a low abuse potential, serious side effects associated with dysphoria, anhedonia, and hallucinations are a limiting factor in the promotion of first-generation kappa-selective analgesics [24].

Nowadays, there are opioid receptor agonists that can exert an antinociceptive effect without causing undesirable psychotropic effects. The possibility of such an activity is explained by a selective activation of signal transduction pathways from the opioid receptor (selective functional signaling). For example, the stimulation of μ-opioid receptors by morphine, and κ-receptors by the selective kappa agonist U 50488, activates two intracellular cascades Gi/0 and β – arrestin [25, 26]. This is realized in the effective pain relief, as well as side effects in the form of euphoria, dependence formation, respiratory depression (for morphine) and dysphoria (for U 50488). Antinociceptive effects upon the activation of any of the opioid receptors, are realized through the activation of the Gi-protein pathway, while the addictive potential, dysphoria, and most other adverse concomitant effects of opioids, are due to the β-arrestin cascade of intracellular signals. Over the past decade, Gi-biased μ- and κ-opioid agonists (PZM21, Oliceridine, Herkinorin), κ-agonists with a β-arrestin-biased antagonistic activity, as well as ligands (Noribogaine) that combine the properties of highly effective Gi- activators and inhibitors of β –arrestin, have been identified [27–29].

The mechanism of dysphoria and aversion formation induced by kappa receptor agonists consists in the activation of mitogen-activated protein kinase p38-MAPK via the β-arrestin signaling pathway [30]. There is a hypothesis that highly selective kappa-receptor agonists, which do not clearly activate or inhibit the p38-MAP-kinase cascade of intracellular signaling reactions, the stimulation of which is realized in kappa-mediated aversion, hyperalgesia and inflammation, will have a more pronounced analgesic effect without any risk of developing dysphoria [31]. This hypothesis served as a prerequisite for the development of a new technology for searching for kappa-selective agonists with the properties of p38-MAPK inhibitors and the prospect of creating competitive analgesics on their basis without respiratory distress syndrome, narcogenic potential and dysphoria. Most of the selective kappa receptor ligands and selective inhibitors of p38-MAPK are derivatives of cyclic nitrogen-containing heterosystems, which also include benzimidazole derivatives and condensed systems based on it [32].

As a result of a targeted complex study of benzimidazole derivatives using *in silico* computer modeling methods, as well as in the course of experimental studies *in vitro* and *in vivo*, the main regularities of kappa-receptor interactions were determined. They were implemented in an integral scaffold (2-p-fluorophenylimidazo [1, 2-a] benzimidazole) [33–35] and made it possible to identify the original molecule – a compound under the laboratory code – RU-1205. The kappa-receptor mechanism of the compound action was laboratory confirmed in an experimental model of the rabbit vas deferens (the inhibition rate of electrically-induced contraction of the isolated duct IC50 = 2 nM), and was also proven using a non-selective opioid antagonist, naloxone and a kappa-selective antagonist, norbinoltorofimine, which blocked the analgesic activity of the test compound in *in vivo* tests [36].

When administered orally at the dose of 50 mg/kg, the maximum concentration of the RU-1205 compound was observed 1 hour after the administration – the time required for penetration through the gastrointestinal tract wall and through the hepatic barrier. The substance under study circulates for a long time in the blood plasma for 12 hours. The long-term nature of the RU-1205 compound elimination, is also confirmed by high values of its half-life and the average hold-time of the RU-1205 molecule in the body. It is noted that the absolute bioavailability of the substance of the RU-1205 compound when administered orally, is 37%. For comparison, the kappa-opioid receptor agonist butorphanol tartrate used in the animal experiment clinic, has a low bioavailability value after the oral administration (it is less than 10%).

The obtained results of the excretion study showed that the extrarenal (metabolic) clearance of the RU-1205 compound significantly prevails over the renal one. This is consistent with the literature data on the excretion of benzimidazole derivatives. Consequently, benzimidazole derivatives undergo intensive metabolic transformations in the body. It has been shown that afobazole (fabomotizole), after the administration by various methods, is registered in urine and feces only in insignificant amounts [37, 38], 80% of the administered dose of omeprazole is excreted in the urine as metabolites and a small part in feces [39].

When evaluating the computer projection of possible RU-1205 metabolites, it should be noted that in all the compounds formed by the radical, the $C^2$ atom retains the fluorophenyl radical, which is presumably in-

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volved in the development of the analgesic effect [33]. All metabolites, except metabolites "E" and "F", are characterized by the preservation of the morpholine radical, which is also involved in the development of pain relief.

On the basis of the computer analysis, it was also found out that in the process of the of RU-1205 metabolism, the detachment of the morpholine radical and, probably, a change in the analgesic activity (metabolite "E"), is possible.

Both in preclinical studies and in the further clinical use of new drugs, it is advisable to search for relationships between pharmacokinetic parameters and drug effects. The discussion of such correlations is important for understanding the system of relationships between pharmacokinetic and pharmacodynamic mechanisms in the action of the future drug. Therefore, the next stage of the research was to study the dependence of pharmacodynamic properties (pain relief) on the pharmacokinetics of the RU-1205 compound.

The previous studies have shown that the compound has a dose-dependent analgesic effect, and the average effective dose of RU-1205 for the oral route of administration is 5 mg/kg [8]. The main parameter characterizing the degree of bioavailability of the drug, the area under the pharmacokinetic curve RU-1205, increases linearly with increasing the dose [40]. Taking into account the sensitivity of the developed HPLC method and the possibility of comparing pharmacokinetic and pharmacodynamic data with linear kinetics, the correlation of the analgesic effects and pharmacokinetics at different doses, was assessed. When comparing the pharmacokinetic and analgesic properties, it was determined that the difference in the maximum analgesic effect and the peak concentration of the substance in the plasma is 3 hours. This difference can be explained by the peculiarities of the compound RU-1205 penetration through the blood-brain and hepatic barriers. With this route of administration, RU-1205 enters the brain at the concentrations below the threshold and is not detected when studying the tissue accessibility. However, a central analgesic effect is observed, and it suggests that biotransformation of RU-1205 may result in the formation of active metabolites possessing analgesic properties. The literature data show that some opioid analgesics are characterized by the formation of active metabolites [41]. For example, as a result of glucuronization of morphine, the main metabolites of morphine are formed. One of them, morphine-6-glucuronide, has pronounced analgesic properties that are superior to morphine itself [42]. Another opioid analgesic (of the mixed action), tramadol, is metabolized by N- and O-demethylation, followed by conjugation with glucuronic acid to form an active metabolite, mono-O-desmethyltramadol, which causes a more pronounced activation of mu-receptors in comparison with tramadol itself [41]. Hydromorphone is a semi-synthetic derivative of morphine, its pharmacological properties are similar to morphine, including its biotransformation. It is also extensively metabolized by glucuronization up to hydromorphone-3-glucuronide; the other minor metabolites include unconjugated and conjugated dihydromorphine and dihydroisomorphine, hydromorphone-3-sulfate, norohydromorphone, and nordihydroisomorphine [41]. Its metabolites, dihydromorphine and norohydromorphone, have the same potency as morphine. Methadone is an opioid used in a number of countries as an analgesic, as well as in the treatment of drug addiction (drug trafficking in Russia is prohibited, according to List I of the RF Government Decree No. 681 dated June 30, 1998 (as amended on 12/03/2020)), is a racemic mixture of R- and S-methadone. Moreover, R-methadone has almost 50 times more powerful analgesic effect than S-methadone. As a result of the oxidative biotransformation, it undergoes stereoselective metabolism (N-demethylation), and with the participation of CYP2C19, metabolites α-(3S6S)-methadol and α-(3S6S)-N-desmethylmethadol are formed from the enantiomer of S-methadone, the latter of which has an analgesic activity, comparable to R-methadone (active enantiomer) [42]. The analgesic oxycodone (in the oral form, until recently considered as an alternative to morphine, due to a less pronounced addictive effect) is metabolized with N-demethylation up to noroxycodone and codeine, O-demethylation up to oxymorphone through CYP3A4 and CYP2D6, respectively [41, 42]. In the 3rd position, Oxymorphone is further metabolized by glucuronidation to oxymorphone-3-glucuronide. Oxycodone and noroxycodone itself have a lower affinity, while oxymorphone has a higher affinity to the μ-opioid receptor [43, 44].

## CONCLUSION

Thus, as a result of the research, the main pharmacokinetic parameters of RU-1205 have been studied at a single oral administration. The processes of the tissue distribution of the studied compound in the organs of the rat organism, as well as its excretion, have been investigated. The value of its absolute bioavailability for this route of administration was 37.3%. Despite the fact that under the developed chromatographic conditions, the RU-1205 compound has not been detected in the brain in the oral route administration, the observed central analgesic effect (realized as a result of penetration through the blood-brain barrier) may indicate a possible presence of active metabolic products with the analgesic activity. The totality of the results obtained suggests that the RU-1205 compound undergoes active biotransformation processes in the body of animals. Based on the revealed pharmacokinetic parameters of the test compound, it is possible to plan an optimal scheme for further clinical trials.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS CONTRIBUTION

Alexander A. Spasov – concept and design of the study, approval of the final version of the article; Lyudmila A. Smirnova – development of the experiment, text editing; Olesya Yu. Grechko – administrative organization of the experimen; Natalya V. Eliseeva – analysis of factual material, text writing; Yulia V. Lifanova – sample preparation, statistical processing; Andrey I. Rashchenko – conducting the experimental series to study pharmacokinetic parameters, results processing; Anatoly S. Morkovnik – synthesis of the compound, text editing; Olga N. Zhukovskaya, Vera A. Anisimova – synthesis of the compound. All authors participated in the discussion of the results.

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# USING QUANTUM-CHEMICAL PARAMETERS FOR PREDICTING ANTIRADICAL (HO•) ACTIVITY OF RELATED STRUCTURES CONTAINING A CINNAMOIL FRAGMENT. IV. STRUCTURE-ACTIVITY RELATIONSHIP BETWEEN UNSATURATION INDICES AND FLAVONE DERIVATIVES WITH FLOROGLUCIN RING "A"

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The quantum-chemical parameters of 52 derivatives related to flavanones, flavanonoles, flavones and flavonoles with a phloroglucinic type of the A ring and containing electron-donating substituents in the B ring were studied.

**The aim** is the analysis of the dynamics of changes in the electron density, bond numbers, free valence indices and unsaturation indices on carbon atoms C-7→C-8 of the vinyl group of the main conjugation chain in relation to the position and number of substituents in the "B" ring and the type of the pharmacological activity.

**Materials and methods**. The quantum-chemical parameters of the 4 analyzed groups of the compounds, have been calculated by the semi-empirical method PM7 (WinMopac 2016 program) on the workstation with an Intel Xeon E5-1620 3.5 GHz processor, 20 GB of RAM.

**Results and discussion.** When comparing the quantum chemical parameters of the analyzed compounds, it was established that when the C-7→C-8 multiple bond is formed, the free valency and unsaturation indices increase on both carbon atoms of the vinylene group in flavones and flavonols compared to the corresponding flavanones and flavanonols. This is explained by the fact that the value of the bond numbers Nμ on these atoms, on the contrary, decreases (Fμ = 4.732-Nμ). The transition from flavanone to flavone is accompanied by the formation of a vinyl group C-7→C-8, and therefore both atoms from the $sp^3$-hybridized state go into the $sp^2$-state. The consequence of this transformation is a change in the electronegativity value and an increase in the unsaturation index of C-7 and C-8 atoms: C $sp^3$ = 2.5; C$sp^2$ = 2.8. At the same time, the transition from flavanone to flavone leads to the formation of a conjugated system with the participation of π-electrons of the aromatic system "B", C-7, C-8 atoms and the carbonyl group, which is commonly called the "main conjugation chain". These structural changes, namely, the transition from a less oxidized flavanone to a more oxidized flavone, contribute to a decrease in the electron density on C-7 and C-8 atoms, and an increase in the total unsaturation of the molecules in general. Mulliken charges on C-7 of all groups of compounds are characterized by a positive value. As for the carbon atoms of the B fragment, the following features are revealed here: in the presence of one substituent –OH or –$OCH_3$ on the carbon atom to which the substituent is bounded, the Mulliken charge is positive; if there are two substituents in the B ring –OH or –$OCH_3$, as well as two -$OCH_3$ groups, then the carbon atoms bonded to the indicated substituents also have a positive Mulliken charge; in the case of trihydroxy substituted in the C-2, C-3 and C-4 B ring, all three carbon atoms are characterized by a positive Mulliken charge; if there are methoxy groups in positions C-2, C-3 and C-4, then the positive Mulliken charge is concentrated only on C-2 and C-4 atoms, and on C-3 atom this charge has a negative value.

**Conclusion.** The above data on the quantum-chemical parameters of the main conjugation chain indicate that the transition of C-7 and C-8 atoms to the $sp^2$-hybrid state, leads to a decrease in the electron density and a decrease in the bond numbers, with a simultaneous increase in the indices of unsaturation and free valence on these atoms. Thus, the trigger mechanism of the anti-radical activity, primarily with respect to the HO• radical, is determined by the fact that this particle, electrophilic in its properties, will attach in the C-8 atom during an initial attack.

**Keywords**: flavanones, flavanonoles, flavones, flavonoles, phloroglucinic type of the A ring

**Abbreviations:** Fμ – free valence indices; IUA – unsaturation index; EO – electronegativity; Nμ – the total values of the bond numbers; Vμ – theoretical valence.

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# ИСПОЛЬЗОВАНИЕ КВАНТОВО-ХИМИЧЕСКИХ ПАРАМЕТРОВ ДЛЯ ПРОГНОЗИРОВАНИЯ АНТИРАДИКАЛЬНОЙ (HO•) АКТИВНОСТИ РОДСТВЕННЫХ СТРУКТУР, СОДЕРЖАЩИХ ЦИННАМОИЛЬНЫЙ ФРАГМЕНТ. IV. ВЗАИМОСВЯЗЬ СТРУКТУРА-АКТИВНОСТЬ МЕЖДУ ИНДЕКСАМИ НЕНАСЫЩЕННОСТИ И ПРОИЗВОДНЫМИ ФЛАВОНА С ФЛОРОГЛЮЦИНОВЫМ КОЛЬЦОМ «А»

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Изучены квантово-химические параметры 52 производных, относящихся к флаванонам, флаванонолам, флавонам и флавонолам с флороглюциновым типом кольца «А», и содержащими электронодонорные заместители в кольце «В».
**Цель.** Анализ динамики изменения электронной плотности, связевых чисел, индексов свободной валентности и ненасыщенности на атомах углерода С-7→С-8 виниленовой группы главной цепи сопряжения во взаимосвязи с положением и числом заместителей в кольце «В» и видом фармакологической активности.
**Материалы и методы.** Квантово-химические параметры анализируемых 4-х групп соединений рассчитаны полуэмпирическим методом PM7 (программа WinMopac 2016) на рабочей станции с процессором IntelXeonE5-1620 3,5 ГГц, 20 Гб оперативной памяти.
**Результаты и обсуждение.** При сопоставлении квантово-химических параметров анализируемых соединений установлено, что при формировании кратной связи С-7→С-8 индексы свободной валентности и ненасыщенности возрастают на обоих углеродных атомах виниленовой группы у флавонов и флавонолов по сравнению с соответствующими флаванонами и флаванонолами. Это объясняется тем, что величина связевых чисел Nμ на этих атомах, наоборот, уменьшается (Fμ= 4,732-Nμ). Переход от флаванона к флавону сопровождается формированием виниленовой группы С-7→С-8, в связи с чем оба атома из $sp^3$-гибридизованного состояния переходят в $sp^2$-состояние. Следствием такой трансформации является изменение значения электроотрицательности и увеличением индекса ненасыщенности атомов С-7 и С-8: С $sp^3$=2,5; С $sp^2$=2,8. Вместе с тем переход от флаванона к флавону приводит к образованию сопряженной системы с участием π-электронов ароматического ядра «В», атомов С-7, С-8 и карбонила что принято называть «главной цепью сопряжения». Указанные структурные изменения, а именно, переход от менее окисленного флаванона к более окисленному флавону способствует уменьшению электронной плотности на атомах С-7 и С-8, и увеличению суммарной ненасыщенности молекул в целом. Малликеновские заряды на С-7 всех групп соединений характеризуются положительным значением. Что касается атомов углерода фрагмента «В», то здесь выявлены следующие особенности: при наличии одного заместителя –ОН или $–OCH_3$ на атоме углерода, с которым связан заместитель, Малликеновский заряд – положительный; если в кольце «В» имеются два заместителя –ОН или $–OCH_3$, а также две $–OCH_3$ группы, то атомы углерода, связанные с указанными заместителями, тоже имеют положительный Малликеновский заряд; в случае тригидроксизамещенных у С-2', С-3' и С-4' кольца «В» все три атома углерода характеризуются положительным Малликеновским зарядом; если в положениях С-2', С-3' и С-4' находятся метоксигруппы, то положительный Малликеновский заряд сосредоточен только на атомах С-2' и С-4', а на С-3' этот заряд имеет отрицательное значение.
**Заключение.** Перечисленные выше данные о квантово-химических параметрах главной цепи сопряжения свидетельствуют о том, что переход атомов С-7 и С-8 в $sp^2$-гибридное состояние приводит к понижению электронной плотности и уменьшению величин связевых чисел, при одновременном увеличении индексов ненасыщенности и свободной валентности на этих атомах. Таким образом, пусковой механизм антирадикальной активности, в первую очередь в отношении радикала HO•, определяется тем, что эта электрофильная по своим свойствам частица при первичной атаке присоединится по положению С-8.
**Ключевые слова:** флаваноны; флаванонолы; флавоны; флавонолы; флороглюциновый тип кольца «А»
**Список сокращений:** Fμ – индексы свободной валентности; IUA – индекс ненасыщенности; ЭО – электроотрицательность; Nμ – суммарные значения связевых чисел; Vμ – теоретическая валентность.

## INTRODUCTION

The final IV-th report summarizes the results of the study of the relationship between the structure of the compounds containing the phloroglucinic type A ring and electron-donating substituents in the B ring with total unsaturation indices (IUA) and electron density.

**THE AIM** of the article is the analysis of the dynamics of changes in the electron density, bond numbers, free valence indices and unsaturation indices on carbon atoms C-7 → C-8 of the vinyl group of the main conjugation chain in relation to the position and number of substituents in the "B" ring and the type of the pharmacological activity.

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## MATERIALS AND METHODS

The quantum-chemical parameters of the 4 analyzed groups of the compounds, have been calculated by the semi-empirical method PM7 (WinMopac 2016 program) on the workstation with an Intel Xeon E5-1620 3.5 GHz processor, 20 GB of RAM.

## RESULTS AND DISCUSSION

The structures of the analyzed compounds and the total values of the listed parameters in C-1→C-9 section of the cinnamoyl fragment are presented in Table 1.

It follows from the Table that, when switching from flavanones to flavanonols, the values of the free valency (Vμ) and unsaturation indices (IUA) change very slightly (the second decimal place is ~0.04) despite the fact that on flavanonol C-8 atom the electron-donating OH-group appears; it contributes to an increase in the electron density on C-7 and a decrease onC-8.

In the flavone-flavonole pair, the introduction of phenolic hydroxyl on C-8 promotes a clear increase in the IUA value, an increase in the electron density on C-7, and its decrease on C-8.

The Vμ value remains almost unchanged, including that of the flavanone-flavanonole pair.

This feature is preserved in all types of compounds presented in Table 1, and for this reason, we will not consider the Vμ parameter further.

After the publication of the pioneering studies of Szent-Györgyi in 1936 about the biological properties of certain flavonoids, the whole subsequent period made it possible to accumulate the extensive information about representatives of this class of natural compounds.

Currently, the structure of approximately 8000 flavonoids [1-6] has been described, and only a very small number of individual substances (approximately 2–3%) from this variety of aglycones and glycosides has been studied in detail from biochemical and pharmacological points of view. Such a low percentage of available information can be explained by the fact that in the absolute majority of plants the content of individual substances is scanty (0.1–2%) and their production in sufficient quantities for the purpose of subsequent biochemical and pharmacological studies is associated with high material costs.

As a rule, detailed information about the biological properties of individual compounds – derivatives of 2-phenyl-benz-γ-pyrone – concerns the substances that can be obtained preparatively from the raw materials (quercetin and rutin from Sophora Japonica, buckwheat herb; taxifolin, or dihydroquercetin, from Lárix sibírica; hesperitin and hesperidin – from the pulp – the spongy part of citrus peels; diosmin – by the oxidation of hesperitin, etc.).

Nevertheless, the most characteristic and perhaps most important are considered the antioxidant properties of flavonoids, the indirect effect of which is manifested by about 50 types of pharmacological activity [7–10].

It should be notified that throughout its evolutionary development, the persons using plant foods, introduce flavonoids into their bodies, and they protect the cells from the oxidative stress and thereby normalize their metabolism.

Thus, flavonoids are a kind of a protective shield of the body's natural antioxidant system, and this is important for preserving the entire cellular system.

The currently used therapeutic and preventive agents based on flavonoids are their total substances – legalon, karsil, silibor, flacumin, etc. (most often) – or individual compounds: rutin, quercetin, flaronin, etc[1]. This treatment is represented not by immediate action drugs, therefore their therapeutic effect is manifested, as a rule, during a long-term administration (detralex, troxevasin). The derivatives of 2-phenyl-benz-γ-pyrone, wide-spread in nature, are represented in the form of glycosides and their aglycones, and glycosides are predominant.

It should be emphasized that the non-carbohydrate residue is a pharmacologically active fragment in flavonoid glycosides, i.e. aglycone, therefore, there is no need to discuss the enormous economic costs that would be necessary for a detailed study of the biological properties of at least one hundred aglycones – derivatives of 2-phenylbenz-γ-pyrone. Such an activity is unproductive, because it is unlikely that new properties of these compounds should be revealed. Moreover, if we compare the known data on the biological activity of the studied flavonones, flavonols and flavononols with the PASS prediction data [11], the most common types of activity for all types of structures are anti-inflammatory, antioxidant, hepatoprotective, choleretic. Besides, they are characterized by such properties as free radical binding, antimutagenic, capillary strengthening and act as apoptosis agonist, membrane integrity agonist, membrane permeability inhibitors.

Individual compounds can be replaced by total flavonoid substances obtained from the corresponding producing plants, because the effect is often preserved, and sometimes exceeds the expected result.

The data about the antiradical (HO•) activity of flavonoids are disorganized and, as a rule, few. Moreover, in the works that are not interconnected, the authors use different methods for generating this radical, which does not make it possible to quantify and compare the results obtained.

The most informative are the works [12] and [13], which provide information on the activity of the representative groups of the compounds.

[1] State Register of Medicines. Available from: https://grls.rosminzdrav.ru/Default.aspx

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**Table 1 – The total values of Nμ (bond numbers), Vμ (theoretical valency), IUA (unsaturation index) and electron density on carbon atoms of the main conjugation chain (C-1→C-9)**

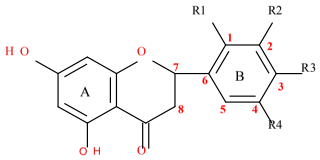
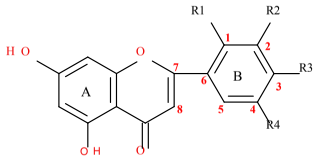
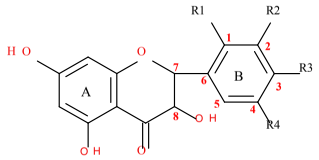
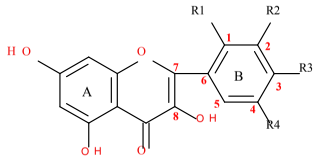
| N | Substituents | | | |  Flavanone 1 | | | |  Flavone 2 | | | |  Flavanonole 3 | | | |  Flavonole 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Nμ | Vμ | IUA | Эл.пл. | Nμ | Vμ | IUA | Эл.пл | Nμ | Vμ | IUA | Эл.пл | Nμ | Vμ | IUA | Эл.пл |
| 1 | H | H | H | H | 34.48 | 35.53 | 1.05 | 36.502 | 34.24 | 35.5 | 1.27 | 36.287 | 34.41 | 35.51 | 1.09 | 36.208 | 34.18 | 35.56 | 1.38 | 36.059 |
| 2 | OH | H | H | H | 34.24 | 35.4 | 1.15 | 36.27 | 34.00 | 35.34 | 1.34 | 36.021 | 34.17 | 35.36 | 1.19 | 35.906 | 33.95 | 35.41 | 1.46 | 35.787 |
| 3 | $CH_3O$ | H | H | H | 34.28 | 35.45 | 1.16 | 36.252 | 34.02 | 35.37 | 1.36 | 36.083 | 34.17 | 35.38 | 1.21 | 35.932 | 34.02 | 35.5 | 1.49 | 35.828 |
| 4 | H | OH | H | H | 34.24 | 35.4 | 1.16 | 36.297 | 34.06 | 35.44 | 1.38 | 36.049 | 34.2 | 35.5 | 1.3 | 35.965 | 33.99 | 35.45 | 1.49 | 35.821 |
| 5 | H | $CH_3O$ | H | H | 34.23 | 35.42 | 1.19 | 36.337 | 34.05 | 35.45 | 1.41 | 36.091 | 34.19 | 35.42 | 1.23 | 36.001 | 33.68 | 35.5 | 1.82 | 35.861 |
| 6 | H | H | OH | H | 34.22 | 35.38 | 1.15 | 36.273 | 33.98 | 35.33 | 1.35 | 36.033 | 34.18 | 35.37 | 1.19 | 35.942 | 33.94 | 35.4 | 1.47 | 35.798 |
| 7 | H | H | $CH_3O$ | H | 34.22 | 35.39 | 1.16 | 36.314 | 33.96 | 35.34 | 1.38 | 36.076 | 34.17 | 35.39 | 1.19 | 35.979 | 33.92 | 35.42 | 1.5 | 35.839 |
| 8 | H | OH | OH | H | 34.14 | 35.41 | 1.26 | 36.025 | 33.93 | 35.41 | 1.48 | 35.786 | 34.1 | 35.4 | 1.3 | 35.691 | 33.88 | 35.47 | 1.59 | 35.552 |
| 9 | H | $CH_3O$ | OH | H | 34.13 | 35.42 | 1.29 | 36.057 | 33.91 | 35.42 | 1.51 | 35.823 | 34.09 | 35.42 | 1.33 | 35.728 | 33.86 | 35.48 | 1.62 | 35.588 |
| 10 | H | OH | $CH_3O$ | H | 34.14 | 35.42 | 1.28 | 36.057 | 33.93 | 35.42 | 1.49 | 35.823 | 34.1 | 35.41 | 1.32 | 35.724 | 33.87 | 35.48 | 1.61 | 35.587 |
| 11 | H | $CH_3O$ | $CH_3O$ | H | 34.14 | 35.42 | 1.29 | 36.084 | 33.9 | 35.43 | 1.53 | 36.861 | 34.09 | 35.43 | 1.34 | 35.764 | 33.85 | 35.49 | 1.64 | 35.624 |
| 12 | H | OH | OH | OH | 33.98 | 35.318 | 1.33 | 35.786 | 33.81 | 35.37 | 1.56 | 35.547 | 33.95 | 35.32 | 1.37 | 35.45 | 33.75 | 35.42 | 1.67 | 35.312 |
| 13 | H | $CH_3O$ | $CH_3O$ | $CH_3O$ | 33.93 | 35.32 | 1.39 | 35.898 | 33.75 | 35.37 | 1.62 | 35.661 | 33.9 | 35.33 | 1.43 | 35.562 | 33.7 | 35.42 | 1.73 | 35.425 |

**Table 2 – Antiradical (HO•) activity of some flavone and flavonole derivatives**

| | Compounds with antiradical (HO•) activity experimentally detected* | Antiradical (HO•) activity established experimentally* A,% | Total values of bond numbers (Nμ), theoretical valency (Vμ), unsaturation indices (IUA) and electron density (E.D.) on carbon atoms of the main conjugation chain | | | |
|---|---|---|---|---|---|---|
| 1 | Flavon (unsubstituted) | 4 | Nμ | Vμ | IUA | E.D. |
| 2 | apigenin(5,7,4'-trihydroxyflavone) | 34 | 34.27 | 35.53 | 1.26 | 36.156 |
| 3 | diosmethine(5,7,3'-trihydroxy-4'-methoxyflavone) | 39 | 33.98 | 35.33 | 1.35 | 36.033 |
| 4 | 5,7-dihydroxy-3', 4', 5'-trimethoxyflavone | 28 | 33.93 | 35.42 | 1.49 | 35.823 |
| 5 | myricetin (3,5,7,3', 4', 5'-hexahydroxyflavone) | 50 | 33.75 | 35.37 | 1.62 | 35.661 |
| 6 | quercetin(3,5,7,3', 4'-pentahydroxyflavone) | 48 | 33.75 | 35.42 | 1.67 | 35.312 |
| 7 | rhamnetin (3,5,3', 4'-tetrahydroxy-7-methoxyflavone) | 46 | 33.88 | 35.47 | 1.59 | 35.552 |
| 8 | morin (3,5,7,2', 4'-pentahydroxyflavone) | 40 | 33.88 | 35.474 | 1.589 | 35.574 |
| 9 | kaempferol (3,5,7,4'-tetrahydroxyflavone) | 20 | 33.641 | 35.173 | 1.532 | 35.564 |
| 10 | Luteolin** (5,7,3',4'-tetrahydroxyflavone) | | 33.93 | 35.41 | 1.48 | 35.786 |

Note: * – compounds 1–9 show the data according to Husaine... [12]; **– There is no available data on the radical (HO•) activity



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**Table 3 – Predicted PASS Activities**

| N | Compounds with antiradical (HO•) activity (A,%) experimentally detected according to [11] | | Activity | Compounds and their probability of predicted activity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | **Flavanone*** (unsubstituted) | 4 | Antimutagenic | 0.795 | 0.921 | 0.943 | 0.931 | 0.963 | 0.940 | 0.952 | 0.947 | 0.948 |
| 2 | Apigenin | 34 | | 0.547 | 0.64 | 0.627 | 0.612 | 0.720 | 0.689 | 0.667 | 0.680 | 0.676 |
| 3 | diosmetin | 39 | | | 0.732 | 0.683 | 0.651 | 0.924 | 0.872 | 0.798 | 0.850 | 0.856 |
| 4 | 5,7-dihydroxy-3', 4', 5'-trimethoxyflavone | 28 | Antiinflammatory | | 0.719 | 0.808 | 0.784 | 0.832 | 0.811 | 0.839 | 0.759 | 0.771 |
| | **Flavononoles** | | Antioxidant | 0.947 | 0.967 | 0.956 | 0.944 | 0.968 | 0.973 | 0.966 | 0.974 | 0.974 |
| 5 | myricetin | 50 | | 0.914 | 0.946 | 0.952 | 0.959 | 0.959 | 0.938 | 0.954 | 0.956 | 0.957 |
| 6 | quercetin | 48 | | 0.720 | 0.847 | 0.851 | 0.860 | 0.915 | 0.887 | 0.877 | 0.886 | 0.881 |
| 7 | rhamnetin | 46 | Freeradicalscavenger | 0.539 | 0.539 | | 0.569 | | | 0.529 | | 0.530 |
| 8 | morin | 40 | | | 0.650 | 0.692 | 0.607 | 0.705 | 0.737 | 0.726 | | 0.705 |
| 9 | kaempferol | 20 | Membrane integrity agonist | 10 | 11 | 12 | 13 | 15 | 16 | 17 | 14 | 18 |
| 10 | Flavanone (unsubstituted) | | | 0.537 | 0.857 | 0.883 | 0.881 | 0.917 | 0.920 | 0.916 | 0.910 | 0.895 |
| 11 | Naringenin (dihydroapigenin) | | Apoptosis agonist | 0.602 | 0.660 | 0.691 | 0.640 | 0.628 | 0.737 | 0.692 | 0.685 | 0.722 |
| 12 | Eriodiktiol (dihydroluteolin) | | | 0.550 | 0.794 | 0.817 | 0.746 | 0.938 | 0.961 | 0.919 | 0.832 | 0.946 |
| 13 | Hesperitin (dihydrodiosmetin) | | Inhibitor membrane permeability | 0.514 | 0.769 | 0.809 | 0.878 | 0.877 | 0.901 | 0.925 | 0.830 | 0.831 |
| 14 | 5,7,3', 4', 5'-pentahydroxyflavanone | | | 0.935 | 0.964 | 0.962 | 0.952 | 0.973 | 0.969 | 0.966 | 0.956 | 0.975 |
| 15 | Dihydroquercetin (Taxifolin) | | | 0.748 | 0.851 | 0.877 | 0.874 | 0.850 | 0.834 | 0.848 | 0.830 | 0.823 |
| 16 | Dihydromyretin (ampelopsin)** | | | 0.520 | 0.709 | 0.790 | 0.780 | 0.795 | 0.835 | 0.785 | 0.800 | 0.766 |
| 17 | 3,5,7,3'-tetrahydroxy-4'-methoxyflavanone | | Capillary fragility treatment | 0.712 | 0.714 | 0.634 | 0.653 | 0.687 | 0.644 | 0.706 | 0.594 | 0.759 |
| 18 | Dihydrokaempferol (aromadendrine) | | | | 0.510 | 0.577 | 0.566 | 0.714 | 0.668 | 0.707 | 0.690 | 0.668 |

Note: * – compounds 2 → 4 are flavone derivatives; ** – Compounds 16–18 are flavanonole derivatives

**Table 4 – PASS-predicted types of activity of some flavanones**

| N | Compounds | Antiviral activity | Antitumoractivity | The total values of the bond numbers (Nμ), theoretical valency (Vμ), unsaturation indices (IUA) and electron density (E.D.) on carbon atoms of the main conjugation chain | | | |
|---|---|---|---|---|---|---|---|
| | | | | Nμ | Vμ | IUA | E.D. |
| 1 | Flavanone(unsubstituted) | Influenza (0.555)<br>Rhinovirus (0.578) | Antineoplastic (0.578) | 34.48 | 35.53 | 1.05 | 36.602 |
| 2 | Naringenin (dihydroapigenin) | Influenza (0.691)<br>Rhinovirus (0.611) | Antineoplastic (0.751)<br>Anticarcinogenic (0.690) | 34.22 | 35.38 | 1.15 | 36.273 |
| 3 | Hesperitin (dihydrodiosmetin) | Influenza (0.673)<br>Rhinovirus (0.564)<br>Herpes (0.503) | Antineoplastic (0.772)<br>Anticarcinogenic (0.783) | 34.14 | 35.42 | 1.28 | 36.057 |
| 4 | 5,7-dihydroxy-3', 4', 5'-trimethoxyflavone | < 0.500 | Antineoplastic (0.628)<br>Anticarcinogenic (0.514) | 33.93 | 35.32 | 1.39 | 35.898 |
| 5 | Dihydromyretin (ampelopsin) | Influenza (0.659)<br>Herpes (0.508) | Antineoplastic (0.781)<br>Anticarcinogenic (0.837) | 33.95 | 35.32 | 1.37 | 35.45 |
| 6 | Dihydroquercetin (Taxifolin) | Rhinovirus (0.503)<br>Influenza (0.620) | Antineoplastic (0.790)<br>Anticarcinogenic (0.690) | 34.1 | 35.4 | 1.3 | 35.691 |
| 7 | Dihydrorhamnetin | Rhinovirus (0.510)<br>Influenza (0.625) | Antineoplastic (0.800)<br>Anticarcinogenic (0.695) | 34.2 | 35.42 | 1.3 | 35.350 |
| 8 | 3,5,7,2',4'--pentahydrohyflavanone | Herpes (0.543)<br>Hepatit B (0.505) | Antineoplastic (0.808)<br>Anticarcinogenic (0.796) | 34.35 | 35.42 | 1.33 | 35.921 |
| 9 | Dihydrokaempferol (aromadendrine) | Rhinovirus (0.528)<br>Influenza (0.617) | Antineoplastic (0.715)<br>Anticarcinogenic (0.792) | 34.18 | 35.37 | 1.19 | 35.942 |
| 10 | Eriodiktiol (dihydroluteolin) | Rhinovirus (0.590) | Antineoplastic (0.763)<br>Anticarcinogenic (0.775) | 34.14 | 35.41 | 1.26 | 36.025 |
| 11 | 3,5,7,3'-tetrahydroxy-4'-methoxyflavanone | Influenza (0.573) | Antineoplastic (0.747)<br>Anticarcinogenic (0.835) | 34.1 | 35.41 | 1.32 | 35.724 |

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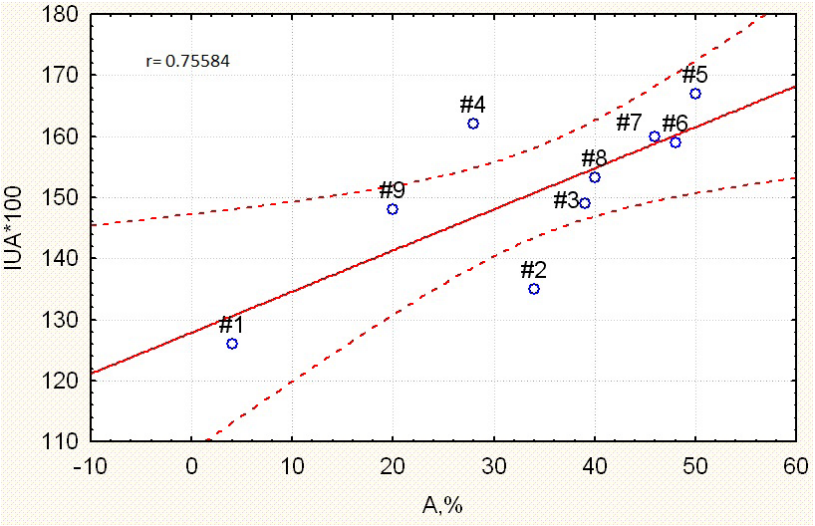


**Figure 1 – Functional relationship between $\sum_{IUA}$ of the cinnamoyl fragment of compounds 1–9 (Table 2) and the level of their antiradical activity (HO•)**

In the article of Husaine et al. [12], the antiradical activity of nine flavone derivatives (Table 2) which are aglycones, has been studied on the same model. As the data of the table show, myricetin is the most active. It is followed by quercetin, rhamnetin and morin with a slight lag. All four aglycons belong to flavonols, however, if the first three substances in the B ring contain an *ortho*-dihydroxy group in position 3', 4' (the activity is 50, 48, 46, respectively), in morin the hydroxy groups in the B ring are on C-2' and C-4', which, apparently, affects its activity.

Kaempferol, also related to flavonoles, contains only one –OH group in position 4', that's why it has a low activity, which is 2.5 times less than that of myricetin.

Apigenin and diosmetin are not flavonols: in positions 5 and 7 they contain hydroxy groups, and apigenin has one –OH group on C-4', and diosmetin has 3 -OH and 4'-$OCH_3$ groups in the B ring. The unsubstituted flavone has a minimum activity, it is 4. Although the information presented in [12] is very limited, while comparing these data it can be argued that the antiradical activity of flavonols is higher than that of flavones; the ortho-substitution in the "B" ring of types 3', 4'-diOH or 3'-OH, 4'-$OCH_3$, enhances the antiradical activity.

In order to expand information about other types of biological activity of the compounds shown in Table 2 and their corresponding flavanone derivatives, the PASS program was used [11]. It allowed to identify other types of activity shown in Tables 3 and 4. Only the species with a probability of occurrence of at least 0.5, are listed here.

The obtained data indicate that for the analyzed derivatives of flavone and flavanone, the most characteristic types of activity are those that were experimentally detected at different times.

The flavonoids listed in Table 4, are also characterized by anticancer (Antineoplastic, Anticarcinogenic, Cytoprotectant) types of activity.

Having such extensive information about various types of biological activity, the functional relationships between one of the quantum-chemical parameters and their levels of activity should be studied. Unfortunately, it is incorrect to identify correlation relationships among such a limited number of compounds that differ in the presence of enol hydroxyl on C-3, because of the 9 compounds, 4 are represented by flavones and 5 by flavonoles.

Special mention should be made about the prognosis of the antiviral activity (Table 4) of the analyzed structures in which the A core is represented by a phloroglucinol fragment: almost all compounds are characterized by the activity against the influenza virus and rhinovirus. Some compounds have activity against the Herpes virus and Hepatitis B virus.

Nowadays and in the near future, the relevant objective will be to find prophylactic drugs against various coronavirus infections. In this regard, the studies using computer technologies, in particular molecular docking, are of particular importance.

The work of Wu et al. [14] presents the results of a study of some natural compounds with antiviral and anti-inflammatory effects. A high affinity of binding to 3CLpro of flavonoids such as chrysin 7-O-glucuronide, hesperidin and neoheperidine has been established. The data obtained indicate that these compounds can be potential inhibitors of 3CLpro and, probably, can be used for the prevention and treatment of infections caused by SARS-CoV2. Likoflavonol (from Glycyrrhizauralensis), cosmosin and mangosteen (from Gurciniamangostana) have also shown similar activities. Moreover, hesperidin can interfere with the interaction of ACE2 with RBD.

The authors have also revealed a high binding affinity of vogonin-7-glucuronide (vagonoside) and vitexin (8-C-glucopyranosidapigenin) with three proteins – Nsp1, Nsp3 and ORF7, which are virulence factors of this

type of coronavirus. The authors have also shown that the highest affinity (of the 3,500 compounds analyzed) for various target proteins is shown by antibacterial, anti-inflammatory and antiviral substances including silybin, hesperidin, neohesperidin, baikalin, campferol-3-rutinoside and rutin. This fact shows that these compounds may be useful for the treatment of SARS-CoV-2 [15–17].

From our point of view, specialist virologists with the appropriate possibilities should pay attention to natural polyphenolic compounds that contain *ortho*-dihydroxy groups in the Baromaticcore. Such substances include caffeic acid, taxifolin (dihydroquercetin), amielopsin (dihydromyreticin), rhamnetin, morin, luteolin, fisetin, robinetin, etc. Of course, it is unlikely that from an economic point of view, in the future, these individual compounds will be available in sufficient quantities.

Earlier, a few our works devoted to the analysis of the quantum chemical characteristics of cinnamic acid derivatives in relation to their antiradical (OH·) activity [13] and possible metabolic pathways, were published. The data obtained were the basis for the prediction and subsequent synthesis of a new derivative of cinnamic acid, which was more active than ascorbic acid (C1/2 = 27.5 μM), caffeic acid (C1/2 = 15.7 μM) by the ability to inhibit the generation of a superoxide anion radical. The resulting new compound (C1/2 = 9.8 μM) is a spatially hindered phenolic OH group located between two *tert*-butyl substituents and is 4-hydroxy-3,5-di-*tert*-butyl cinnamic acid. [18] It is also shown here that there is a linear relationship between the level of antioxidant activity and the total degree of unsaturation of the studied derivatives of cinnamic acid with a correlation coefficient of 0.911.

## CONCLUSION

The remainder of cinnamic acid in the structure of flavonoids constitutes the main conjugation chain and, as our studies have shown, the quantum chemical characteristics of the substituted cinnamic acid practically coincide with those of the cinnamic fragment of flavones, flavonoles and flavanones with similar substituents and with the same type of substitution in the B ring [19].

Despite a small number of the related structures in Table 2 (1-4-flavones, 5-9 flavonoles), a linear relationship is observed between the total amount of unsaturation and the type of activity; compounds 2, 3, 5, 6, 7, 8 are located on a straight line (Fig. 1).

The graph indicates that the correlation coefficient is 0.75, it is quite acceptable for biological experiments [20].

Based on the analysis and comparison of the quantum-chemical parameters presented in [13, 18–19], we believe that the unsaturation index (IUA) may be the most reliable criterion for performing correlation analyzes of the antiradical activity (OH•) in the ranks of derivatives of flavone, flavanone, flavonole and flavonole. It is possible, first of all, because the hydroxyl radical, characterized by high electrophilicity, is attached in the C-8 position of the cinnamic fragment[2], where the highest electron density is concentrated.

Nevertheless, this parameter is also convenient for a qualitative analysis of patterns of the structure-activity.

It should be also notified that the transition of the flavone nucleus to flavanone is accompanied by the reduction of the vinylene fragment C-8→C-7 and, accordingly, the C-8 and C-7 atoms pass into the $sp^3$-hybridized state. Quantum-chemical parameters change as follows: the total values of the bond numbers (Nμ) for flavanones increase, the theoretical valency remains unchanged, the electron density also increases, and the unsaturation index for flavanones decreases compared to the corresponding flavones.

The latest studies on the search for the so-called small molecules able of binding to the coronavirus S-protein, deserve special attention. They will apparently be useful both for prevention and, possibly, for alleviating the infection caused by COVID-19.

Eriodiktiol, chrysin, rutin, hesperidin, quercetin, neohesperidin and others containing the phloroglucinic type of the A ring, as well as ortho-dihydroxy or ortho-methoxy-hydroxy substituents in the B ring, were referred to such “small molecules” by the authors.

Finally, it is possible to carry out chemical modification by introducing specific functional groups into the molecule, depending on the ultimate goal of a synthetic chemist, in case the structure of aglycon, flavonoid and their biochemical and pharmacological properties are known.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHORS’ CONTRIBUTION

E.T. Oganesyan – search and analysis of literature, interpretation of results, writing the text of the manuscript;
S.S. Shatokhin – search and analysis of literature, performance of quantum-chemical calculations.

[2] The numeration of carbon atoms of the cinnamoyl fragment generated by WinMopac 2016 program, is kept to.

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