Научно-практический журнал
Scientific and Practical Journal

ISSN 2307-9266
e-ISSN 2413-2241

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

PHARMACY & PHARMACOLOGY

2026 Том / Volume XIV

№ 2

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Scientific and Practical Journal

PHARMACY & PHARMACOLOGY

(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Frequency of 6 issues per year

Volume XIV, Issue 2, 2026

The mass media registration certificate
ПИ № ФС77–67428 от 13.10.2016

ISSN 2307-9266 e-ISSN 2413-2241

Founder: Volgograd State Medical University. 1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131
Publisher and editors office address: 11, Kalinin Ave., Pyatigorsk, Russia, 357532
Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University
Phone number: +7(8793) 32-44-74. E-mail: pharmjournal@mail.ru
www.pharmpharm.ru
Union catalogue. Russian Press / Newspapers an journals. Code 94183
A4 size, 1000 issues circulation. Price free

Journal "Pharmacy & Pharmacology" is recommended International Comittee Of Medical Journal Editors and included in Higher Attestation Commission, Scopus, Web of Science (ESCI), Russian citation database, eLibrary, ARISTI (All-Russian Institute of Scientific and Technical Information), RSL (Russian State Library), CyberLeninka, Socionet, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Printed in the LLC "Buro novostey" in accord with provided materials. 278A, Serova Str., Stavropol, 355000

Научно-практический журнал

ФАРМАЦИЯ И ФАРМАКОЛОГИЯ

(PHARMACY & PHARMACOLOGY)

Периодичность 6 номеров в год

Том 14, Выпуск 2, 2026

Журнал зарегистрирован Федеральной службой
по надзору в сфере связи, информационных технологий
и массовых коммуникаций (Роскомнадзор):
Свидетельство регистрации СМИ ПИ № ФС77–67428 от 13.10.2016 г.

ISSN 2307-9266 e-ISSN 2413-2241

Учредитель: Федеральное государственное бюджетное образовательное учреждение высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации (сокращенное наименование: ФГБОУ ВО ВОЛГГМУ МИНЗДРАВА РОССИИ).
400131, Россия, г. Волгоград, площадь Павших Борцов, д. 1
Адрес издательства и редакции: 357532, Россия, г. Пятигорск, пр-кт Калинина, д. 11
Пятигорский медико-фармацевтический институт – филиал ФГБОУ ВО ВолгГМУ Минздрава России
Телефон: +7 (8793) 32-44-74. E-mail: pharmjournal@mail.ru
www.pharmpharm.ru
Объединенный каталог. Пресса России. Газеты и журналы. Индекс 94183
Формат А4, тираж 1000 экз. Цена свободная. Подписано в печать 29.04.2026; выход в свет 15.05.2026

Журнал «Фармация и фармакология» включен в перечень рецензируемых научных изданий, входящих в международные реферативные базы данных и системы цитирования, и в соответствии с пунктом 5 правил формирования перечня рецензируемых научных изданий, в которых должны быть опубликованы основные научные результаты диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук (Перечень ВАК), Scopus, Web of Science (ESCI), РИНЦ, eLibrary, ВИНИТИ, РГБ, Киберленинка, Соционет, EMBASE, Chemical Abstracts (CAS), Directory of Open Access Journals (DOAJ), EBSCO Discovery Service, RNMJ, University of CAMBRIDGE, Ulrich'sWeb, Google Scholar, Biefeld Academic Search Engine (BASE), Directory of Open Access Scholarly Resources (ROAD), Research Bible, Open Archives Initiative, Academic Keys, JournalTOCs, WorldCat, OpenAIRE, University of Oxford, The British Library, Universitait Gent, Université de Montréal, University of Saskatchewan.

Отпечатано в соответствии с предоставленными материалами в ООО «Бюро новостей», 355000, Россия, г. Ставрополь, ул. Серова, д. 278А

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REVIEW

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Senolytic effects of first and second generation BCL-xL/BCL-2 dual degraders

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Received 25 Aug 2025 After peer review 24 Dec 2025 Accepted 03 Apr 2026

The aim. To conduct a literature review of current data on the senolytic effects of dual BCL-xL/BCL-2 degraders, including available molecules, their mechanism of action, efficacy, and safety.

Materials and Methods. Literature search was performed in PubMed, Science Direct, and SciELO databases using the keywords: “senolytics”, “BCL-xL/BCL-2 dual degraders”, “proteolysis targeting chimeras”, “753b”, “WH244”. In the eLIBRARY.ru database were used the next keywords: «сенолитики», «двойные деградаторы BCL-xL/BCL-2», «протеолиз-направленные химеры», «753b», «WH244».

Results. The accumulation of a small number of senescent cells in the body, due to their release of the senescence-associated secretory phenotype (SASP), contributes to the elimination of old and damaged cells. However, when the number of senescent cells becomes large, SASP triggers a chronic inflammatory process that accelerates aging and leads to the development of age-related diseases such as cancer, diabetes mellitus, atherosclerosis, etc. Therefore, there is a need to develop senolytics — drugs aimed to eliminate senescent cells. One possible way to achieve this is through the pharmacological induction of apoptosis. According to literature data, a chimeric molecule, 753b, was created using PROTACs technology. One end of it binds to an E3 ligase, the other to anti-apoptotic proteins (BCL-xL or BCL-2). As a result, all these molecules are brought together in space, forming a ternary complex. Due to proximity, the E3 ligase attaches ubiquitin molecules to the anti-apoptotic proteins, after which the proteasome destroys them. When BCL-xL and BCL-2 are degraded, apoptosis of senescent cells occurs. The molecule 753b is classified as a first-generation dual BCL-xL/BCL-2 degrader. Its anti-senescence and anti-tumor efficacy has been demonstrated in preclinical studies without the development of significant thrombocytopenia. Based on molecule 753b, a more potent analog was developed through two modifications — molecule WH244, which is classified as a second-generation dual BCL-xL/BCL-2 degrader.

Conclusion. Considering the data on efficacy and safety presented in the literature sources, further comprehensive research on molecules 753b, WH244, and/or their derivatives is required, including in clinical studies.

Keywords: senolytics; proteolysis targeting chimeras; BCL-xL/BCL-2 dual degraders; 753b; WH244

Abbreviations: SASP — senescence-associated secretory phenotype; SMIs — small molecule inhibitors; FDA — US Food and Drug Administration; PROTACs — Proteolysis Targeting Chimeras; UPS — ubiquitin-proteasome system; POI — protein of interest; VHL — von Hippel-Lindau protein; Ub — ubiquitin; SCLC — small cell lung cancer; MAFLD — metabolically associated fatty liver disease; MASH — metabolically associated steatohepatitis; AML — acute myeloid leukemia.

Сенолитические эффекты первого и второго поколения двойных деградаторов BCL-xL/BCL-2

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Получена 25.08.2025 После рецензирования 24.12.2025 Принята к печати 03.04.2026

For citation: E.S. Berezhnaya, A.V. Savustyanenko. Senolytic effects of first and second generation BCL-xL/BCL-2 dual degraders. *Pharmacy & Pharmacology.* 2026;14(2):136-146. **DOI:** 10.19163/2307-9266-2026-14-2-136-146

Для цитирования: Е.С. Бережная, А.В. Савустьяненко. Сенолитические эффекты первого и второго поколения двойных деградаторов BCL-xL/BCL-2. *Фармация и фармакология.* 2026;14(2):136-146. **DOI**: 10.19163/2307-9266-2026-14-2-136-146

Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОБЗОР
DOI: 10.19163/2307-9266-2026-14-2-136-146

Цель. Провести литературный обзор современных данных о сенолитических эффектах двойных деградаторов BCL-xL/BCL-2, включая доступные молекулы, механизм их действия, эффективность и безопасность.

Материалы и методы. Поиск литературы выполнен в базах PubMed, Science Direct, SciELO по ключевым словам: «senolytics», «BCL-xL/BCL-2 dual degraders», «proteolysis targeting chimeras», «753b», «WH244». В базе eLIBRARY.ru использовали следующие ключевые запросы: «сенолитики», «двойные деградаторы BCL-xL/BCL-2», «протеолиз-направленные химеры», «753b», «WH244».

Результаты. Накопление в организме небольшого количества стареющих (сенесцентных) клеток благодаря высвобождению ими сенесцентно-ассоциированного секреторного фенотипа (SASP) способствует уничтожению старых и повреждённых клеток. Однако, когда сенесцентных клеток становится много, SASP запускает хронический воспалительный процесс, который ускоряет старение и ведет к развитию возраст-связанных заболеваний, таких как рак, сахарный диабет, атеросклероз и пр. Следовательно, возникает необходимость в разработке сенолитиков — лекарственных препаратов, направленных на уничтожение сенесцентных клеток. Один из возможных путей достижения этого сводится к фармакологической индукции апоптоза. По данным литературы с помощью технологии PROTACs была создана химерная молекула 753b. Один ее конец связывается с Е3-лигазой, другой с антиапоптотическими белками (BCL-xL или BCL-2). В результате все эти молекулы сближаются в пространстве, формируя тройной комплекс. Благодаря близости Е3-лигаза присоединяет молекулы убиквитина к антиапоптотическим белкам, после чего протеосома их разрушает. Когда BCL-xL и BCL-2 разрушены, происходит апоптоз сенесцентных клеток. Молекулу 753b относят к первому поколению двойных деградаторов BCL-xL/BCL-2. В доклинических исследованиях была продемонстрирована ее антисенесцентная и противоопухолевая эффективность, без развития выраженной тромбоцитопении. На базе молекулы 753b за счёт двух модификаций был разработан ее более сильный аналог — молекула WH244, которую относят ко второму поколению двойных деградаторов BCL-xL/BCL-2.

Заключение. Учитывая представленные в литературных источниках данные по эффективности и безопасности, требуется дальнейшее всестороннее исследование молекул 753b, WH244 и/или их производных, в том числе и в клинических исследованиях.

Ключевые слова: сенолитики; протеолиз-направленные химеры; двойные деградаторы BCL-xL/BCL-2; 753b; WH244

Список сокращений: SASP — сенесцентно-ассоциированный секреторный фенотип; SMIs — маломолекулярные ингибиторы; FDA — Управление по контролю за качеством пищевых продуктов и лекарственных средств США; PROTACs — протеолиз-направленные химеры; UPS — убиквитин-протеосомная система; POI — целевой белок; pVHL — белок фон Хиппеля-Линдау; Ub — убиквитин; SCLC — мелкоклеточный рак лёгкого; МАЖБП — метаболически ассоциированная жировая болезнь печени; МАСГ — метаболически ассоциированный стеатогепатит; ОМЛ — острый миелоидный лейкоз.

INTRODUCTION

The term “cellular senescence” was proposed by L. Hayflick and P.S. Moorhead based on the observation that normal cells have limited proliferative capacity—after a long period of cultivation, they exit the cell cycle and enter a state of stable growth arrest [1, 2]. This type of senescence was named “replicative senescence” and is associated with telomere shortening and/or dysfunction [3, 4].

Later, the phenomenon of “premature cellular senescence,” not related to telomere shortening, was discovered. Its development is caused by the influence of various endogenous and exogenous stressors on the cell, such as inadequate cell culture conditions, oncogenic factors, genotoxic factors, chemotherapy, radiotherapy, etc. [5–8].

The transient accumulation of a limited number of senescent cells in the body, by any of the above-mentioned methods, has a positive physiological significance. The biologically active substances they produce—the so-called senescence-associated secretory phenotype (SASP)—attract immune system cells, which destroy old and damaged cells, and stimulate the proliferation and differentiation of stem cells and progenitor cells, allowing for the replenishment of cell populations in damaged tissues. In particular, this is one of the important mechanisms in the fight against carcinogenesis [9].

However, the prolonged accumulation of a large number of senescent cells leads to the opposite effect. The SASP released by them triggers a chronic inflammatory process [10, 11], which accelerates aging

and leads to the development of age-related diseases such as cancer, diabetes mellitus, atherosclerosis, and others [12–14].

Based on their positive physiological role, researchers initially made efforts to artificially induce cellular senescence [15]. When the negative effects of an excess of senescent cells became clear, attention shifted to finding ways to reduce their number and/or activity [16]. To date, four groups of such agents with the aforementioned effect are known [17–19]:

- senolytics (destroy senescent cells);
- senomorphs (suppress SASP release);
- senoblockers (block the transition of normal cells into a senescent state);
- senoreversers (enhance the exit of senescent cells from this state).

Until relatively recently, it was unclear whether senescent cells were causally involved in age-related dysfunction and whether their elimination would have a positive effect. However, it was demonstrated that genetic ablation of p16Ink4a-positive senescent cells using INK-ATTAC, which functions as a suicide transgene, along with the administration of the recombinant dimerization protein AP20187, prolonged the lifespan of mice and delayed the onset of many age-related diseases and disorders in mice with accelerated and normal aging. These data confirm the expediency and effectiveness of the first group of drugs mentioned above—senolytics [9, 20, 21]. The other groups were not the focus of our article.

Since the action of senolytics is directed at senescent cells, it is important to note a number of characteristics that distinguish them from normal cells. In particular, they have an increased size and irregular shape, altered cell membrane composition, increased content of mitochondria and lysosomes, structural remodeling and destabilization of the nuclear membrane, their cell cycle is usually arrested in the G1 phase, they lack apoptosis, etc. [15]. Pharmacological induction of apoptosis is one of the important pathways for eliminating an excess number of senescent cells by senolytic agents [22–25].

Thus, considering the scientific and practical significance of the topic, we have dedicated this review to senolytics that induce apoptosis of senescent cells by influencing BCL-xL and BCL-2 molecules. In this context, we have focused primarily on those that achieve this by degrading BCL-xL and BCL-2.

THE AIM. To conduct a literature review of current data on the senolytic effects of BCL-xL/BCL-2 dual degraders, including available molecules, their mechanism of action, efficacy, and safety.

MATERIALS AND METHODS

Literature search was performed in the PubMed, Science Direct, and SciELO databases using the keywords: “senolytics”, “BCL-xL/BCL-2 dual degraders”, “proteolysis targeting chimeras”, “753b”, “WH244”. In the eLIBRARY.ru database (in Russian), the following search queries were used: «сенолитики», «двойные деградаторы BCL-xL/BCL-2», «протеолиз-направленные химеры», «753b», «WH244». None of the molecules belonging to the class of BCL-xL/BCL-2 dual degraders have been included in clinical studies. Consequently, this review is based on preclinical studies of these molecules. Original articles in full text or abstract form were considered, excluding conference materials, short communications, etc.

During the search in the PubMed database with all keywords, 24 studies were found. Of these, 18 were excluded because the molecule of interest, 753b, belonging to the first generation of BCL-xL/BCL-2 dual degraders, has the same spelling as the V-79-753B cell line, which represents Chinese hamster lung fibroblasts and is used in radiation biology and toxicology to study DNA damage, repair, and mutation. Furthermore, one study was a preprint that had not undergone peer review in a scientific journal. The final version of this article (after peer review) was present separately in the search results and was considered by us. Thus, 5 articles from the PubMed database were included in this review.

A similar search in the Science Direct database using all keywords revealed 60 studies. Following the approach described above, 59 studies were excluded as not matching the specified objective. Only one work was included in this review, but it was among the 5 articles found in the PubMed database.

No publications were found in the eLIBRARY.ru and SciELO databases using the keywords.

Consequently, a total of 5 preclinical studies were found across all databases, which were ultimately included in this review. The small number of articles is related to the novelty of the molecules being studied

ОБЗОР
DOI: 10.19163/2307-9266-2026-14-2-136-146

and, in general, this scientific direction—all works were performed between 2021 and 2025.

The chemical formulas of molecules 753b and WH244 are borrowed from reference [26]. To facilitate the reader's comprehension, the following designations are made on them: ligand binding to VHL (E3 ligase); intermediate linker; ligand binding to BCL-xL/BCL-2. Red arrows in the WH244 molecule indicate the 1,4-dimethylpiperazine fragment and the site where a bridging carbon atom is added to the morpholine group. A detailed explanation is provided in the text.

Since molecules 753b and WH244 are created using PROTACs technology, the advantages of this technology are listed in this review based on additional literature sources.

RESULTS AND DISCUSSION

First generation of BCL-xL/BCL-2 dual degraders

History of the creation of molecule 753b

Members of the BCL-2 family of proteins are key regulators of cellular apoptosis and include both anti-apoptotic (BCL-2, BCL-xL, MCL-1, etc.) and pro-apoptotic (BAD, BIM, PUMA, BAK, BAX, etc.) proteins. In cancer cells, their balance is shifted towards anti-apoptotic proteins, which contributes to tumor initiation, progression, and the development of drug resistance [26]. Initially, so-called small molecule inhibitors (SMIs) that can directly inhibit the function of anti-apoptotic proteins came into researchers' focus. Among them, venetoclax (ABT199) can selectively inhibit BCL-2 and is the only anti-tumor drug targeting BCL-2 family members that has been approved by the FDA (US Food and Drug Administration). It is also approved for use in the Russian Federation[1]. In general, it is used to treat hematological malignancies whose survival depends more on BCL-2. However, venetoclax is poorly effective in treating solid tumors, whose survival depends more on BCL-xL [26–29].

An effect on both hematological and solid tumors was observed with navitoclax (ABT263), a non-selective small molecule inhibitor that preceded venetoclax and directly inhibited BCL-2 and BCL-xL. However, during its trials, a serious side effect was discovered—thrombocytopenia, as BCL-xL proved important for the survival of circulating platelets. Due to this side effect, navitoclax was not introduced for clinical practice [26, 30].

Researchers then changed their strategy and, instead of small molecule inhibitors (SMIs), began using proteolysis-targeting chimeras (PROTACs), which do not inhibit but rather degrade proteins. Accordingly, the molecule DT2216 was created based on navitoclax (ABT263). However, researchers were again met with an unpleasant surprise: although DT2216 bound to both BCL-2 and BCL-xL *in vitro* in a cell-free system, it degraded only BCL-xL, but not BCL-2, within cells. One problem was solved—due to the specific characteristics of PROTAC technology, thrombocytopenia did not develop. However, the much-needed simultaneous effect on both anti-apoptotic proteins (BCL-xL and BCL-2) was lost [26].

Further refinement of the DT2216 molecule, while maintaining the same PROTAC technology, led to the creation of a new molecule represented by two mixed stereoisomers, 753a and 753b. After their separation, the S-enantiomer (753a) only partially degraded BCL-xL and did not degrade BCL-2, whereas the R-enantiomer (753b) effectively degraded both BCL-xL and BCL-2 [31].

Thus, the molecule 753b became the first representative of the BCL-xL/BCL-2 dual degrader class. Due to the specific characteristics of PROTAC technology, its application does not lead to the development of thrombocytopenia.

Mechanism of action of molecule 753b

The functioning of the ubiquitin-proteasome system (UPS) is the primary intracellular non-lysosomal mechanism responsible for protein homeostasis, as it degrades old and damaged proteins, misfolded proteins, and regulatory proteins that have reached the end of their lifespan. During the enzymatic cascade, a ubiquitin-activating enzyme (E1) activates ubiquitin, a ubiquitin-conjugating enzyme (E2) captures and transfers ubiquitin, and a ubiquitin ligase (E3) attaches ubiquitin to the target protein. Subsequently, the proteasome degrades it [32–35].

PROTAC technology involves the creation of heterobifunctional molecules composed of two

[1] Venetoclax. LP-No. (004567)-(RG-RU). The State Register of Medicines of Russian Federation. Available from: https://grls.minzdrav.gov.ru/Grls_View_ v2.aspx?routingGuid=719240d6-b494-446a-b1a6-28c1faa3adba. Russian

ligands connected by an intermediate linker [36–38]. One ligand is specific for binding to an E3 ligase, and the other ligand is specific for binding to the protein of interest (POI). A typical representation of such a molecule is shown in Figure 1 [39–41].

Accordingly, after administration into the body, PROTACs, through their ligands, bind to the E3 ligase and the target protein, forming a ternary complex that spatially brings them close together (see Fig. 1). Under these conditions, the E3 ligase attaches multiple ubiquitin molecules to the target protein, a process called polyubiquitination (pink color in Figure 1), after which the proteasome degrades it [36].

As noted above, molecule 753b was created using PROTAC technology. One of its ligands is designed to bind to an E3 ligase, and the other to the target protein, which is BCL-xL or BCL-2. As a result, the proteasome degrades both anti-apoptotic proteins, which is why 753b is classified as a BCL-xL/BCL-2 dual degrader [31].

It is important to clarify that the ligand of 753b that binds to the E3 ligase specifically binds to the VHL (von Hippel-Lindau) protein, which is part of the E3 ubiquitin ligase complex along with other proteins. The VHL protein is practically absent in platelets, which is why 753b does not degrade BCL-xL in them and, consequently, cannot cause significant thrombocytopenia [41, 42].

The chemical structure of molecule 753b and the arrangement of its ligands are presented in Figure 1.

Results of preclinical studies of molecule 753b

A review of electronic databases revealed 3 preclinical studies of molecule 753b.

S. Khan et al. [43] evaluated the anti-tumor effects of 753b on BCL-xL/BCL-2 co-dependent SCLC cell lines and H146 xenograft models. (In both cases, this refers to small cell lung cancer, but experiments with SCLC were performed *in vitro*, while H146 was injected subcutaneously into mice with subsequent tumor growth; co-dependence means that the survival of SCLC cells is ensured by inhibiting apoptosis by both BCL-xL and BCL-2 molecules simultaneously). The study found that 753b degraded BCL-xL and BCL-2 in both SCLC cells and H146 cells. Importantly, 753b proved to be a more potent molecule than DT2216, navitoclax, or the DT2216+venetoclax combination in reducing the viability of BCL-xL/BCL-2 co-dependent SCLC cell lines in cell culture *in vitro*. Weekly administration of 5 mg/kg 753b led to a significant delay in tumor growth in H146 xenograft models *in vivo* ($p < 0.0001$), similar to the DT2216+venetoclax combination. Additionally, administration of 5 mg/kg 753b every 4 days caused tumor regression. At this dose, 753b was well-tolerated in mice, without the development of severe thrombocytopenia (observed with navitoclax) and without changes in mouse weight. The obtained results indicate that 753b, a BCL-xL/BCL-2 dual degrader, may be an effective and safe therapeutic agent in SCLC patients. This fact requires confirmation in future clinical studies.

In their work, Y. Yang et al. [44] evaluated the anti-senescence and anti-tumor effects of 753b. It was found that administration of this agent selectively reduced the number of senescent cells in the livers of old mice and STAM mice, partly due to their sequestration in the liver. (STAM™ mice are a commercial model for pharmacological evaluation of drugs for liver fat damage and associated tumor growth). Moreover, 753b effectively ($p < 0.05$) reduced the progression of metabolic-associated fatty liver disease (MAFLD) and the development of hepatocellular carcinoma in STAM mice, even after the mice developed significant metabolic-associated steatohepatitis (MASH) and liver fibrosis. The obtained data suggest that 753b could become a potential therapeutic agent for MAFLD, helping to reduce the incidence of MASH-induced hepatocellular carcinomas.

In the work by Y. Jia et al. [42], the anti-senescence and anti-tumor effects of 753b were evaluated. It was found that administration of this molecule significantly ($p < 0.05$) reduced cell viability and induced dose-dependent degradation of BCL-xL and BCL-2 in a subpopulation of hematopoietic cell lines, primary samples of acute myeloid leukemia (AML) *in vitro*, and patient-derived AML xenograft models *in vivo*. In the latter case, the authors noted the absence of thrombocytopenia. Additionally, the senolytic activity of 753b was demonstrated, enhancing the efficacy of chemotherapy by reducing the severity of chemotherapy-induced cellular senescence ($p < 0.01$). The obtained results provide preclinical justification for the use of 753b in AML therapy. They also suggest that administering 753b in combination with chemotherapy may provide an additional therapeutic effect by combating chemoresistance caused by cellular senescence.

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DOI: 10.19163/2307-9266-2026-14-2-136-146

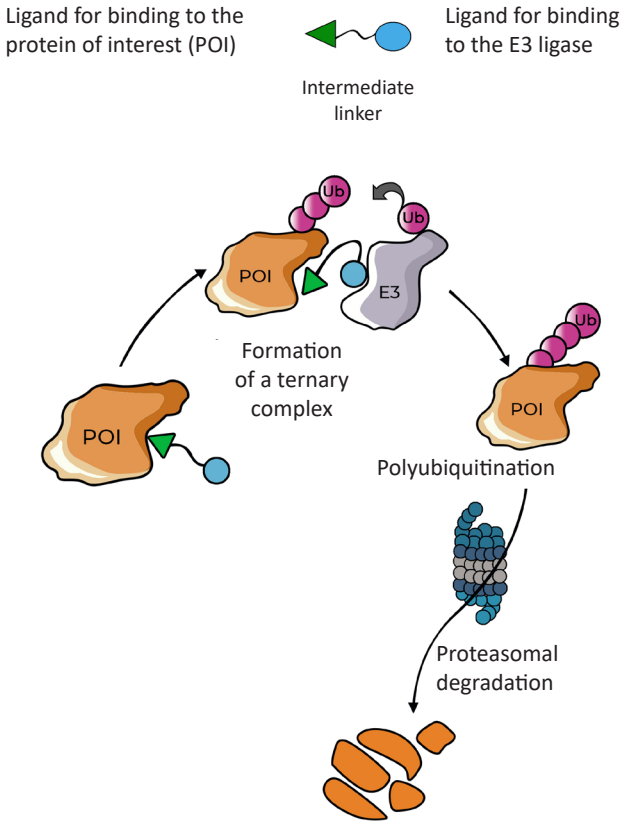

Figure 1 — Structure of PROTAC molecules (top) and their mechanism of action (bottom).
Note: POI — protein of interest, E3—E3 ligase, Ub — ubiquitin (pink color), PROTACs — proteolysis-targeting chimeras.
Figure adapted from source [36] under the Creative Commons Attribution (CC BY 4.0) license.

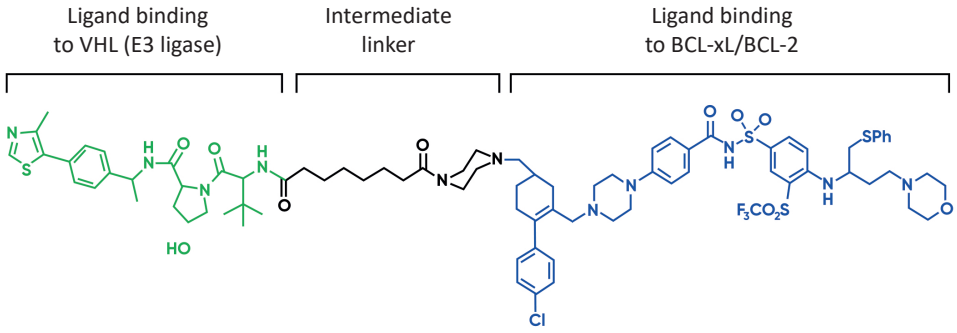

Figure 2 — Structure of molecule 753b.

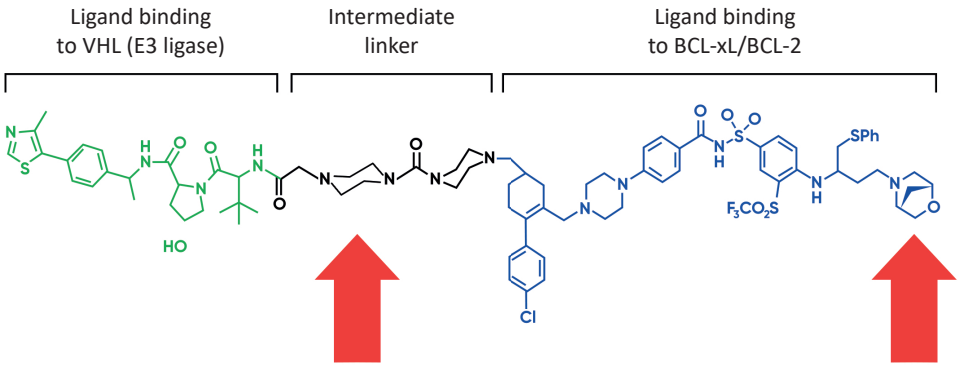

Figure 3 — Structure of molecule WH244.

Thus, the preclinical studies presented have confirmed the anti-senescence and anti-tumor effects of 753b, without the development of significant thrombocytopenia. Consequently, molecule 753b and/or its derivatives are good candidates for future clinical studies.

Second-generation BCL-xL/BCL-2 dual degraders

The researchers who developed molecule 753b studied the crystal structure of the ternary complexes VHL (E3 ligase)/753b/BCL-xL and VHL (E3 ligase)/753b/BCL-2. This allowed for several useful modifications and the synthesis of a new molecule, WH244 (Fig. 3) [26].

Specifically, in the intermediate linker, the 6-carbon alkyl chain (753b) was replaced with a 1,4-dimethylpiperazine fragment (WH244), and in the ligand that binds to BCL-xL or BCL-2, a bridging carbon atom was added to the morpholine group (753b) (WH244). This group is the last in the ligand and directly binds to BCL-xL or BCL-2.

The introduction of the aforementioned 1,4-dimethylpiperazine fragment into the intermediate linker of WH244 provided the following advantages:

- Increased rigidity of the intermediate linker (unlike the flexible 6-carbon alkyl chain), which is important for maintaining the overall structure of the ternary complex;
- Improved electrostatic interaction between the ionizable amino group of 1,4-dimethylpiperazine and BCL-2, which is important for maintaining the ternary complex.

The introduction of a bridging carbon atom into the morpholine group of WH244 resulted in stronger binding of the corresponding ligand to BCL-xL/BCL-2, which is important for maintaining the ternary complex.

Maintaining an optimal ternary complex structure promotes closer proximity between the E3 ligase and BCL-xL/BCL-2, thereby stimulating their degradation.

Based on the results of cellular experiments [26], WH244 induced greater death of Jurkat cancer cells (co-dependent on BCL-xL/BCL-2) compared to small molecule inhibitors (SMIs), DT2216, and 753b. The HiBiT degradation assay in live cells also confirmed superior performance of WH244 compared to 753b.

Advantages of PROTAC technology

Since both molecules discussed (753b, WH244) are created using PROTAC technology, it is worth noting the advantages of this technology.

Traditional small molecule inhibitors (SMIs) demonstrate so-called "occupancy-driven pharmacology": they bind to proteins and directly inhibit their activity. This requires the constant presence of SMIs at a sufficient concentration [45–47]. PROTACs operate on the principle of "event-driven pharmacology": the drugs initiate a cellular event, such as protein degradation, which leads to a decrease in the target protein concentration. These effects last for some time even after drug withdrawal, as cells require time for protein resynthesis [48, 49].

PROTACs act substoichiometrically—one molecule can degrade many molecules of the target protein (after degrading one molecule of the target protein, PROTACs are released to bind and degrade another molecule of the target protein, and so on) [50–52]. For this reason, the concentration of PROTACs should be lower compared to the concentration of SMIs to achieve a therapeutic effect [53, 54].

PROTACs can selectively degrade target proteins in a tissue/cell-specific manner [26, 55].

Target proteins can mutate, leading to the development of drug resistance. However, PROTACs degrade the protein regardless of mutations, thus overcoming drug resistance [56, 57].

Proteins that lack catalytic activity and/or have catalytically independent functions are considered "difficult-to-target" due to the absence of active sites for binding. However, PROTACs degrade the entire protein and do not require such binding sites, consequently reaching these very "difficult-to-target" proteins [61–63].

Thus, the presented literature data indicate a number of advantages of PROTAC technology, which justifies the development of new drug candidates.

Review Limitations

This review, dedicated to the senolytic effects of first and second-generation dual BCL-xL/BCL-2 degraders, was conducted in the form of a simple descriptive review. At the same time, in recent decades, systematic reviews and meta-analyses, originally developed for clinical research, are increasingly being used to analyze preclinical experimental data. This allows for the generalization of results, identification of knowledge gaps, and improvement of the translation of laboratory data to the human body. We could not utilize these more complex assessment methods due to the small number of initial (primary) studies.

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DOI: 10.19163/2307-9266-2026-14-2-136-146

One limitation is related to the study selection methodology. Typically, several authors independently search for publications in databases. After that, they compare and combine the search results, and discuss controversial articles. In our review, we considered it sufficient for one author to perform the search work, given the novelty of the topic and the associated small number of works.

In each of the publications dedicated to molecules 753b and WH244, approximately 10–20 researchers from different scientific centers participated. Some researchers changed, while others were represented by the same individuals. This suggests that, to some extent, all articles were written by the same team of authors, which may introduce an error into the presented data.

The WH244 molecule is a new development and currently the sole representative of the second generation of dual BCL-xL/BCL-2 degraders. Only one pilot study was dedicated to it, which is insufficient to draw definitive conclusions about its efficacy.

CONCLUSION

The development of molecules 753b and WH244 marks an important stage not only in oncology but also in the fight against aging, owing to their pronounced senolytic effect. The main problem with earlier drugs, such as navitoclax, was the development of severe thrombocytopenia. Since platelets critically depend on the BCL-xL protein, its direct inhibition led to their mass death. Due to this side effect, navitoclax was never approved for clinical use.

Dual degraders (PROTACs) work differently. Instead of simply inhibiting the active center of the protein, molecules 753b and WH244 bind BCL-xL and BCL-2 for subsequent destruction by the cellular disposal system. The key to safety here lies in tissue specificity. Preclinical data show that degradation mechanisms in platelets hardly occur. This creates a wide therapeutic window: a powerful senolytic effect—clearing the body of defective cells—is achieved without a critical drop in platelet levels.

The transition from the first generation of degraders (753b) to the second (WH244) has further increased affinity to targets and improved pharmacokinetics. WH244 demonstrates deeper protein degradation at lower concentrations, making it a promising candidate for the therapy of age-dependent pathology and systemic tissue rejuvenation resistant to standard geroprotectors.

Moreover, the innovative architecture of molecules 753b and WH244 allows for more effective overcoming of pathological cell survival mechanisms. Unlike traditional small molecule inhibitors, which can be displaced by increased concentrations of anti-apoptotic proteins, degraders operate on a catalytic principle: one drug molecule can sequentially destroy multiple target proteins. This ensures a prolonged senolytic effect even after the drug concentration in blood plasma decreases.

Collectively, all of the above transforms BCL-xL/BCL-2 degraders into a powerful tool in biogerontology, capable of fundamentally changing the approach to treating chronic diseases and extending active longevity. Their expected synergy with other regenerative medicine methods should be noted separately.

Thus, the advantages of PROTAC technology are evident, and the new molecules mentioned in this review (753b and WH244), as well as their future derivatives, require further comprehensive study, including clinical trials. Particular attention should be paid to optimizing pharmacokinetic properties and assessing long-term safety. A deep understanding of the mechanisms of selective target protein degradation will expand the therapeutic arsenal, opening unique opportunities for the targeted treatment of complex pathologies that were previously considered practically incurable.

FUNDING

This study did not have financial support from third-party organizations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Elizaveta S. Berezhnaya — conceptualization, visualization, writing—original draft, writing—review & editing; Andrey V. Savustyanenko — formal analysis, visualization, writing—original draftю All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept and preparation of the article, read and approved the final version before publication).

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОБЗОР

DOI: 10.19163/2307-9266-2026-14-2-147-160

Phacelia tanacetifolia as a Promising Object for Pharmacognostic Research

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Received 19 Jan 2025 After peer review 12 Dec 2025 Accepted 08 Feb 2026

Currently, there is an increasing interest in studying the chemical composition of the above-ground part of *Phacelia tanacetifolia Benth*., honey based on the plant, individual biologically active compounds, and results of studying the pharmacological activity of this promising species are emerging.

The aim. To conduct a review and systematization of scientific data on the chemical composition, and application in medicine and pharmacy of the promising plant *Phacelia tanacetifolia.*

Materials and Methods. For searching scientific literature, data posted in the electronic databases elibrary.ru, Cyberleninka, Google Scholar, and PubMed were used. Publication search was conducted for the period from January 2001 to January 2026. The final number of works included in this review was 69.

Results. As a result of analyzing scientific literature data, the work characterizes the Boraginaceae family, the Phacelia genus, and the *Phacelia tanacetifolia* species; the main groups of biologically active compounds and the chemical composition of the studied object. The main aspects of studying *Phacelia tanacetifolia* as a honey-producing plant and green manure are presented. The results of the pharmacological activity of phacelia honey are presented. The potential pharmacological activity of *Phacelia tanacetifolia* is formulated.

Conclusion. A comprehensive search for information on *Phacelia tanacetifolia* abroad and in Russia has been conducted. Based on the results of the work, the expediency of a deeper study of the selected plant object for its application in medicine and pharmacy is justified.

Keywords: *Phacelia tanacetifolia* Benth.; phenolic compounds; chlorogenic acid; hydroxycinnamic acids; flavonoids; phenolamides; obesity; hyperlipidemia

Abbreviations: BACs — biologically active compounds; MPRMs — medicinal plant raw materials; RD — regulatory document; HPLC — high-performance liquid chromatography; DHBA — dihydroxybenzoic acid; DOPAC — dihydroxyphenylacetic acid; HBA — hydroxybenzoic acid; CA — caffeic acid; HA — hippuric acid; HPA — hydroxypicolinic acid; HVA — 4-hydroxy-3-methoxyphenylacetic acid; HPPA — hydroxyphenylpyruvic acid; FA — ferulic acid, BA — benzoic ACID; ERC — eriocitrin; ERI — eriodictyol; FIS — fisetin; HSD — hesperidin; HST — hesperetin; NAR — naringenin; NARG — naringin; NHSD — neohesperidin; NRI — narirutin; PIN — pinocembrin; QUE — quercetin; R-ERI — R-enantiomer of eriodictyol; R-NAR — R-enantiomer of naringenin; RUT — rutin; S-ERI — S-enantiomer of eriodictyol; S-HST — S-enantiomer of hesperetin; S-NAR — S-enantiomer of naringenin; TAX — taxifolin, LDLs — low-density lipoproteins.

For citation: P.A. Sheykhmagomedova, O.I. Popova, I.V. Popov. *Phacelia tanacetifolia* as a Promising Object for Pharmacognostic Research. *Pharmacy & Pharmacology.* 2026;14(2):147-160. **DOI:** 10.19163/2307-9266-2026-14-2-147-160

Для цитирования: П.А. Шейхмагомедова, О.И. Попова, И.В. Попов. Фацелия пижмолистная как перспективный объект фармакогностического исследования. *Фармация и фармакология.* 2026;14(2):147-160. **DOI**: 10.19163/2307-9266-2026-14-2-147-160

REVIEW

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Фацелия пижмолистная как перспективный объект фармакогностического исследования

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Получена 19.01.2025 После рецензирования 12.12.2025 Принята к печати 08.02.2026

На сегодняшний день наблюдается увеличение интереса к изучению химического состава надземной части фацелии пижмолистной (*Phacelia tanacetifolia* Benth.), мёда на основе растения, к отдельным биологически активным соединениям, а также появляются результаты изучения фармакологической активности этого перспективного вида.

Цель. Провести обзор и систематизацию научных данных о химическом составе, применении в медицине и фармации перспективного растения — фацелии пижмолистной (*Phacelia tanacetifolia* Benth.).

Материалы и методы. Для поиска научной литературы использовали данные, размещённые в электронных базах elibrary.ru, Киберленинка, Google Академия и PubMed. Поиск публикаций проводили за период с января 2001 по январь 2026 гг. Итоговое число работ, включённых в настоящий обзор, составило 69.

Результаты. В результате анализа данных научной литературы в работе охарактеризованы семейство бурачниковые, род Фацелия, вид фацелия пижмолистная; основные группы биологически активных соединений и химический состав изучаемого объекта. Приведены основные аспекты изучения фацелии пижмолистной как растения-медоноса и сидерата. Представлены результаты фармакологической активности фацелиевого мёда. Сформулирована потенциальная фармакологическая активность фацелии пижмолистной.

Заключение. Проведён всесторонний поиск информации о фацелии пижмолистной за рубежом и в России. На основании результатов работы обоснована целесообразность более глубокого изучения выбранного растительного объекта для применения его в медицине и фармации.

Ключевые слова: фацелия пижмолистная; *Phacelia tanacetifolia* Benth.; фенольные соединения; хлорогеновая кислота; гидроксикоричные кислоты; флавоноиды; феноламиды; ожирение; гиперлипидемия

Список сокращений: БАС — биологически активные соединения; ЛРС — лекарственное растительное сырьё; НД — нормативный документ; ВЭЖХ — высокоэффективная жидкостная хроматография; DHBA —дигидроксибензойная кислота; DOPAC — дигидроксифенилуксусная кислота; HBA — гидроксибензойная кислота; CA — кофейная кислота; HA — гиппуридная кислота; HPA —гидроксипиколиновая кислота; HVA — 4-гидрокси-3-метоксифенилуксусная кислота; HPPA — гидроксифенилпировиноградная кислота; FA — феруловая кислота, BA — бензойная кислота; ERC — эриоцитрин; ERI — эриодиктиол; FIS — физетин; HSD — гесперидин; HST — гесперетин; NAR — нарингенин; NARG — нарингин; NHSD — неогесперидин; NRI — нарирутин; PIN — пиноцембрин; QUE — кверцетин; R-ERI — R-энантиомер эриодиктиола; R-NAR — R-энантиомер нарингенина; RUT — рутин; S-ERI — S-энантиомер эриодиктиола; S-HST — S-энантиомер гесперетина; S-NAR — S-энантиомер нарингенина; TAX — дигидрокверцетин, ЛПНП — липопротеины низкой плотности.

INTRODUCTION

Disorders of lipid and carbohydrate metabolism are leading pathogenetic factors of metabolic syndrome (obesity, dyslipidemia, diabetes mellitus, fatty liver disease, cardiosclerosis, ischemic heart disease [IHD]), which reduce both the duration and quality of life of modern humans [1].

Obesity and overweight are a widespread problem worldwide. More than 2 billion adults (approximately 30 % of the global population) are overweight or obese. In Russia, according to literature data, the number of people with excess in 2021 was 40.3 % [2, 3]. Furthermore, overweight and obesity are established risk factors for IHD. Data from a meta-analysis on the prevalence of overweight (from 3.9 % to 29.1 %) and obesity (1.2 % to 25.3 %) in the Russian Federation in the pediatric population are particularly concerning. Exogenous-constitutional obesity is the most common form in adolescence [4].

Modern research has established that

ОБЗОР
DOI: 10.19163/2307-9266-2026-14-2-147-160

lipotoxicity accelerates apoptosis and causes inflammatory reactions, leading to chronic liver disease. In the pathogenesis of obesity, as a chronic proinflammatory disease, macrophages, oxidative stress, and hereditary predisposition play a significant role. Additionally, oxidized lipids and proteins can be cytotoxic, cause damage to membranes and membrane-bound receptors, provoke enzymatic dysfunction, disrupt signaling cascades, trigger asthma development, and activate proinflammatory processes [5, 6].

Currently, Russian scientific medicine uses about 300 species of medicinal plants. In addition to species already in demand in medicine and pharmacy, the importance of new, little-studied plant sources of biologically active compounds (BACs), including those promising for disorders of lipid and carbohydrate metabolism, should be noted [7, 8]. In this regard, plant raw materials containing phenolic compounds are of interest, as the etiology of many diseases (cardiovascular diseases, diabetes mellitus, atherosclerosis) is associated with hyperlipidemia. Hyperlipidemia is a leading factor determining the development of non-alcoholic fatty liver disease, leading to dysfunction and disruption of other bodily systems and metabolic processes [9].

According to L.V. Vasileva, et al. [10], chlorogenic and caffeic acids are involved in the regulation of adipocyte differentiation and metabolism, and chlorogenic acid is characterized by effects leading to weight loss, suppression of lipogenesis, and reduction of liver steatosis [11].

Identifying promising medicinal plants and their BACs that can exhibit hypolipidemic, hypocholesterolemic, hypoglycemic, and other types of activity is relevant. Such plants include *Phacelia tanacetifolia* Benth. (*P. tanacetifolia)*, from the *Boraginaceae* family, which is a cultivated species in the Russian Federation [12].

This plant has acclimatized in Russia and has effectively become “native,” which is due to its abundant self-seeding. One of the features of *P. tanacetifolia* is its short vegetation period. It can be grown in conditions of a short summer, therefore this plant is widely cultivated in various regions of Russia—the Omsk region and even in Siberia [13, 14].

Recently, *P. tanacetifolia* has begun to be cultivated in the North Caucasus, and especially in the Stavropol territory, where it has started being actively used as a popular honey plant [15].

In the North Caucasus, including the Caucasian Mineral Waters region, under conditions of a long, almost six-month frost-free period, *P. tanacetifolia* can yield several harvests: early sowings are possible in early April, the main vegetation and flowering period occurs in the first half of summer, late sowings are possible in June, with the main vegetation and flowering period in July–August [16].

Previously, research on this plant primarily focused on studying its morphological diagnostic features, allelopathic potential, herbicidal properties, and the use of phacelia as a honey and fodder crop. Currently, there is an increasing interest in studying the chemical composition of the above-ground part of phacelia, honey based on the plant, individual BACs contained within them, and results of studying the pharmacological activity of this promising species are emerging. In recent years, data have been obtained and published confirming the presence of aromatic acids, phenolic compounds (flavonoids, anthocyanins, hydroxycinnamic acids, tannins), amino acids (tyrosine, phenylalanine), phenylamides, and some essential minerals in the chemical composition of *Phacelia tanacetifolia*. Results have been obtained on the study of the antioxidant activity of phacelia honey [14, 17].

Given the trend of studying *P. tanacetifolia* by scientists from around the world and the discovery of new properties of the plant, we believe it is relevant to analyze the obtained information and summarize the accumulated research experience found in both scientific literature and our own work in this review.

No studies on the pharmacognostic examination of *P. tanacetifolia* cultivated in Russia have been found. The potential of using *P. tanacetifolia* from the *Boraginaceae* family as a source of medicinal plant raw material (MPRM) makes it relevant to investigate the biochemical characteristics of this plant for introduction purposes.

The use of cultivated plants as sources of MPRM ensures a stable raw material base, less variability in chemical composition, and the possibility of using mechanized sowing, processing, and harvesting.

No less important for introduced species, including *P. tanacetifolia*, is reproduction by seed, as the required amount of plant material can be obtained in the year of sowing. According to literature data and our own research, the yield of raw phytomass is established at 280–300 c/ha, and seeds at 4.5–5.5 c/ha [18–20].

The revival of medicinal plant cultivation in Russia is well-founded, as the country has significant ecologically clean territories suitable for growing about 70 % of medicinal plants for the production of phytopreparations under industrial conditions. This

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
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issue is of particular relevance in accordance with the Strategy for the Development of the Pharmaceutical Industry of the Russian Federation for the period up to 2030[1], approved by the Government of the Russian Federation, the main vector of which is the development and implementation of domestically produced medicinal drugs.

We also consider it necessary to note that the organic farming system, which is given great importance in Russia, will prevent risks of contamination of *P. tanacetifolia* raw material. The availability of domestic agricultural technology will allow influencing the quality of the harvest of the above-ground mass of *P. tanacetifolia*, the accumulation of secondary metabolites, and protect the plant from pests and diseases.

The noted characteristics allow us to consider *P. tanacetifolia* as a promising plant for industrial production.

THE AIM. To review and systematize scientific data on the chemical composition, use in medicine and pharmacy, and to justify the need for further study of the promising plant *Phacelia tanacetifolia* Benth.

MATERIALS AND METHODS

For this review, texts from available scientific information sources, located in the electronic databases eLibrary, CyberLeninka, Google Scholar, and PubMed, were analyzed. The search for publications was conducted for the period from January 2001 to January 2026, with a search depth of 25 years. The period of search and analysis of scientific literature was 23 months, with the first query made in March 2024 and the last in January 2026. To identify relevant publications, combinations of terms in Russian and English were used. During the analysis, searches were conducted using the following keywords: фацелия, Phacelia, *Phacelia tanacetifolia* Benth., фацелия пижмолистная, виды фацелии, фенольные соединения, phenolic compounds, «гидроксикоричные кислоты, флавоноиды, flavonoids, феноламиды, фенилпропаноиды, гидроксициннаматы, ожирение, гиперлипидемия.

Using the main keyword «Phacelia» in the PubMed database, with the filter set for data from 2021–2026, 103 articles were found. During the process, articles were excluded after specifying the query to «*Phacelia tanacetifolia*» (*n* = 53), removing duplicates (*n* = 10), and excluding non-full-text articles (*n* = 21). A total of 19 articles were included in the review. In the CyberLeninka database, 391 articles were found. After specifying the query («*Phacelia tanacetifolia*»), 320 articles were excluded, duplicates were removed (*n* = 15), and works not relevant to the given keyword query were discarded (*n* = 7). A total of 49 articles were selected for acceptability assessment, of which 5 sources were included in the review. For the same keyword query in the eLibrary, 1602 scientific works were found (2001–2026). After specifying the query («*Phacelia tanacetifolia*»), 1263 articles were excluded due to irrelevance to the given query (*n* = 267), duplicates were removed (*n* = 34), and non-full-text articles were excluded (*n* = 22). A total of 16 articles were studied, of which 2 scientific articles were included in the review. In the Google Scholar database, 15,300 works were found for the query «Phacelia». During the process, articles were excluded after specifying the query to «*Phacelia tanacetifolia*» (*n* = 9290), removing duplicates (*n* = 2096), discarding sources not relevant to the given query (*n* = 3150), and excluding non-full-text articles (*n* = 456). A total of 308 scientific works were analyzed, 11 of which were included in the review.

The process of selecting literature sources and preparing the review was conducted according to the PRISMA 2020[2]. Figure 1 shows a flowchart reflecting the publication search strategy.

A total of 6 492 sources of information were found, in accordance with our specified search query («фацелия» and *«Phacelia»*) after it was refined to the query *«Phacelia tanacetifolia»,* and the final number of works included in this review was 37. Data from our own research on the study of the phytochemical properties, quantitative determination of the main groups of biologically active substances of *Phacelia tanacetifolia*, and its pharmacological activity were also used; a total of 10 works were included in the review. The remaining 22 sources were used to justify the relevance of studying *Phacelia tanacetifolia*, its potential pharmacological activity, and its possible application for the treatment and prevention of diseases associated with excess body weight.

[1] Government Decree No. 1495-r dated June 7, 2023 “Strategy for the Development of the Pharmaceutical Industry of the Russian Federation for the period up to 2030”. Available from: http://static.government.ru/media/files/HqCzKkoTf7fzVdKSYbhNiZHzWTEAAQ3p.pdf. Russian

[2] The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Available from: https://www.equator-network.org/reporting-guidelines/prisma/

ОБЗОР
DOI: 10.19163/2307-9266-2026-14-2-147-160

RESULTS AND DISCUSSION

Characteristics of the families *Hydrophyllaceae* and *Boraginaceae*

To date, the status of the family *Hydrophyllaceae* is not defined and is debatable.

According to the classification of A.L. Takhtajan (1982)[3], it is an independent family belonging to the order *Polemoniales*, subclass *Asteridae*. In later classifications by A.L. Takhtajan, the family was assigned to the order *Solanales*, subclass *Lamiidae*, then to the order *Boraginales* of the same subclass[4].

Following Western systems of flowering plant classification, the family *Hydrophyllaceae* is either not distinguished at all, or is distinguished at the rank of subfamily within the family *Boraginaceae*, order *Boraginales*[5]. Plants of the World[6] also adheres to this status. Representatives of the family *Hydrophyllaceae* are exclusively American species, not found in the natural flora of the Old World.

Previously, in our works, we indicated *Phacelia tanacetifolia* as a representative of the family *Hydrophyllaceae*. However, the APG IV (Angiosperm Phylogeny Group, 4th ed.) system of flowering plants is currently considered current, according to which *Phacelia tanacetifolia* belongs to the family *Boraginaceae* [21].

Characteristics of the genus *Phacelia* Juss.

The genus *Phacelia* is the most numerous genus of the family *Boraginaceae*, including up to 209 species (according to Plants of the World).

All phacelias are annual or perennial herbs (biennials are possible) with a height of 60 cm to 120 cm. The habitat of Phacelia extends from Alaska in the north to Argentina in the south and is more concentrated in the western half of the American continent. At the same time, phacelia is not found on the Labrador Peninsula (Canada) and in the tropical forests of Brazil, Venezuela, and Colombia. The natural habitats of Phacelia are dry forests, steppes, and semi-deserts, including mountainous areas [22].

Phacelia are characterized by a cymose inflorescence—a scorpioid cyme or helicoid cyme, collected in an umbellate thyrse. The flowers are regular, the perianth is double, pentamerous, the corolla is often brightly colored in blue, purple, or pink. There are 5 stamens; in some species, the stamens protrude from the throat of the corolla. The fruit is a capsule [13, 17]. The leaves of different species of *Phacelia* vary from simple rounded leaves to complex odd-pinnately dissected leaves.

Some species of phacelia have been introduced into cultivation in Russia. Currently, 5 species are cultivated in our country: *Phacelia sericea* (Graham) A.Gray, *Phacelia campanularia* A.Gray, *Phacelia congesta* Hook., *Phacelia purshii* Buckley, and *Phacelia tanacetifolia* Benth. The first four species are known only as ornamental and are not widely cultivated [23].

Characteristics of the *Phacelia tanacetifolia* Benth.

The native region of *Phacelia tanacetifolia* is the western part of North America: the states of California, Arizona (USA), Baja California and Sonora, and adjacent territories (Mexico) [23, 24]. It grows in a dry subtropical climate. It ascends into the mountains (1500 meters above sea level). The plant collector David Douglas (1798–1834) brought the plant to Scotland from his travels in North America and California in 1832. In 1837, it was described by the English botanist G. Bentham. From England, Phacelia soon reached Germany and then spread throughout Europe, including Russia [24].

Despite the fact that the natural range of *Phacelia tanacetifolia* is located in the subtropical geographical zone, the plant is easily introduced into cultivation in countries with a temperate climate, up to the Arctic Circle. Currently, *Phacelia tanacetifolia* is distributed almost throughout the USA and most of Canada [24, 25]. It has been introduced into cultivation in almost all European countries.

Phacelia tanacetifolia is an annual herbaceous plant, the height of which can vary from 60 cm to 120 cm. The plant is completely covered with dense short and sparse long white hairs. It has an erect stem, branching in the upper part. The leaves are alternate, pinnately dissected, 8–9 cm long, 4–5.5 cm wide, with unevenly serrated-dentate margins [26, 27].

The leaves, in their shape and dissection, resemble the leaves of common tansy, which led to the specific name [28]. The inflorescence of *Phacelia tanacetifolia* can be characterized as cymose, which is typical for

[3] Plant Life: In 6 volumes. Vol. 5; part 2. Flowering plants; A.A. Fedorov, chief editor; A.L. Takhtajyan, editor. Moscow: Prosveshchenie; 1981. 511 p. Russian
[4] Takhtajan System of Angiosperm Classification; 1997.
[5] The Plant List (2013). Version 1.1. Available from: http://www.theplantlist.org/
[6] POWO (2025). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Available from: https://powo.science.kew.org/

plants of the family *Hydrophyllaceae*. The inflorescence is a thyrse of large spike-like scorpioid cymes [17, 29].

The flowers are collected in a dense one-sided inflorescence—a spike-like scorpioid cyme. The flowers are actinomorphic, sometimes slightly zygomorphic in inflorescences, the perianth is double [29]. The calyx is gamosepalous, 6–7 mm long, consisting of five sepals. The corolla is gamopetalous, 8 mm long, bell-shaped, with auricles, five petals, the corolla color is light blue-violet. It can change slightly depending on the flowering phase. There are 5 clearly visible, long stamens. The color of the stamens is similar to the color of the petals, which can be a characteristic diagnostic feature of this species [13, 17, 28, 29]. The gynoecium is syncarpous, consisting of two carpels forming a pistil. The fruit is a bivalve capsule, spherical or ovoid [13, 29].

The habit of the plants is important for raw material harvesting. *Phacelia tanacetifolia* has an erect stem, which allows for mechanized harvesting on an industrial scale. Characterizing its biological features, the plant is very productive; in our climatic conditions, it is possible to harvest raw materials twice in one summer season, as the seeds of *Phacelia tanacetifolia* have high germination energy. The high viability of the plant in the conditions of southern Russia is ensured by self-seeding, which we have repeatedly observed in the Botanical Garden of the Pyatigorsk Medical Pharmaceutical Institute — branch of Volgograd State Medical University [30].

Our study of the morphological and anatomical characteristics of the raw material—Phacelia tanacetifolia herb—allowed us to establish the main macro- and microscopic diagnostic features [31].

Chemical composition of *Phacelia tanacetifolia*

Until recently, searching for information on the chemical composition of the plant in scientific literature was a difficult task. Due to the expansion of areas where *P. tanacetifolia* is grown, not only abroad but also in Russia, researchers are increasingly studying the chemical composition of various parts of the plant.

Thus, the study of the composition of roots, leaves, stems, and flowers of *P. tanacetifolia* using high-performance liquid chromatography (HPLC) established the presence of phenolic compounds: some phenolic acids and flavonoids [32]. As a result, researchers S. Bajkacz, et al. discovered phenolic acids: gallic, caffeic, 4-hydroxybenzoic, 3,4-dihydroxybenzoic; and flavonoids: rutin, quercetin, hesperidin, naringin, eriocitrin (Fig. 2) [32]. The detection of phenolic compounds was carried out using alcoholic extracts obtained from the flowers, leaves, stems, and roots of *P. tanacetifolia*. The amount of phenolic compounds (phenolic acids and flavonoids) in the *P. tanacetifolia* extracts was 21.9 μg/g.

In all studied samples of phacelia analyzed by HPLC-MS/MS, rutin (flavonoid) and 4-hydroxybenzoic acid (phenolic acid) predominated; however, no significant difference was found in their content in flowers and leaves. The highest content of flavonoids was found in the flowers of phacelia (from 0.16 ng/g to 13.922 ng/g). The compounds with the highest concentrations in the flower samples were rutin, followed by hesperidin and neohesperidin. A higher content of phenolic acid (4-hydroxybenzoic acid) was also observed in the flowers of phacelia (from 0.80 ng/g to 4784 ng/g). Data obtained for hippuric acid, 3-hydroxybenzoic acid, and 3-hydroxypicolinic acid mainly indicated lower concentrations compared to other acids [32].

J. Kruk, et al. in their study investigated the distribution of some flavanones (eriodictyol, liquiritigenin, naringenin, hesperetin)—enantiomers in free form and bound to glycosides in different parts of *P. tanacetifolia* (Fig. 3).

As a result, the highest content of hesperetin was determined in the leaves of phacelia (0.38 μg/g), where it was present as a glycoside and only as the (S)-enantiomer [33].

The results of the analysis of the chemical composition of different parts of *P. tanacetifolia* are presented in Table 1.

Thin-layer chromatography confirmed the presence of flavonoids (rutin, quercetin), phenolic acids (caffeic, gallic, chlorogenic) in the herb of *P. tanacetifolia*. Hydrolyzable tannins and flavonoids were detected by qualitative reactions [34]. Quantitative spectrophotometric analysis allowed for the determination of the main active substances in the herb of the studied plant: flavonoids (2.3 % ± 0.17), phenolic acids (2.9 % ± 0.15), anthocyanins in flowers (0.53 % ± 0.09) [35, 36].

HPLC analysis of *P. tanacetifolia* herb was used to identify and quantify phenolic compounds (hydroxycinnamic acids—gallic (2.02 mg%), chlorogenic (9.48 mg%), ferulic (7.50 mg%), caffeic (4.27 mg%); flavonoids—quercetin (1.4 mg%), luteolin, apigenin, rutin (0.9 mg%), hyperoside; coumarins—umbelliferone) [37, 38].

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DOI: 10.19163/2307-9266-2026-14-2-147-160

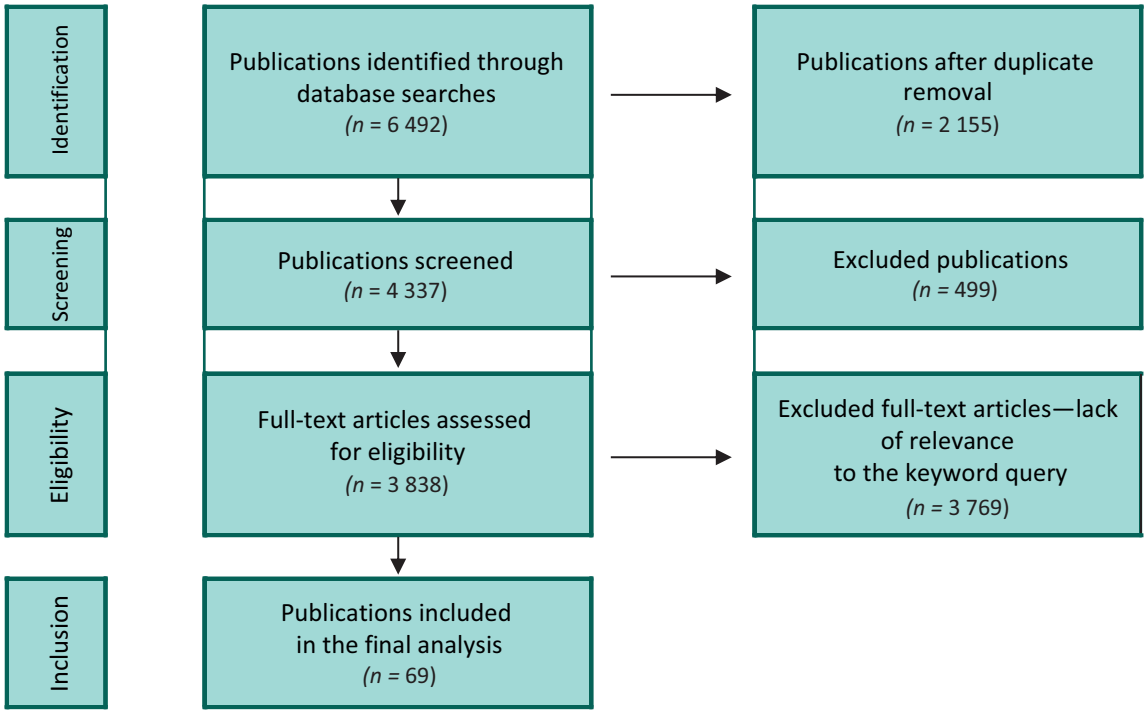

Figure 1 — Flowchart of literature source selection.

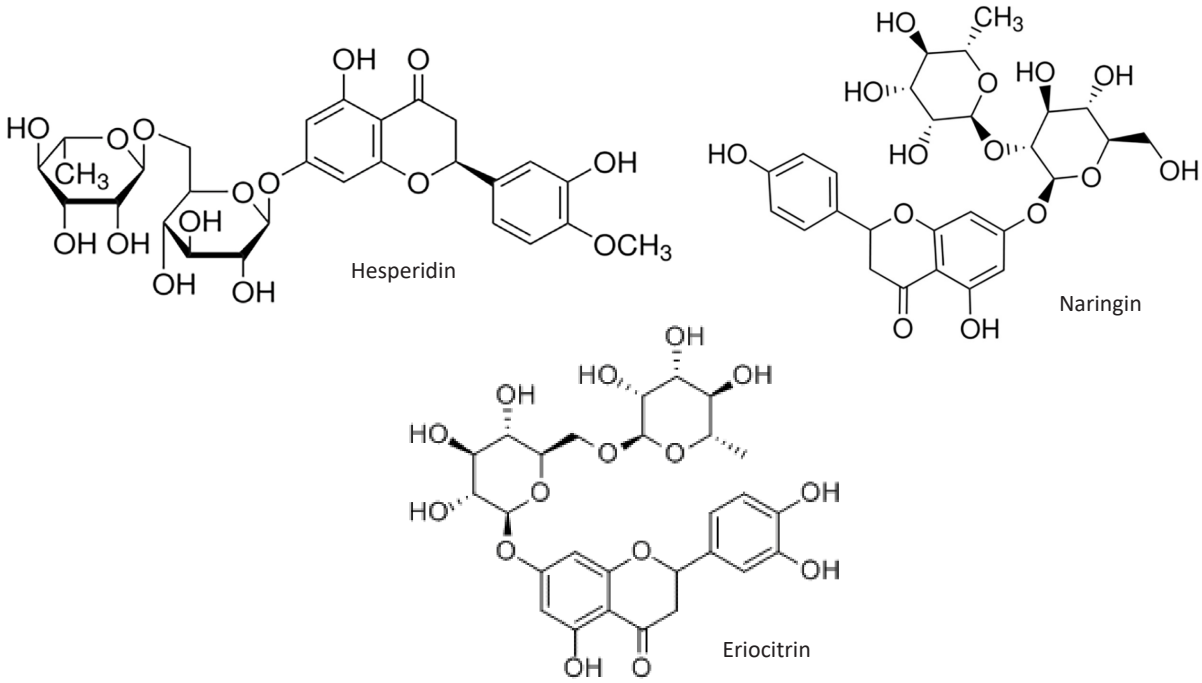

Figure 2 — Structural formulas of hesperidin, naringin, eriocitrin.

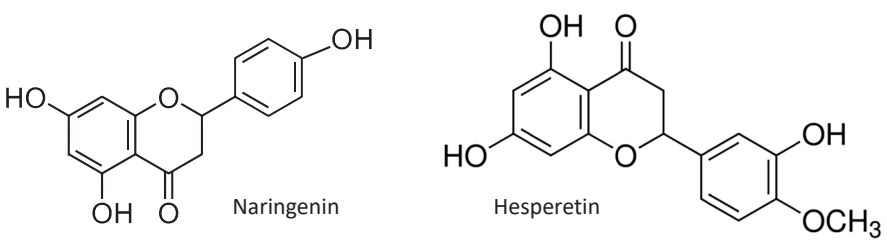

Figure 3 — Structural formulas of naringenin, hesperetin.

ISSN 2307-9266 e-ISSN 2413-2241

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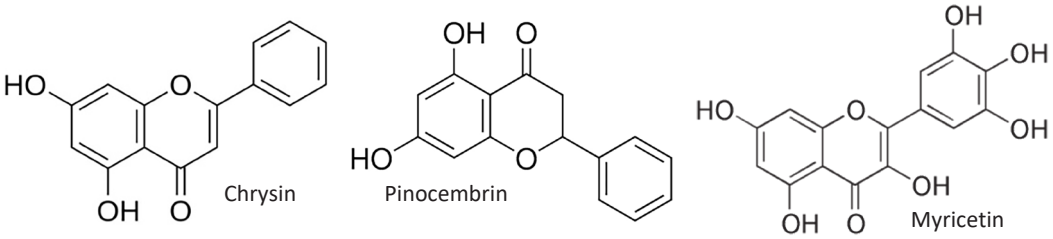

Figure 4 — Structural formulas of chrysin, pinocembrin, myricetin.

Table 1 — Content of chemical substances in different parts of *P. tanacetifolia*

Chemical composition	Concentration, ng/g^{-1}			
	Roots	Stems	Leaves	Flowers
		Aromatic acids		
3,4-DHBA (3,4-dihydroxybenzoic)	211.90 (B)	212.90 (B)	230.20 (B)	347.50 (B)
DOPAC (3,4-dihydroxyphenylacetic)	380.60 (B)	1199.0 (B)	1956.0 (B)	868.90 (B)
4-HBA (4-hydroxybenzoic)	4049.0 (B)	3809.0 (B)	3915.0 (B)	4784.0 (B)
CA (caffeic acid)	1444.0 (B)	873.0 (B)	911.90 (B)	404.10 (B)
HA (hippuric acid)	0.73(B)	0.76 (B)	27.10 (B)	0.80 (B)
3-HBA (3-hydroxybenzoic)	2.47(B)	0.27 (B)	0.34 (B)	21.60 (B)
3-HPA (3-hydroxypicolinic)	0.22 (B)	0.20 (B)	1.37 (B)	7.90 (B)
HVA (4-hydroxy-3-methoxyphenylacetic)	43.50 (B)	25.0 (B)	13.0 (B)	24.10 (B)
3,4-HPPA (4-hydroxyphenylpyruvic)	237.20 (B)	141.40 (B)	161.50 (B)	77.90 (B)
p-CA (para-caffeic acid)	324.80 (B)	11778.0 (B)	297.90 (B)	173.40 (B)
FA (ferulic acid)	823.80 (B)	608.10 (B)	610.10 (B)	385.60 (B)
BA (бензойная кислота)	197.30 (B)	170.90 (B)	360.90 (B)	648.50 (B)
		Flavonoids		
ERC (eriocitrin)	–	–	3,09 (B)	3,09 (B)
ERI (eriodictyol)	0.19 (B)	2.50 (B)	25.80 (B)	21.30 (B)
FIS (fisetin)	–	–	–	14.70 (B)
HSD (hesperidin)	0.97 (B)	57.60 (B)	66.50 (B)	93.10(B)
HST (hesperetin)	0.42 (B)	–	0.67 (B)	0.16 (B)
NAR (naringenin)	1.00 (B)	0.99 (B)	1.88 (B)	1.60 (B)
NARG (naringin)	–	0.31 (B)	–	0.78 (B)
NHSD (neohesperidin)	0.52 (B)	34.10 (B)	22.20 (B)	62.10 (B)
NRI (narirutin)	4.75 (B)	7.60 (B)	3.60 (B)	7.80 (B)
PIN (pinocembrin)	–	–	0.33 (B)	0.24 (B)
QUE (quercetin)	0.27 (B)	74.30 (B)	58.10 (B)	20.60 (B)
R-ERI (R-enantiomer of eriodictyol)	–	365.0 (K)	2574.0 (K)	1502.0 (K)
R-NAR (R-enantiomer of naringenin)	–	141.0 (K)	230.0 (K)	311.0 (K)
RUT (rutin)	2336.0 (B)	1129.0 (B)	10296.0 (B)	13992.0 (B)
S-ERI (S-enantiomer of eriodictyol)	–	460.0 (K)	4752.0 (K)	4461.0 (K)
S-HST (S-enantiomer of hesperetin)	–	210.0 (K)	380.0 (K)	244.0 (K)
S-NAR (S-enantiomer of naringenin)	–	724.0 (K)	1298.0 (K)	1656.0 (K)
TAX (dihydroquercetin)	4.00 (B)	1.59 (B)	5.90 (B)	0.79 (B)

Note: The table is compiled according to S. Bajkacz et al. [32] — (B), and J. Kruk et al. [33] — (K).

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Scientific works on the study of *P. tanacetifolia* by Russian researchers are increasingly encountered. For example, D.N. Olennikov, et al., using HPLC analysis with a photodiode detector and a time-of-flight mass detector (HPLC-DAD-TOF-MS), discovered flavonoids, hydroxycinnamates, and phenolidamides in the herb of *P. tanacetifolia* [14]. Among the flavonoids, tiffaneoside, kaempferol 3-O-neohesperidoside, calendoflavoside, isoquercitrin, nicotiniflorin, narcissin, astragalin, isorhamnetin 3-O-glucoside, isoorientin, cosmosiin, and quercetin 3'-O-glucoside were identified for the first time. The hydroxycinnamates found in the herb of *P. tanacetifolia* were cinnamoylquinic acids (monocaffeoylquinic: 1-O-caffeoylquinic (trans-), 4-O-caffeoylquinic (trans-), 5-O-caffeoylquinic (trans-), 3-O-caffeoylquinic (trans-), 5-O-caffeoylquinic (cis-) and monoferuloylquinic: 1-O-feruloylquinic (trans-), 4-O-feruloylquinic (trans-), 5-O-feruloylquinic (trans-), 5-O-feruloylquinic (cis-), 3-O-feruloylquinic (cis-). Among the identified phenolidamides, spermidine derivatives (safflospermidins A and B) can be distinguished, and a new phenolidamide, phaceliazide, was isolated, which had the highest content [14].

Quantitative assessment of these groups of substances in 8 domestic varieties of Phacelia tanacetifolia allowed for the determination of the content of flavonoids (from 0.99 to 3.61 mg/g), phenylpropanoids (from 1.53 to 15.69 mg/g), hydroxycinnamates (from 0.57 to 5.71 mg/g), and phenolidamides (from 0.89 to 9.5 mg/g) [14].

Phacelia tanacetifolia is a honey plant

Phacelia honey is highly valued for its aroma and taste. The valuable properties of phacelia honey are known worldwide. According to research on this product, the main natural compounds responsible for its therapeutic activity have been identified, its mineral composition is known, and the properties of phacelia honey have been described in detail [39, 40–42].

The antioxidant, antibacterial, antiviral, anti-inflammatory, antithrombotic, and antiallergic properties of honey are explained by many factors, such as pH, sugar content, hydrogen peroxide level, and phenolic compound content, most of which are present as flavonoids [26]. The beneficial effect of flavonoids on human health is due to their antioxidant activity against divalent transition metal cations involved in radical formation processes [26, 43, 44]. Foreign researchers have studied the antioxidant activity of *P. tanacetifolia* pollen [45]. Using spectrophotometry, they determined the total content of phenols and flavonoids and calculated the index of relative antioxidant capacity. The study found that phacelia pollen exhibits a high level of antioxidant activity, which the authors attribute to the presence of phenolic compounds [45]. The main compounds responsible for the antioxidant activity of honey are flavonoids (chrysin, pinocembrin, quercetin, galangin, kaempferol, hesperidin, and myricetin), phenolic acids (caffeic, coumaric, ellagic, ferulic, and chlorogenic acids), ascorbic acid, catalase, peroxidase, and carotenoids (Fig. 4) [43, 44].

Studies of the chemical composition of honey were conducted in Poland. In 2019, an analysis of the chemical composition of phacelia honey was carried out, which qualitatively and quantitatively detected phenolic acids: gallic, caffeic, ferulic, chlorogenic, as well as flavonoids: quercetin, kaempferol, myricetin, naringenin, apigenin [13]. The compounds with the highest content in honey were quercetin (0.293 ± 0.008 mg) and kaempferol (0.304 ± 0.036 mg) per 100 g of honey. They also studied the antioxidant activity of phacelia honey using a spectrophotometric method based on the interaction of antioxidants with the stable chromogen radical 2,2-diphenyl-1-picrylhydrazyl. Ultra-HPLC method allowed to identify the following chemical substances in phacelia honey: 6 nitrogen compounds, including aromatic amino acids (tyrosine [14.66 ± 10.22 mg/kg], phenylalanine [20.41 ± 11.99 mg/kg]), purine derivatives (adenine [18.45 ± 4.63 mg/kg], xanthine [10.53 ± 2.98 mg/kg]), nucleoside uridine (42.84 ± 9.26 mg/kg) [39].

The main component of Hungarian phacelia honey is the flavanone hesperidin, and the mineral composition is represented by potassium (102–130 mg/kg), magnesium (4.09–5.16 mg/kg), calcium (9.12–12.5 mg/kg), and sodium (3.02–3.81mg/kg) [41–43].

The use of phacelia honey is mentioned in Chinese traditional medicine. It was used there as a diuretic, disinfectant, and for the treatment of burns [46]. Phacelia honey has estrogenic effects, a strong rejuvenating effect, and also maintains blood cholesterol levels [46–48]. Honey based on phacelia is especially valued by healers in Western Siberia. Phacelia honey is used in folk medicine for the treatment of gastrointestinal diseases, cardiovascular system diseases, for the normalization of metabolic processes, strengthening immunity, and as a general tonic [49–51].

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
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Potential pharmacological activity of *Phacelia tanacetifolia*

No scientific data on the pharmacological activity of *P. tanacetifolia* has been found. However, based on the available information on the study of the antioxidant activity of phacelia honey, as well as data on the use of honey in folk medicine as a remedy for cardiovascular diseases and for lowering cholesterol levels, it can be stated that *P. tanacetifolia* is a potential source of BACs with hypolipidemic and antioxidant activity.

It has been established by many scientists that the most pronounced antioxidant, hepatoprotective, and antitoxic effects are characteristic of a complex of BACs from MPRMs where phenolic compounds, including hydroxycinnamic acids and flavonoids, are the dominant components [44, 52, 53]. The mechanism of the therapeutic effect of chlorogenic acid in animals with progressive alcoholic steatohepatitis (administration of ethanol at a dose of 4 g/kg for 8 weeks) and a high-fat diet has been established due to its antioxidant and anti-inflammatory effects [54].

It is known that chlorogenic acid, by reducing the level of malondialdehyde in blood plasma and in low-density lipoproteins (LDL), reduces the susceptibility of LDL to oxidation, thereby lowering the risk of cardiovascular diseases [55, 56].

There is information that various flavonoids reduce LDL oxidation, inhibit platelet aggregation, slow the rate of atherosclerotic plaque formation, reduce the expression of adhesion molecules in endothelial cells, cause vasodilation, and lower blood pressure [57, 58].

These assumptions about the potential pharmacological activity of *P. tanacetifolia* are confirmed by pharmacological studies [59]. It has been shown that prophylactic administration of *P. tanacetifolia* extract in a model of acute hyperlipidemia induced by ethanol demonstrates a hypolipidemic effect, due to a decrease in triglycerides and total cholesterol in blood serum and liver homogenate [60]. *P. tanacetifolia* extract has an anti-atherogenic effect, inhibiting the humoral manifestation of athero-arteriosclerosis: it reduces hyperlipidemia, prevents the activation of lipid peroxidation, apparently by enhancing the antioxidant system and providing a protective effect on local vascular mechanisms of atherogenesis [60].

Excess body weight and obesity in humans can reflect modern negative processes—early disqualification for certain professions, an expanding spectrum and rejuvenation of diseases, and a reduction in active professional activity. Based on the foregoing, it is advisable to use current approaches of modern medicine, the concept of occupational health, which is based on the principle of prevention, contributing to the solution of the main task—extending professional longevity.

In this regard, the study of *P. tanacetifolia* as a potential source of pharmaceutical substances with hypolipidemic and hepatoprotective activity is of both scientific and practical interest. Information about the potential pharmacological activity of *P. tanacetifolia* can be the basis for the development of medicines as well as dietary supplements (DSs), the market for which is continuously growing [61].

Phacelia tanacetifolia is a green manure

As noted by A. Schappert, et al., *P. tanacetifolia* covers the soil more effectively when grown as a monoculture rather than in mixtures, which greatly influences the reduction of surface erosion [62]. Furthermore, it suppresses weed growth [63] and improves soil structure [64]. N. Tursun, et al. proved that *P. tanacetifolia* as a cover crop in apricot orchards eradicates weeds by almost 75 % [65]. Live phacelia is less effective than, for example, glyphosate or mechanical weed control, but after mowing or plowing, it is more effective than these treatments[7]. Some authors indicate that a plot after *P. tanacetifolia*, compared to white mustard (*Sinapis alba* L.), is characterized by a greater number and biodiversity of associated plants, for example, in organic oat cultivation [66]. Based on the study of drought stress in plants in a greenhouse experiment, it was found that *P. tanacetifolia* has much higher resistance to reduced water content compared to *Sinapis alba* L. and *Avena strigosa* Schreb. (bristly oat) [67, 68]. M. Handlířová, et al. found that in an agroecosystem with a high average annual temperature and low rainfall, *P. tanacetifolia* achieves higher and more stable yields compared to buckwheat (*Fagopyrum esculentum* Moench.) [69].

[7] Lipinski M. Pożytki pszczele. Zapylanie i miododajność roślin; PWRiL: Warszawa, Poland; 2010.

DOI: 10.19163/2307-9266-2026-14-2-147-160

CONCLUSION

MPRMs containing phenolic compounds, as well as preparations based on them, are of particular importance in phytotherapy due to their wide range of applications and broad spectrum of therapeutic activity. According to the scientific data studied and summarized in the article, *P. tanacetifolia* is a promising plant for study. The effectiveness of extracts obtained from Phacelia MPRMs has been proven, namely antioxidant, hypolipidemic, and hepatoprotective activities. *P. tanacetifolia* can become a valuable source of BACs used for the treatment of major cardiovascular diseases (atherosclerosis, etc.) and liver diseases. Furthermore, the plant's value as a honey plant and green manure indicates the need for further study of *P. tanacetifolia*.

Considering the information from the provided literature, it should be noted that the chemical composition and spectrum of biological activity of the main BAS of *P. tanacetifolia* can serve as a basis for the development of new medicines with predictable pharmacological properties for the prevention and treatment of socially significant diseases.

FUNDING

This study did not have financial support from third-party organizations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Patimat A. Sheykhmagomedova — data curation, formal analysis, visualization, writing—original draft;
Olga I. Popova — conceptualization, data curation, formal analysis, visualization, writing—review & editing;
ivan V. Popov — data curation, formal analysis, visualization, writing—original draft, writing—review & editing.
All authors made an equivalent and equal contribution to the preparation of the publication. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept and preparation of the article, read and approved the final version before publication).

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2026-14-2-161-174

Antidepressant activity of extracts from the herbs *Astragalus varius* and *Astragalus testiculatus* in the "Tail suspension test"

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Received 21 Nov 2024 After peer review 28 Dec 2025 Accepted 02 March 2026

The aim. To determine the content of flavonoids in aqueous and aqueous-alcoholic extracts from the herbs of *Astragalus varius* and *Astragalus testiculatus* and to investigate the antidepressant effect of the extracts *in vivo*.

Materials and methods. The objects of the study were dried and ground herbs of *Astragalus varius* S.G. Gmel. and *Astragalus testiculatus* Pall., collected in the Saratov region during the period of mass flowering (May–June 2021). Aqueous (1:10) and aqueous-alcoholic (1:10, extractant 70% ethanol) extracts were obtained from the raw material. The flavonoid content was determined by differential spectrophotometry at an analytical wavelength of 410 nm in quartz cuvettes with *l*=1 on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan). The study of antidepressant activity was conducted on male mice weighing 32–38 g and aged 2–3 months using the Tail Suspension Test (TST). The animals received the studied extracts at a dose of 100 mg/kg, and amitriptyline at a dose of 10 mg/kg served as the comparison drug. For data evaluation, the Mann-Whitney U-test and Bonferroni correction (p <0.01) were used. To study the strength of the linear relationship between antidepressant activity and flavonoid content, correlation analysis was used (Spearman correlation coefficient at p <0.05).

Results. The flavonoid content in aqueous and aqueous-alcoholic extracts from the herb of *Astragalus varius* was 2.54 ± 0.04% and 9.31 ± 0.07%, respectively, and in extracts from the herb of *Astragalus testiculatus* — 1.06 ± 0.05% and 10.34 ± 0.05%, respectively. The aqueous-alcoholic extract of *Astragalus testiculatus* demonstrated a pronounced effect reliably similar to amitriptyline (p = 0.01) both after a single oral administration and throughout the entire experimental period (21 days). The aqueous-alcoholic extract of *Astragalus varius* did not show an antidepressant effect after a single administration; however, on days 8, 15, and 21 of administration, a significant (p = 0.01) effect was observed in the animals. Upon administration of the aqueous extract of *Astragalus varius*, an antidepressant effect was observed on days 1, 15, and 21 of the study (p = 0.01); however, the effect was absent on day 8 of the experiment. The aqueous extract of *Astragalus testiculatus*, both after single and chronic oral administration of the extract to animals, showed no activity in the experiment (p >0.01).

Conclusion. Aqueous-alcoholic extracts from the herbs of both species exhibited a more pronounced antidepressant effect compared to aqueous extracts. Correlation analysis established that the identified antidepressant activity is associated with the flavonoid content in the studied extracts.

Keywords: extract; flavonoids; antidepressant activity; *Astragalus varius* S.G. Gmel.; *Astragalus testiculatus* Pall.

Abbreviations: TST — Tail Suspension Test; WHO — World Health Organization; BACs — biologically active compounds; SPh RF XV ed. — State Pharmacopoeia of the Russian Federation XV edition; PhM — pharmacopoeial monograph; SS — standard sample; ROSs — reactive oxygen species; SOD — superoxide dismutase; HPA axis — hypothalamic-pituitary-adrenal axis.

For citation: U.A. Matvienko, A.Yu. Karetnikova, N.A. Durnova. Antidepressant activity of extracts from the herbs *Astragalus varius* and *Astragalus testiculatus* in the "Tail suspension test". *Pharmacy & Pharmacology.* 2026;14(2):161-174. **DOI:** 10.19163/2307-9266-2026-14-2-161-174

Для цитирования: У.А. Матвиенко, А.Ю. Каретникова, Н.А. Дурнова. Антидепрессивная активность экстрактов из травы астрагала изменчивого и астрагала яйцеплодного в тесте «Подвешивание за хвост». *Фармация и фармакология.* 2026;14(2):161-174. **DOI**: 10.19163/2307-9266-2026-14-2-161-174

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Антидепрессивная активность экстрактов из травы астрагала изменчивого и астрагала яйцеплодного в тесте «Подвешивание за хвост»

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Получена 21.11.2024 После рецензирования 28.12.2025 Принята к печати 02.03.2026

Цель. Определить содержание флавоноидов в водных и водно-спиртовых экстрактах из травы астрагала изменчивого и астрагала яйцеплодного и *in vivo* исследовать антидепрессивный эффект экстрактов.

Материалы и методы. Объектами исследования были высушенная и измельчённая трава астрагала изменчивого (*Astragalus varius* S.G. Gmel.) и астрагала яйцеплодного (*Astragalus testiculatus* Pall.), заготовленная на территории Саратовской области в период массового цветения (май–июнь 2021 года). Из сырья были получены водные (1:10) и водно-спиртовые (1:10, экстрагент 70% этанол) извлечения. Содержание флавоноидов определяли методом дифференциальной спектрофотометрии при аналитической длине волны 410 нм в кварцевых кюветах *l*=1 на спектрофотометре Shimadzu UV-1800 (Shimadzu, Япония). Исследование антидепрессивной активности проводили на мышах-самцах массой 32–38 г и возрастом 2–3 мес с помощью теста «Подвешивание за хвост/Tail Suspesion Test» (TST). Исследуемые экстракты животные получали в дозе 100 мг/кг, препаратом сравнения служил амитриптилин в дозе 10 мг/кг. Для оценки данных использовали U-критерия Манна-Уитни, поправку Бонферрони (p <0,01). Для изучения тесноты линейной связи между антидепрессивной активностью и содержанием флавоноидов использовали корреляционный анализ (коэффициент корреляции Спирмена при p <0,05).

Результаты. Содержание флавоноидов в водных и водно-спиртовых экстрактах из травы астрагала изменчивого составило 2,54±0,04 и 9,31±0,07% соответственно, а в экстрактах из травы астрагала яйцеплодного — 1,06±0,05 и 10,34±0,05% соответственно. Водно-спиртовой экстракт астрагала яйцеплодного демонстрировал достоверно аналогичный амитриптилину выраженный эффект (p=0,01) как после однократного перорального введения, так и на протяжении всего периода эксперимента (21 день). Водно-спиртовой экстракт астрагала изменчивого не проявлял антидепрессивный эффект после однократного введения, однако на 8, 15 и 21 сутки приёма наблюдалось достоверное (p=0,01) проявление эффекта у животных. При введении водного экстракта астрагала изменчивого наблюдался антидепрессивный эффект на 1, 15 и 21 сутки исследования (p=0,01), однако эффект отсутствовал на 8 день эксперимента. Водный экстракт астрагала яйцеплодного, как после однократного, так и после хронического перорального введения экстракта животным не показал активности в эксперименте (p >0,01).

Заключение. Водно-спиртовые экстракты из травы обоих видов обладали более выраженным антидепрессивным эффектом по сравнению с водными. С помощью корреляционного анализа установлено, что выявленная антидепрессивная активность связана с содержанием флавоноидов в исследуемых извлечениях.

Ключевые слова: экстракт; флавоноиды; антидепрессивная активность; *Astragalus varius* S.G. Gmel.; *Astragalus testiculatus* Pall.

Список сокращений: TST — тест «Подвешивание за хвост»; ВОЗ — Всемирная организация здравоохранения; БАС — биологически активные соединения; ГФ РФ XV изд. — Государственная фармакопея Российской Федерации XV издания; ФС — фармакопейная статья; СО — стандартный образец; АФК — активные формы кислорода; СОД — супероксиддисмутаза; ГГНС — гипоталамо-гипофизарно-надпочечниковая система.

INTRODUCTION

Globally, approximately 350 million people suffer from depression, and Russia ranks 4th in the world in terms of disease prevalence (38 % of the population across different age groups), according to an assessment by the World Health Organization (WHO) as of 2021. Synthetic antidepressants are used for the therapy of depressive disorders, but their use

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DOI: 10.19163/2307-9266-2026-14-2-161-174

often leads to the development of not only therapeutic but also undesirable effects [1–3]. It has been found that pharmacotherapy with antidepressants is ineffective in one-third of patients due to emerging side effects [4–6].

Phytopreparations can be used for the treatment of mild to moderate depressive states [2, 7]. Medicines of St. John's Wort (*Hypericum perforatum* L.) have shown the greatest efficacy in treating mild depression in adults [8, 9]. Given that biologically active components isolated from St. John's Wort herb are well-tolerated, drugs based on them can be recommended for long-term use, including for maintenance therapy [8]. Antidepressant activity has also been identified in other plants, for example, in *Crataegus submollis* Sarg. [9], *Anisum vulgare* Goerth. [10], *Rhodiola rosea* L. [11], *Magnolia grandiflora* L. [12], and others.

One of the promising sources of biologically active compounds (BACs) are plants of the genus *Astragal*, which comprises over 3000 species. The most studied is *Astragalus membranaceus* (Fisch.) Bunge. Drugs from the roots of *Astragalus membranaceus* are included in the State Pharmacopoeia of China and are used for various diseases as immunomodulatory, cardioprotective, and antitumor agents [13–16].

Individual components and complex extracts obtained from some *Astragalus* species exhibit neuroprotective activity [17]. The neuroprotective effect of a methanolic extract from the shoots of *Astragalus spinosus* against bisphenol A-induced anxiety and depression in a rat model of postnatal Schizophrenia is known [18]. The combination of anxiolytic and potential antidepressant effects of *Astragalus membranaceous var. A. mongholicus* was similar to the action of a benzodiazepine derivative—alprazolam, but demonstrated some differences from alprazolam, including the absence of sedative effects and amnesia [19]. A comprehensive assessment of animal behavior parameters in the "Suck Test" showed that administration of an extract from the herb *Astragalus vulpinus* Willd under conditions of informational stress had a corrective effect on the psychoemotional status, which manifested in the activation of the orienting-exploratory component of behavior, as well as in the elimination of anxiety-depressive behavioral disorders in white rats [20].

Despite the active study of plants of the genus *Astragal*, scientific data is currently insufficient. Furthermore, the lack of data on the chemical composition and biological activity of most representatives of the genus provides a basis for their research and defines the relevance of this direction.

Astragalus varius S.G. Gmel. and *Astragalus testiculatus* Pall. are widely distributed in the Volga region. Extracts from *Astragalus varius* and *Astragalus testiculatus* have shown pronounced antimicrobial activity against *S. aureus, E. coli, P. aeroginosa* [21] and antioxidant activity based on the level of inhibition of adrenaline hydrochloride auto-oxidation in an alkaline medium [22], as well as low toxicity in in vivo experiments [23]. The diuretic effect of an infusion of *Astragalus varius* herb in a 4-hour experiment exceeded the diuretic effect of furosemide at a threshold dose of 1 mg/kg, and in a 24-hour experiment was slightly lower than that of hydrochlorothiazide at a medium therapeutic dose of 20 mg/kg [24].

Phytochemical studies have revealed the presence of flavonoid compounds with potential antidepressant activity. Thus, isoquercitrin, rutin, hyperoside, narcissin, cynaroside, and astragalin were found in the herb of *Astragalus varius*, and rutin, cynaroside, and astragalin were found in the herb of *Astragalus testiculatus* [25]. It was previously established that the antidepressant effect of St. John's Wort herb-based medicines is primarily associated with the action of flavonoids—hyperoside and bisapigenin [9]. Additionally, antidepressant properties of another flavonoid glycoside—astragalin—are known [26]. The antidepressant activity of extracts from the herbs *Astragalus varius* and *Astragalus testiculatus* has not been previously studied, which is of interest for investigation.

THE AIM. To determine the flavonoid content and investigate the antidepressant activity of aqueous and aqueous-alcoholic extracts from the herbs *Astragalus varius* and *Astragalus testiculatus*.

MATERIALS AND METHODS

Experimental design

The experimental design is presented in Figure 1.

Preparation of active substances

For the study of biological activity, aqueous and aqueous-alcoholic extracts from the herb of two species of *Astragalus* were selected: *Astragalus varius (A. varius* S.G. Gmel.) and *Astragalus testiculatus* (*A. testiculatus* Pall.).

The plant raw material was collected during the period of mass flowering in the Saratov region in May–June 2021. Drying was carried out by air-shade method to a residual moisture content of no more than 12 %.

Aqueous extracts (1:10) were prepared to the method described in GPhM.1.4.1.0018 "Infusions and Decoctions"[1] of the State Pharmacopoeia of the Russian

[1] GPhM.1.4.1.0018 "Infusions and decoctions". State Pharmacopoeia of the Russian Federation XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-4/1-4-1-lekarstvennye-formy/nastoi-i-otvary/. Russian

Federation, XV edition (SPh RF XV ed.); aqueous-alcoholic extracts (70 % ethanol) were obtained by maceration for 6 days in a raw material-to-extractant ratio of 1 : 10. The extracts were filtered, concentrated to the state of a thick extract, after which the residue was dried in a drying oven at 40 °C to constant weight.

To assess antidepressant activity, the obtained dry residues were dissolved in distilled water to obtain a concentration of 100 mg/mL.

Phytochemical analysis

To assess the content of the sum of flavonoids, the method of differential spectrophotometry calculated as rutin [27] was used. For this, 0.1 g of dry residue was dissolved in 10 mL of 70 % ethyl alcohol in a 25 mL volumetric flask (solution A). Then, 1 mL of solution A was placed in a 25 mL volumetric flask, 5 mL of 5% aluminum (III) chloride solution and 0.5 mL of acetic acid solution were added. The analysis was performed after 30 min on a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan) in quartz cuvettes with a layer thickness of 10 mm at an analytical wavelength of 410 nm relative to a reference solution (1 mL of solution A without the addition of a complexing agent). Under similar conditions, the optical density of a rutin standard sample of 0.05 % was determined.

Preparation of Rutin Solution. Approximately 0.05 g (exact weight) of rutin standard (≥ 95 %, No. 89270, lot No. 66853802, PhytoLab, Germany) was placed in a 100 mL volumetric flask, 85 mL of 70 % alcohol was added, and heated in a water bath until completely dissolved. Then, it was cooled, the volume of the solution was brought to the mark with the same solvent, and mixed. The shelf life of the solution is 30 days when stored in a well-sealed container, in a cool, light-protected place.

The content of flavonoids calculated as rutin was determined by the formula:

$$X(\%) = \frac{A \times 25 \times m_0 \times 100}{A_0 \times m \times (100 - W)},$$

where A is the optical density of the test solution; A_0 is the optical density of the rutin standard solution; m is the mass of the extract, g; m_0 is the mass of the rutin standard, g; W is the loss on drying, %.

Experimental animals

Antidepressant activity was determined on 36 outbred male mice housed in the vivarium of the common use center for experimental oncology of the Saratov State Medical University named after V.I. Razumovsky, weighing 32–38 g and aged 2–3 months. The animals were kept under standard vivarium conditions with a 12-hour light cycle, constant temperature and humidity, with free access to food and water.

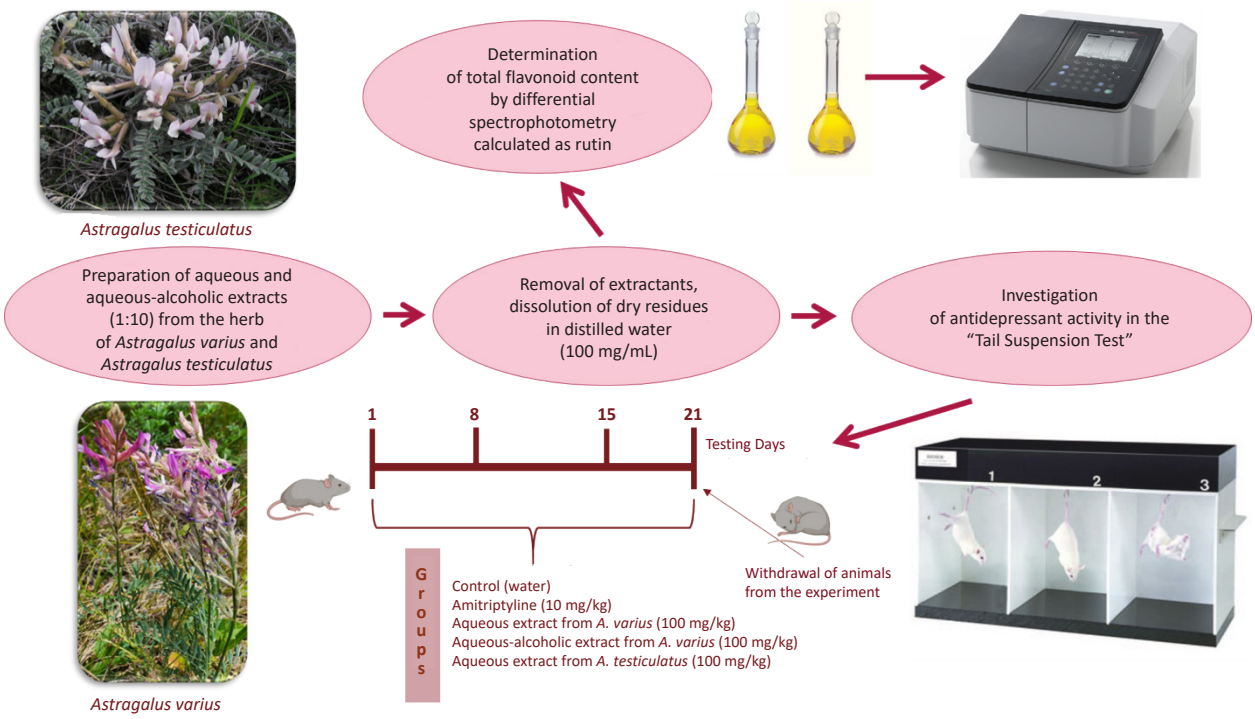

Figure 1 — Experimental design.

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The design of the experimental study was approved by the Local Ethics Committee of the Saratov State Medical University named after V.I. Razumovsky (Protocol No. 4 dated Feb 01, 2022).

The study groups are presented in Table 1 (each group received a daily intragastric administration of the test substance solution using a probe). Amitriptyline was chosen as the comparison drug at a therapeutic dose of 10 mg/kg (average 0.35 mg per day; OZON PHARM LLC, expiry date 12.2023) [28], and the test extracts were used at a dose of 100 mg/kg (average 3.5 mg per day). The choice of dose for the test extracts is due to the fact that antimicrobial and diuretic activity were previously found in extracts from the herb of *Astragalus varius* and *Astragalus testiculatus* at the indicated dose [25, 28]. We previously identified the effect of an aqueous extract from the herb of *Astragalus membranaceus* at a dose of 100 mg/kg on the cognitive functions of rats in the "eight-arm radial maze" test, in which the infusion of *Astragalus membranaceus* caused activation of working and long-term spatial memory [29].

Antidepressant activity

To assess the antidepressant activity of the test substances, the "Tail Suspension Test" (TST; OpenScience, Russia) was used, which was conducted on days 1, 8, 15, and 21 of the experiment. During the test, mice were suspended by their tails on sticky tape; the testing duration was 3 minutes.

To assess behavior, the following parameters were recorded: total time of activity maintenance (s), total duration of immobility (s), latent period of the first episode of immobility (s) [30].

Laboratory animals were euthanized at the end of the 21st day by overdose of anesthetic drugs — an intraperitoneal combination of Zoletil (tiletamine 250 mg and zolazepam 250 mg; (Virbac, France) and Xylanit (xylazine; Nita-Pharm, Russia) at a dose of 0.1 mg/kg.

Statistical analysis

Statistical analysis of the results was carried out using the Statistica 10.0 software package (StatSoft Inc., USA). Sample normality of distribution was checked using the Shapiro-Wilk test. During statistical processing of the study results, the distribution of trait values differed from normal, therefore, the Mann-Whitney U-test was used to evaluate the data with recalculation of the significance level (Bonferroni correction was applied) considering multiple comparisons (5)— $p < 0.01$. For each indicator, the median (Me) and interquartile range [Q1; Q3] were calculated. To study the strength of the linear relationship between indicators, correlation analysis was used—the Spearman correlation coefficient was calculated. With positive r values, a direct relationship was identified; with negative values, an inverse relationship; with 0, no relationship. The strength of the relationship was assessed by the values of the r coefficient (from 0 to 0.3—very weak, from 0.3 to 0.5—moderate, 0.5–0.7—medium, 0.7–0.9—high, 0.9–1.0—very high). Results were considered significant at $p < 0.05$.

RESULTS

Phytochemical analysis

The study of the electronic spectra of aqueous and aqueous-alcoholic extracts from the herb of *Astragalus* species (Fig. 1) showed the presence of two absorption bands with maxima at 270 nm and 330 nm, characteristic of flavonoids. Upon addition of 5 % aluminum (III) chloride solution, a bathochromic shift to the long-wavelength region of approximately 70 nm was observed, and under differential spectrophotometry conditions, the maximum absorption of the long-wavelength band was recorded in the range of 402–409 nm (Fig. 2). Rutin (λmax = 410 ± 0.2 nm) was chosen as the standard sample.

The results of determining the flavonoid content calculated as rutin are presented in Table 2.

It was found that the flavonoid content in the aqueous-alcoholic extract from *Astragalus ovatus* herb (10.34 ± 0.05 %) is higher than in the aqueous-alcoholic extract from Astragalus varius herb (9.31 ± 0.07 %). The sum of flavonoids in the aqueous extract of *Astragalus ovatus* (1.06 ± 0.05 %) is 2 times less than in the aqueous extract of *Astragalus varius* (2.54 ± 0.04 %).

Antidepressant activity

The results of the study of antidepressant activity over 21 days of the experiment are presented in Tables 3–6.

In animals in the group receiving amitriptyline (10 mg/kg) throughout the 21-day experiment (Group 2), a pronounced antidepressant effect was observed. The time of activity maintenance was longer than in mice in the control group: day 1 by 98.5% ($p = 0.02$) (see Table 3), day 8 by 105.9% ($p = 0.004$) (see Table 4), day 15 by 91.6 % ($p = 0.004$) (see Table 5), day 21 by 139.1 % ($p = 0.011$) (see Table 6), and the duration of immobilization was shorter: day 1 by 59.4 % ($p = 0.025$) (see Table 3), day 8 by 93.2 % ($p = 0.004$) (see Table 4), day 15 by 78.5 % ($p = 0.004$) (see Table 5), day 21 by 80.6 % ($p = 0.01$) (see Table 6). The latent period of the first episode of immobilization did not show statistically significant differences from the control group ($p > 0.01$).

Table 1 — Experimental animal groups in the "Tail Suspension Test"

Group No.	1	2	3	4	5	6
Name	Control (Water)	Amitriptyline	Aqueous extract of *A. varius*	Alcoholic extract of *A. varius*	Aqueous extract of *A. testiculatus*	Alcoholic extract of *A. testiculatus*
Dose, mg/kg	–	10	100	100	100	100
Dose per day, mg	–	0.35	3.5	3.5	3.5	3.5

Table 2 — Results of determining the flavonoid content in extracts from *Astragalus varius* and *Astragalus ovatus* herbs in % (P=0.95; *n*=3)

Extract name	Average value, $\overline{X}$ (%)	Variance, S^2	Standard deviation (S_x), *SD*	Standard deviation of the mean result, $S_{\overline{x}}$	Relative standard deviation, RSD (%)	Deviation from the average value, $\Delta\overline{X}$ (%)	Relative error, ε (%)
Aqueous *Astragalus varius*	2.54	0.001233333	0.03512	0.02028	1.384	0.04	3.44
Aqueous-alcoholic *Astragalus varius*	9.31	0.001600000	0.04000	0.02309	0.430	0.07	1.07
Aqueous *Astragalus ovatus*	1.06	0.000400000	0.02000	0.01155	1.887	0.05	4.68
Aqueous-alcoholic *Astragalus ovatus*	10.34	0.000400000	0.02000	0.01155	0.193	0.05	0.48

Table 3 — "Tail Suspension Test", Day 1 of the experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	68.00 [54.75; 75.75]	–	–	112.00 [97.75; 119.75]	–	–	7.00 [4.75; 13.75]	–	–
Amitriptyline, 10 mg/kg	135.00 [103.00; 167.25]	0.02	–	45.50 [14.25; 75.75]	0.02	–	46.00 [26.00; 48.50]	0.13	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	113.50 [109.25; 122.25]	0.01	0.33	66.50 [47.25; 70.25]	0.01	0.66	32.50 [16.25; 43.00]	0.13	0.52
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	86.00 [66.00; 101.00]	0.20	0.19	94.00 [53.00; 102.00]	0.20	0.20	25.50 [0.00; 59.25]	0.83	0.66
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	99.50 [74.25; 114.75]	0.08	0.28	80.50 [47.75; 93.25]	0.08	0.39	16.50 [8.00; 25.00]	0.39	0.14
Aqueous-alcoholic extract of *Astragalus ovatus*, 100 mg/kg	132.00 [113.25; 146.00]	0.01	0.83	48.00 [24.00; 60.25]	0.01	1.0	65.50 [26.00; 92.75]	0.03	0.29

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

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Table 4 — "Tail Suspension Test", Day 8 of the experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	84.50 [76.25; 89.00]	–	–	95.50 [89.00; 99.25]	–	–	16.00 [9.50; 21.50]	–	–
Amitriptyline, 10 mg/kg	174.50 [136.75; 178.75]	0.003	–	6.50 [0.75; 32.00]	0.004	–	44.00 [27.00; 137.50]	0.14	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	98.50 [78.50; 120.50]	0.45	0.02	100.00 [73.75; 177.25]	0.59	0.01	33.50 [21.25; 45.50]	0.1	0.45
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	136.50 [130.00; 144.25]	0.01	0.13	43.50 [27.25; 48.00]	0.01	0.16	11.50 [2.50; 27.25]	0.45	0.29
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	117.00 [82.00; 128.50]	0.14	0.03	63.00 [44.50; 80.00]	0.13	0.03	15.50 [6.00; 22.00]	0.75	0.08
Aqueous-alcoholic extract of *Astragalus ovatus*, 100 mg/kg	144.00 [112.50; 157.75]	0.01	0.13	36.00 [18.75; 54.50]	0.01	0.14	58.00 [19.75; 72.75]	0.16	0.83

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

Table 5 — "Tail Suspension Test", Day 15 of the experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	77.50 [72.50; 93.00]	–	–	102.50 [81.75; 104.50]	–	–	16.50 [12.25; 19.25]	–	–
Amitriptyline, 10 mg/kg	148.50 [108.50; 158.50]	0.004	–	22.00 [15.00; 39.50]	0.003	–	23.50 [2.25; 40.75]	1.00	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	127.50 [109.50; 144.00]	0.01	0.52	52.50 [30.00; 67.50]	0.01	0.08	38.50 [14.75; 33.75]	0.14	0.68
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	122.50 [101.50; 128.75]	0.01	0.27	57.50 [41.75; 65.50]	0.01	0.06	19.00 [2.75; 37.50]	0.83	0.92
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	85.50 [64.00; 109.25]	1.00	0.05	94.50 [66.25; 116.00]	1.00	0.01	5.50 [0.00; 13.25]	0.16	0.28
Aqueous-alcoholic extract of *Astragalus ovatus*, 100 mg/kg	131.00 [119.50; 133.50]	0.01	0.39	49.00 [43.50; 53.50]	0.01	0.09	36.50 [24.75; 50.75]	0.01	0.39

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

Table 6 — Tail Suspension Test, Day 21 of the Experiment

Group of animals	Indicator								
	Total time of activity maintenance, sec			Total duration of inactivity, sec			Latent period of the first episode of immobilization, sec		
	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2	Me [Q1; Q3]	p_1	p_2
Control	66.50 [51.25; 90.75]	–	–	113.50 [81.25; 124.25]	–	–	11.00 [6.50; 13.25]	–	–
Amitriptyline, 10 mg/kg	159.00 [122.50; 174.00]	0.01	–	22.00 [3.50; 35.00]	0.013	–	19.00 [0.00; 48.00]	0.71	–
Aqueous extract of *Astragalus varius*, 100 mg/kg	109.50 [107.00; 118.50]	0.01	0.14	70.5 [48.50; 73.00]	0.01	0.14	30.00 [18.00; 37.50]	0.03	0.80
Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	136.50 [126.00; 147.75]	0.01	0.27	43.50 [24.75; 52.00]	0.01	0.33	16.00 [0.00; 39.50]	0.91	1.00
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	91.00 [88.75; 101.50]	0.19	0.03	89.00 [57.50; 89.75]	0.19	0.03	5.00 [2.75; 11.50]	0.19	0.80
Aqueous extract of *Astragalus ovatus*, 100 mg/kg	143.50 [122.75; 157.75]	0.01	0.46	36.50 [12.75; 49.75]	0.01	0.46	48.50 [26.25; 88.50]	0.02	0.14

Note: significance of differences by Mann-Whitney criterion (at $p < 0.01$); "p_1" — difference from control; "p_2" — difference from amitriptyline.

Table 7 — Correlation of the content of flavonoids and the antidepressant activity of aqueous and aqueous-alcoholic extracts of the *Astragalus varius* and *Astragalus ovatus* herbs

Indicator	Day of experiment	Group of animals			
		Aqueous extract of Astragalus varius, 100 mg/kg	Aqueous-alcoholic extract of *Astragalus varius*, 100 mg/kg	Aqueous extract of *Astragalus ovatus*, 100 mg/kg	Aqueous extract of *Astragalus ovatus*, 100 mg/kg
Total time of activity maintenance, sec	1	$r = 0.5$*	$r = -0.5$*	$r = -0.5$*	$r = -0.5$*
	8	$r = -1$*	$r = 0.6$*	$r = -0.5$*	$r = 0.5$*
	15	$r = -0.5$*	$r = 0.5$*	$r = -0.5$*	$r = 0.5$*
	21	$r = -0.9$*	$r = 0.6$*	$r = -0.5$*	$r = 0.5$*
Total duration of inactivity, sec	1	$r = -0.5$*	$r = 0.5$*	$r = 0.5$*	$r = 0.5$*
	8	$r = 1.0$*	$r = 0.6$*	$r = 0.5$*	$r = 0.5$*
	15	$r = 0.5$*	$r = 0.5$*	$r = 0.5$*	$r = 0.5$*
	21	$r = 0.9$*	$r = 0.6$*	$r = 0.5$*	$r = 0.5$*
Latent period of the first episode of immobilization, sec	1	$r = 0.5$	$r = 0$	$r = -0.5$	$r = -0.5$
	8	$r = -0.5$	$r = -0.3$	$r = -1$	$r = -0.5$
	15	$r = -0.5$	$r = -1$	$r = -1$	$r = -0.5$
	21	$r = -0.5$*	$r = 0.3$*	$r = 0.5$*	$r = 0.5$*

Note: * — $p < 0.05$ (Spearman correlation coefficient).

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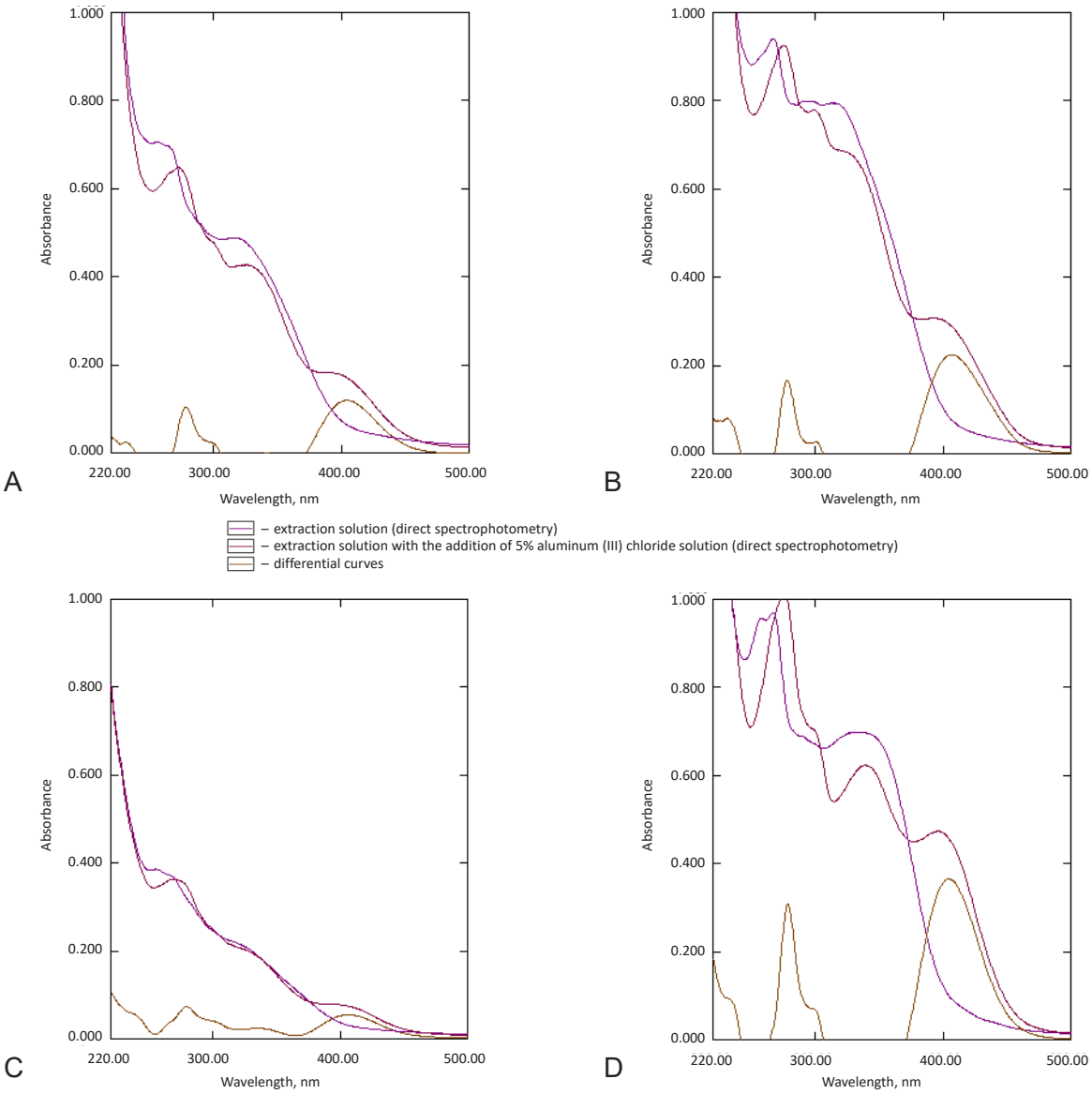

Figure 2 — Electronic spectra of extracts from the herb.
Note: A — *Astragalus ovatus*, aqueous; B — *Astragalus ovatus*, aqueous-alcoholic; C — *Astragalus varius*, aqueous; D — *Astragalus varius*, aqueous-alcoholic.

In mice of the 3rd experimental group, receiving aqueous extract of *Astragalus varius* (100 mg/kg), an antidepressant effect was observed with chronic administration of the extract. Thus, on days 15 and 21, the time of activity maintenance (p = 0.01) and the duration of the immobilization period (p = 0.01) were comparable to the values of the 2nd experimental group receiving amitriptyline (see Tables 5, 6). On day 1 of the experiment, a statistically significant antidepressant effect was also detected (p = 0.01) (see Table 3); however, on day 8 of the experiment, the effect was absent (p > 0.01), which requires further research (see Table 4). The latent period of the first episode of immobilization also did not show statistically significant differences from the control group and the group receiving amitriptyline (p > 0.01).

In animals of the 4th experimental group, receiving aqueous-alcoholic extract of *Astragalus varius* (100 mg/kg), an antidepressant effect was detected after a week of extract administration. On days 8, 15, and 21 of the experiment, the time of activity maintenance (p = 0.01) and the total duration of inactivity (p = 0.01) were comparable to the group receiving amitriptyline (Tables 4–6). On day 1 of testing, the indicators did not statistically differ from the values in the control group (p = 0.02) (see Table 3). The latent period of the first episode of immobilization also did not show statistically

significant differences from the control group and the group receiving amitriptyline ($p > 0.01$).

In animals of the 5th experimental group, receiving aqueous extract of *Astragalus ovatus* (100 mg/kg), an antidepressant effect was absent throughout the experiment ($p > 0.01$).

In animals of the 6th experimental group, receiving alcoholic extract of *Astragalus ovatus* (100 mg/kg), a pronounced antidepressant effect was detected both after a single administration and throughout the entire experiment. From day 1 of the experiment, the time of activity maintenance and the duration of the immobilization period were comparable to the indicators of animals in the control group. On day 1, the time of activity maintenance was higher than in the control group by 49.5% ($p = 0.01$), and inactivity was lower by 2.3 times ($p = 0.01$) (see Table 3). On day 8, activity exceeded the control by 41.7% ($p = 0.01$), and the time of inactivity decreased by 62.2 % ($p = 0.01$) (see Table 4). On day 15, animals maintained activity by 40.9 % ($p = 0.011$) compared to the control (see Table 5). On day 21, the activity of the experimental group was 2.2 times higher than that of the control group ($p = 0.01$), and the indicators of inactivity time were lower by 67.9 % ($p = 0.01$) (see Table 6). The latent period of the first episode of immobilization on days 1, 8, and 21 did not show statistically significant differences from the control group and the group receiving amitriptyline ($p > 0.01$) (see Tables 3, 4, 6), and on day 15 it was higher than in the control group ($p = 0.01$) (see Table 5).

Correlation analysis was performed to determine the relationship between flavonoid content and the antidepressant activity of aqueous and aqueous-alcoholic extracts. For the aqueous extract of *Astragalus varius* herb, a significant moderate positive correlation was observed between flavonoid content and total activity maintenance time on day 1 of the experiment ($r = 0.5$), while no correlation was found on days 8, 15, and 21 of the experiment. The correlation between flavonoid content and total inactivity duration was very high on day 8 ($r = 1$), moderate on day 15 ($r = 0.5$), and high on day 21 (Table 7).

For the aqueous extract of *Astragalus ovatus* herb, there was no correlation between total flavonoid content and total activity maintenance time; however, a significant moderate correlation was found between total flavonoid content and total inactivity duration on days 1, 8, 15, and 21 of the experiment ($r = 0.5$).

For the aqueous-alcoholic extracts of *Astragalus varius* and *Astragalus ovatus* herbs, a direct significant correlation was observed between flavonoid content and total activity maintenance time on days 8, 15, and 21 of the experiment, and a direct significant moderate correlation between total flavonoid content and total inactivity duration on days 1, 8, 15, and 21 of the experiment (see Table 7).

The correlation between flavonoid content and the latency period of the first episode of immobilization was insignificant for almost all extracts (see Table 7).

Analysis of the obtained data showed that alcoholic extracts from the herbs of both “Astragalus” species exhibited a more pronounced antidepressant effect compared to aqueous extracts, which is attributed to the flavonoid content in the extracts: the sum of flavonoids in alcoholic extracts is significantly higher than in aqueous extracts (see Table 2), which is confirmed by the correlation relationships. The aqueous-alcoholic extract of *Astragalus ovatus* demonstrated a significantly similar pronounced effect to amitriptyline ($p = 0.01$) after both single oral administration and throughout the entire experimental period (21 days). The alcoholic extract of *Astragalus varius* did not show an antidepressant effect after single administration; however, after 1 week of administration, a significant ($p = 0.01$) effect was observed on days 15 and 21 in animals.

After single administration of the aqueous extract of *Astragalus varius*, an antidepressant effect was observed, which was nullified after a week of extract administration. When the extract was administered to animals for 2 weeks, a significant ($p = 0.01$) activity was observed. The aqueous extract of *Astragalus ovatus*, after both single and chronic oral administration to animals, showed no activity in the experiment.

Thus, the most pronounced antidepressant properties were found in the aqueous-alcoholic extract of *Astragalus ovatus*, the least in the aqueous extract of *Astragalus varius*, and they were entirely absent in the aqueous extract of *Astragalus ovatus*.

DISCUSSION

The data on the study of the antidepressant activity of extracts from *Astragalus varius* and *Astragalus ovatus* herbs have been obtained by us for the first time. Comparison of the activity of extracts from *Astragalus varius* and *Astragalus ovatus* herbs with extracts from other “Astragalus” species is difficult due to differences in experimental conditions. Under similar conditions, we have studied extracts from *Astragalus*

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DOI: 10.19163/2307-9266-2026-14-2-161-174

membranaceus herb, which is used in traditional medicine for its diuretic and hypotensive effects. Antidepressant properties of aqueous and aqueous-alcoholic extracts from *Astragalus membranaceus* herb were not detected [31], which may be due to the absence or low content of BACs capable of affecting the central nervous system.

It has been established that stress exposure contributes to the development of anxiety-depressive disorders, which require correction. For these purposes, medicinal preparations are widely used, including herbal preparations containing flavonoids [32, 33].

Flavonoids are a group of polyphenolic compounds produced by plants as secondary metabolites. They often occur in glycosylated or esterified forms, have a basic 15-carbon skeleton consisting of C3 and C6 rings linked by a single bond, namely rings A and B connected by a third carbon ring [34].

A meta-analysis showed that flavonoids have a significant overall effect on depression (p = 0.004, Hedges' g = -0.487, 95% confidence interval from -0.814 to -0.160). Subgroup analysis showed that depressive symptoms significantly decreased when the flavonoid dose was 50–100 mg per day or the treatment duration was more than 8 weeks [35].

Various factors are involved in the pathogenesis of depressive disorder. The "monoamine hypothesis of depression" is a well-known theory explaining depressive disorder, which states that a decrease in the level of monoaminergic neurotransmitters in the brain, particularly serotonin and norepinephrine, is the primary cause of depression. However, current data indicate the involvement of several neural and hormonal pathways in the development of depressive disorder. Other factors include increased activation of the hypothalamic-pituitary-adrenal (HPA) axis, reduced regulation of brain-derived neurotrophic factor (BDNF), as well as dopaminergic and glutamatergic systems [36].

Many flavonols possess antidepressant and anxiolytic activity, possibly by increasing 5-HT and decreasing 5-hydroxyindoleacetic acid (5-HIAA) in the brain [37]. Quercetin inhibits hepatocarcinogenesis mediated by reactive oxygen species (ROS) by upregulating enzymatic (catalase, superoxide dismutase (SOD), glutathione peroxidase, paraoxonase) and non-enzymatic (total glutathione) antioxidant defense systems [38]. Astragalin significantly improved behavioral deficits in a chronic unpredictable mild stress (CUMS) model, promoted SIRT1 expression, and reduced levels of NF-κB p65, NLRP3, cleaved caspase-1, IL-1β, and gasdermin D proteins in the hippocampus [25]. It has been established that the biological effects of apigenin (a flavone) are related to gene transcription, protein expression, and enzyme activity levels, as well as a decrease in the loss of antioxidant enzymes in cells treated with streptozotocin [39]. Isoflavones, which are abundantly found in legumes, particularly formononetin and calycosin, are quite specific to plants of the *Astragalus* genus and can improve cognitive functions and alleviate depressive symptoms [40].

The obtained data indicates the need for more in-depth study of the mechanisms of antidepressant action of the biologically active compounds of the analyzed *Astragalus* species. The observed effect of the aqueous extract of *Astragalus varius* herb, combined with its diuretic action [24], may be recommended for the correction of chronic cardiovascular diseases.

Study Limitations

This study was conducted on male mice using a single-dose regimen for the analyzed aqueous and aqueous-alcoholic extracts, as well as the reference drug (amitriptyline). Therefore, further research on animals of both sexes is necessary, as well as an expanded range of doses to establish therapeutic efficacy.

CONCLUSION

The study determined the flavonoid content in aqueous and aqueous-alcoholic extracts from *Astragalus varius* herb (2.54 % and 9.31 %, respectively) and *Astragalus ovatus* herb (1.06 % and 10.34 %, respectively). An antidepressant effect of the aqueous-alcoholic extract of *Astragalus ovatus* was established with single and chronic administration at a dose of 100 mg/kg over 21 days. The aqueous-alcoholic extract of *Astragalus varius* demonstrated an antidepressant effect with chronic administration from days 8 to 21 of the experiment. The aqueous extract of *Astragalus varius* showed an antidepressant effect with chronic oral administration from days 15 to 21 of the experiment, while the aqueous extract of *Astragalus ovatus* showed no antidepressant activity. The antidepressant effect of the analyzed extracts from *Astragalus varius* and *Astragalus ovatus* herbs is likely due to the flavonoid content in them, which is confirmed by correlation relationships. Determining the mechanism of action of the BASs in the analyzed extracts requires further research.

FUNDING

The study was carried out with the financial support of the Saratov State Medical University of the Scientific Project No. SSMU-2022-007.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Ulyana A, Matvienko — conceptualization, invwstigation, data analysis of research, writing a draft manuscript, revision and editing of the text of the article; Alyona Yu. Karetnikova — definition of the concept, validation; Natalia A. Durnova — definition of the concept, writing a draft manuscript, revision and editing of the text of the article. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2026-14-2-175-188

Prediction, *in silico* antioxidant activity, and targeted synthesis of sterically hindered phenol azomethine derivatives

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Received 27 June 2025 | After peer review 09 Jan 2026 | Accepted 15 March 2026

Molecular design and synthesis of a new series of biologically active azomethines containing a sterically hindered phenolic fragment were carried out. Within the scope of the study, 8 compounds were synthesized, and their antioxidant activity was evaluated under *in vitro* conditions. To establish the mechanism of action, molecular docking was used to model the interaction of the synthesized ligands with the active site of glutathione peroxidase-4 (GPx-4). The conducted analysis revealed key structural features determining antioxidant efficacy and established a correlation between molecular structure and biological activity.

The aim. Synthesis, computer screening, and investigation of the antioxidant properties of new azomethines based on sterically hindered phenol, as well as establishing structure–activity relationships.

Materials and methods. A new series of 2,6-di-tert-butyl-4-[C-alkyl-(aryl)-(N-phenyl)-azomethine]phenols was synthesized by the condensation of corresponding ketones with aromatic amines in the presence of catalytic amounts of p-toluenesulfonic acid. The structure and purity of the obtained compounds were confirmed by a complex of physicochemical methods, including IR spectroscopy, H NMR spectroscopy, and elemental analysis. For the initial assessment of the biological potency of the synthesized compounds, computer prediction (*in silico*) of their antioxidant, antiradical, and cardiotonic properties was performed using the online platform PASS Online. Molecular modeling of potential inhibitory activity against human glutathione peroxidase-4 (GPx-4) was carried out using the Autodock 4.0 program. The conformational mobility of the ligands was taken into account, for which optimal torsion angles were previously determined and set. Experimental study of antioxidant activity (AOA) was conducted in two model systems: induction of lipid peroxidation (LPO) in a complex of corn oil fatty acids under UV irradiation; and the Fenton system (H_2O_2/Fe^{2+}). To compare efficacy, ubiquinone and bottled hydroxytoluene (BHT, the active substance of the drug dibulin), representing the class of sterically hindered phenols, were used as reference standards.

Results. The spectrum of biological activity of the studied compounds was predicted *in silico* using the PASS Online service. As it was expected, all substances have cardiotonic, membrane-stimulating, and antioxidant potential. The presence of AOA and the ability to scavenge free radicals allows these molecules to be classified as antiradical agents. Experimental verification of AOA was carried out in two model systems: based on photooxidation (UV irradiation) of a complex of fatty acids from corn oil (system No. 1) and on the Fenton system (H_2O_2/Fe^{2+}, system No. 2). In all the cases, the studied compounds demonstrated high efficacy, inhibiting lipid peroxidation LPO by 42–48%. This result significantly exceeds the activity of standard antioxidants — ubiquinone (11%) and BHT (39%) — in the same conditions.

Conclusion. The results of molecular docking indicate a high affinity of the new ligands to the GP-4 protein, with the calculated binding energy for the most promising structures being comparable to that of known standards—ubiquinone, dibulin (hydroxybutylated toluene), and mexidol. *In vitro* experimental data confirmed the pronounced antioxidant activity of the synthesized compounds. “Lead” structures were identified that surpass classical antioxidants—ubiquinone and dibulin — in efficacy.

Keywords: azomethines; azomethine phenols; sterically hindered phenols; antioxidant activity; lipid peroxidation; PASS Online

Abbreviations: SHPs — sterically hindered phenols; TLC — thin-layer chromatography; LPO — lipid peroxidation; AOA — antioxidant activity; UV irradiation — ultraviolet irradiation; PhAs — phenolic antioxidants; WSOM — water, alcohol and oil mixture; TCA — trichloroacetic acid; Pa — probability of activity manifestation.

For citation: O. Tsakulova, I.P. Kodonidi, A.S. Chiriapkin, F.N. Bidarova, M.T. Kisieva, L.A. Usmanova. Prediction, *in silico* antioxidant activity, and targeted synthesis of sterically hindered phenol azomethine derivatives. *Pharmacy & Pharmacology.* 2026;14(2):175-188. **DOI:** 10.19163/2307-9266-2026-14-2-175-188

Для цитирования: Т.В. Цакулова, И.П. Кодониди, А.С. Чиряпкин, Ф.Н. Бидарова, М.Т. Кисиева, Л.А. Усманова. Прогноз, антиоксидантная активность *in silico* и целенаправленный синтез азометиновых производных пространственно-затруднённого фенола. *Фармация и фармакология.* 2026;14(2):175-188. **DOI**: 10.19163/2307-9266-2026-14-2-175-188

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Прогноз, антиоксидантная активность *in silico* и целенаправленный синтез азометиновых производных пространственно-затруднённого фенола

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Получена 27.06.2025 После рецензирования 09.01.2026 Принята к печати 15.03.2026

Проведено молекулярное конструирование и синтез нового ряда биологически активных азометинов, содержащих пространственно-затруднённый фенольный фрагмент. В рамках исследования 8 соединений, для которых оценена антиоксидантная активность в условиях *in vitro*. Для установления механизма действия методом молекулярного докинга выполнено моделирование взаимодействия синтезированных лигандов с активным центром глутатионпероксидазы-4 (GPx-4). Проведённый анализ позволил выявить ключевые структурные особенности, определяющие антиоксидантную эффективность, и установить корреляционную связь между строением молекул и их биологической активностью.

Цель. Синтез, компьютерный скрининг и исследование антиоксидантных свойств новых азометинов на основе пространственно-затруднённого фенола, а также установление корреляций «структура–активность».

Материалы и методы. Методом конденсации соответствующих кетонов с ароматическими аминами в присутствии каталитических количеств *п*-толуолсульфокислоты был осуществлен синтез нового ряда 2,6-*ди*-*трет*-бутил-4-[С-алкил-(арил)-(N-фенил)-азометино]фенолов. Структура и чистота полученных соединений подтверждены комплексом физико-химических методов, включая ИК-спектроскопию, ^{1}Н-ЯМР-спектроскопию и элементный анализ. Для первичной оценки биологической потенции синтезированных соединений проведено компьютерное прогнозирование (*in silico*) их антиоксидантных, антирадикальных и кардиотонических свойств с использованием онлайн-платформы PASS Online. Молекулярное моделирование потенциальной ингибирующей активности в отношении глутатионпероксидазы-4 (GPx-4) человека выполнялось в программе Autodock 4.0. При этом учитывалась конформационная подвижность лигандов, для которых были предварительно определены и заданы оптимальные торсионные углы. Экспериментальное изучение антиоксидантной активности (АОА) проводилось в двух модельных системах: индуцирование перекисного окисления липидов (ПОЛ) в комплексе жирных кислот кукурузного масла под действием УФ-облучения; система Фентона (H_2O_2/Fe^{2+}). Для сопоставления эффективности в качестве референтных стандартов были использованы убихинон и бутилированный гидрокситолуол (БГТ, действующее вещество препарата дибулин), представляющий класс экранированных фенолов.

Результаты. Спектр биологической активности исследованных соединений предсказан *in silico* с помощью сервиса PASS Online. Согласно прогнозу, все вещества обладают кардиотоническим, мембраностимулирующим и антиоксидантным потенциалом. Наличие АОА и способности захватывать свободные радикалы позволяет отнести данные молекулы к классу антирадикальных агентов. Экспериментальная проверка АОА была проведена в двух модельных системах: на основе фотоокисления (УФ-облучение) комплекса жирных кислот кукурузного масла (система № 1) и на системе Фентона (H_2O_2/Fe^{2+}, система № 2). Во всех случаях исследуемые соединения продемонстрировали высокую эффективность, ингибируя ПОЛ на 42–48%. Данный результат существенно превышает активность стандартных антиоксидантов — убихинона (11%) и БГТ (39%) — в аналогичных условиях.

Заключение. Результаты молекулярного докинга свидетельствуют о высоком сродстве новых лигандов к белку ГП-4, причем расчётная энергия связывания для наиболее перспективных структур сопоставима с таковой для известных эталонов — убихинона, дибулина (гидроксибутилированного толуола) и мексидола. Экспериментальные данные *in vitro* подтвердили выраженную антиоксидантную активность синтезированных соединений. Выделены «лидерные» структуры, превосходящие по эффективности классические антиоксиданты — убихинон и дибулин.

Ключевые слова: азометины; азометинофенолы; пространственно-затруднённые фенолы; антиоксидантная активность; перекисное окисление липидов; PASS Online

Список сокращений: ПЗФ — пространственно-затруднённые фенолы; ТСХ — тонкослойная хроматография; ПОЛ — перекисное окисление липидов; АОА — антиоксидантная активность; УФО — ультрафиолетовое облучение; ФАО — фенольные антиоксиданты; ВСМ — водно-спиртово-масляная смесь; ТХУК — трихлоруксусная кислота; Ра — вероятность проявления активности.

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DOI: 10.19163/2307-9266-2026-14-2-175-188

INTRODUCTION

The modern view on the role of lipid peroxidation (LPO) indicates that an imbalance in this process is a key pathogenetic link in numerous pathologies, especially cardiovascular diseases. In particular, recent studies have led to the concept that LPO significantly contributes to the development of venous thrombosis and thromboembolism in patients with heart failure. This is based on oxidative stress, characterized by excessive generation of reactive oxygen species, which ultimately increases the thrombogenic potential of blood [1–3].

In the context of developing new drugs, one of the current strategies in pharmaceutical science is the creation of molecules with combined action, capable of simultaneously affecting multiple biological targets. Such polypharmacological effects are achieved through the rational design of "hybrid" main compounds and the pharmacophore fragments of so-called "privileged molecules" are integrated into their structure [4–6]. Azomethines, considered as a basis for antiplatelet drugs targeting thrombosis [7–9], are a promising chemical class for such design. The synergistic effect, combining antioxidant and antiradical action, can be enhanced by incorporating a free radical scavenger—a structural element of a sterically hindered phenol (SHP)—into the azomethine molecular scaffold [10, 11].

Among polyfunctional phenolic antioxidants (PhAs), derivatives of 4-methyl-2,6-diisobornylphenol, which exhibit antiplatelet and antithrombotic activity [12], are the most thoroughly studied and used. However, the range of such polyfunctional compounds on the market is extremely limited, which is due to the following main problems:

The multi-step nature of known synthetic compound preparation methodologies, economic costs, and poor adaptability for industrial scaling.

The lack of systematized knowledge and data on the relationship between the chemical structure of polyfunctional PhAs and their antioxidant efficacy ("structure–activity") hinders the targeted synthesis of compounds superior to existing analogs.

Thus, up-to-date task is the targeted search and study of new highly effective and safe compounds that combine the properties of antioxidants, antiradical, and antithrombotic agents. A promising direction in this field is the investigation of a series of azomethines containing a sterically hindered phenol fragment, followed by an assessment of their antioxidant potential *in vitro.*

THE AIM. Synthesis of new sterically hindered phenol azomethine derivatives, *in silico* prediction of compounds with optimal pharmacokinetic parameters, *in vitro* study of antioxidant activity, and identification of structure-activity relationship patterns.

MATERIALS AND METHODS

Prediction

The structural formulas of the modeled compounds were constructed using BIOVIA Draw 17.2. Computer analysis of the biological activity of virtual compounds was performed using the PASS program, based on the analysis of structural descriptors of multilevel atomic neighborhoods of known substances. The obtained results were presented as a list of biological activities with calculated probabilities of activity manifestation (Pa) for each constructed substance [13].

Molecular docking was performed using the freely distributed program Autodock 4.0 [14]. Molecular modeling was carried out considering the conformational mobility of ligands, whose torsion angles were set and defined in this program. The charges of all atoms in the modeled system were calculated using the Gasteiger algorithm. The program was set to search for 200 energetically favorable conformations of molecular complex formation between the studied compounds and the protein target using the Lamarckian genetic algorithm scoring function for calculating interaction energy (Lamarckian GA 4.2). The grid spacing was 0.364 Å. A three-dimensional model of the enzyme for computational experiments was selected from the RCSB Protein Data Bank (www.rcsb.org) [15].

Virtual structures of the modeled compounds were built in HyperChem 8.0.4 and then geometrically optimized using the AbInitio method with the STO-3G basis set [16].

The conversion of the .hin format to .pdb, required for molecular modeling, was performed in Open Babel 2.4.1 [17].

As a target for predicting the inhibitory activity of compounds against glutathione peroxidase-4 (GP-4), a virtual model of the human enzyme with identification number 6HKQ was used. This structure contains an inhibitor of this enzyme—ML162 ((2S)-2-[2-chloroanilino(3-chloro-4-methoxyphenyl)amino]-*N*-(2-phenylethyl)-2-thiophenylacetamide) [18–20]. The computational experiment region is a cube centered at the following coordinates: x = -22.487, y = 9.200,

z = 2.438. The number of points along the x and y axes is 40, and along the z axis is 26.

Analysis

Melting point determination was performed on a PTP (M) TU 92–891 (Russia) instrument. IR spectra were recorded on an FSM 1201 FT-IR spectrometer (InfraSpec LLC, Russia) in a KBr pellet. 1H NMR spectra were recorded on a Bruker instrument (Germany) at a working frequency of 400 MHz in DMSO-d6 or deuterated chloroform (CDCl3) solutions, using the solvent as an internal standard. Reaction progress was monitored by thin-layer chromatography using "Sorbfil" plates. The mobile phase used was a chromatographic system of n-butanol–acetic acid–water (4 : 1 : 2). Substance spots were detected under UV light. Elemental analysis was performed on a Flash EA 1112 CHNSO analyzer (Thermo Scientific, USA).

Synthesis

General procedure for the preparation of 2,6-di-tert-butyl-4-[C-alkyl-(aryl)-(*N*-phenyl)-azomethine]-phenol derivatives. To a solution containing equimolar amounts (0.01 mol) of the corresponding 1-(3,5-di-tert-butyl-4-hydroxyphenyl)-alkyl-(aryl)-ketone and a primary aromatic amine in 50 mL of anhydrous toluene, a catalytic amount of *p*-toluenesulfonic acid was added. The mixture was boiled for 4 hours, cooled, and the precipitated solid was washed with petroleum ether, dried, and recrystallized from aqueous methanol.

4-[*N*-(4-bromophenyl)-C-methyl-azomethine]-2,6-di-tert-butyl-phenol (3a). The reaction product is a fine crystalline, odorless beige substance. Yield—76 %. M.p. = 145–147 °C (recrystallization from methanol). IR spectrum (in KBr pellet): 3560 (OH), 3052 (CH_{arom}), 2851 (t-Bu), 1663 (C = N) cm^{-1}. 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.35, 1.46 (2s, 1H each, C(3) tert-Bu, C(5) tert-Bu, J = 4.91); 5.72 (s, 1H, C(4) OH, J = 4.83); 7.61 (s, 2H, Ar, J = 10.05); 7.40, 7.43 (2d, 1H each, H(5, 6), J = 5.14, J = 5.17). Elemental analysis data (%) for $C_{22}H_{28}BrNO$ (402.34): Calculated: C, 65.67; H, 7.01; Br, 19.86; N, 3.48; O, 3.98. Found: C, 65.81; H, 7.06; Br, 20.10; N, 3.53; O, 4.05.

4-[*N*-(2-aminophenyl)-C-ethyl-azomethine]-2,6-di-tert-butyl-phenol (3b). Yield—74 %. M.p. = 132–134 °C (recrystallization from methanol). IR spectrum (in KBr pellet): 3562 (OH), 3044 (CHarom), 2847 (t-Bu), 1662 (C = N) cm^{-1}. 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.38, 1.48 (2s, 1H each, C(3) tert-Bu, C(5) tert-Bu, J = 4.92); 5.78 (s, 1H, C(4) OH, J = 4.90); 7.58 (s, 2H, Ar, J = 10.02); 7.42, 7.44 (2d, 1H each, H(5, 6), J = 5.10, J = 5.16). Elemental analysis data (%) for $C_{23}H_{32}N_2O$ (352.48): Calculated: C, 78.36; H, 9.15; N, 7.95; O, 4.54. Found: C, 78.81; H, 9.22; N, 8.02; O, 4.05.

2,6-di-tert-butyl-4-[C-methyl-*N*-(p-tolyl)-azomethine]-phenol (3c). Yield—80 %. M.p. 142–144 °C (recrystallization from methanol). IR spectrum (in KBr pellet): 3600 (OH), 3050 (CH_{arom}), 2865 (t-Bu), 1663 (C = N) cm^{-1}. 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.36, 1.42 (2s, 1H each, C(3) tert-Bu, C(5) tert-Bu, J = 4.88); 5.80 (s, 1H, C(4) OH, J = 4.91); 7.65 (s, 2H, Ar, J = 10.02); 7.40, 7.48 (2d, 1H each, H(5, 6), J = 5.14, J = 5.16). Elemental analysis data (%) for $C_{23}H_{31}NO$ (337.47): Calculated: C, 81.85; H, 9.26; N, 4.15; O, 4.74. Found: C, 81.74; H, 9.18; N, 3.98; O, 4.05.

***N*-[4-[1-(3,5-di-tert-butyl-4-hydroxy-phenyl)-ethylideneamino]-phenyl]-acetamide (3d).** Yield—80 %. M.p. = 147–149 °C (recrystallization from methanol). IR spectrum (in KBr pellet): 3600 (OH), 3050 (CH_{arom}), 2865 (t-Bu), 1663 (C = N) cm^{-1}. 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.40, 1.46 (2s, 1H each, C(3) tert-Bu, C(5) tert-Bu, J = 4.90); 5.72 (s, 1H); (s, 1H, C(4) OH, J = 4.82); 7.62 (s, 2H, Ar, J = 10.05); 7.44, 7.49 (2d, 1H each, H(5, 6), J = 5.17, J = 5.12). Elemental analysis data (%) for $C_{24}H_{32}N_2O_2$ (380.49): Calculated: C, 75.75; H, 8.48; N, 7.36; O, 8.41. Found: C, 78.81; H, 8.18; N, 7.88; O, 8.05.

1-[4-[1-(3,5-di-tert-butyl-4-hydroxy-phenyl)-ethylideneamino]-phenyl]-ethanone (3e). Yield—78 %. M.p. = 100–102 °C (recrystallization from methanol). IR spectrum (in KBr pellet): 3600 (OH), 3050 (CH_{arom}), 2865 (t–Bu), 1663 (C = N) cm^{-1}. 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.38, 1.48 (2s, 1H each, C(3) tert-Bu, C(5) tert-Bu, J = 4.94); 5.74 (s, 1H, C(4) OH, J = 4.88); 7.63 (s, 2H, Ar, J = 10.02); 7.38, 7.42 (2d, 1H each, H(5, 6), J = 5.15, J = 5.10). Elemental analysis data (%) for $C_{24}H_{31}NO_2$ (365.48): Calculated: C, 78.86; H, 8.55; N, 3.83; O, 8.76. Found: C, 78.92; H, 8.18; N, 3.72; O, 8.05.

2,6-di-tert-butyl-4-[C-(4-chlorophenyl)-*N*-phenylazomethine]-phenol (3f). Yield—72 %. M.p. = 185–187 °C (recrystallization from methanol). IR spectrum (in potassium bromide pellet): 3600 (OH), 3050 (CH_{arom}), 2865 (t-Bu), 1663 (C = N) cm^{-1}. 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.34, 1.42 (2s, 2H, C(3,5)-tert-Bu, J = 4.90); 5.80 (s, 1H, C(4) OH, J = 4.92); 7.61 (s, 2H, Ar, J = 10.00); 7.40, 7.48 (2d, 1H each, H(5, 6), J = 5.14, J = 5.09). Elemental analysis data (%) for $C_{27}H_{30}ClNO_2$ (419.95): Calculated: C, 77.21; H, 7.20; Cl, 8.55; N, 3.34; O, 3.81. Found: C, 77.92; H, 7.55; Cl, 8.19; N, 3.72; O, 8.014.

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DOI: 10.19163/2307-9266-2026-14-2-175-188

***N*-[4-[1-(3,5-di-tert-butyl-4-hydroxy-phenyl)-propylideneamino]-phenyl]-acetamide (3g).** Yield—76 %. M.p. = 128–130 °C (recrystallization from methanol). IR spectrum (in KBr pellet): 3600 (OH), 3050 (CH_{arom}), 2865 (t–Bu), 1663 (C = N) cm^{-1}. 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.40, 1.52 (2s, 1H each, C(3) tert-Bu, C(5) tert-Bu, J = 4.94); 5.74 (s, 1H, C(4) OH, J = 4.86); 7.60 (s, 2H, Ar, J = 10.04); 7.42, 7.48 (2d, 1H each, H(5, 6), J = 5.18, J = 5.14). Elemental analysis data (%) for $C_{25}H_{34}N_2O_2$ (394.52): Calculated: C, 76.21; H, 8.69; N, 7.10; O, 8.11. Found: C, 76.92; H, 8.55; N, 7.21; O, 8.17.

2,6-di-tert-butyl-4-[C-methyl-*N*-(4-nitrophenyl)-azomethine]-phenol (3h). Yield—80 %. M.p. = 132–134 °C (recrystallization from methanol). IR spectrum (in potassium bromide pellet): 3600 (OH), 3050 (CH_{arom}), 2865 (t-Bu), 1663 (C = N) cm^{-1}, 1520 (vas NO_2), 1360 (vs NO_2). 1H NMR spectrum (400 MHz, CDCl3), δ, ppm: 1.38, 1.45 (2s, 1H each, C(3) tert-Bu, C(5) tert-Bu, J = 4.91); 5.78 (s, 1H, C(4) OH, J = 4.80); 7.63 (s, 2H, Ar, J = 10.02); 7.40, 7.48 (2d, 1H each, H(5, 6), J = 5.15, J = 5.10). Elemental analysis data (%) for $C_{23}H_{31}N_2O_3$ (383.47): Calculated: C, 72.03; H, 8.15; N, 7.30; O, 12.52. Found: C, 72.44; H, 8.05; N, 7.52; O, 12.36.

In vitro study of the antioxidant activity of compounds 3a–3h

The antioxidant properties of the synthesized azomethine phenols were evaluated in model systems based on corn oil containing a complex of saturated and unsaturated fatty acids. Lipid oxidation was initiated both physically (ultraviolet irradiation, UVI) and chemically, using the Fenton system (H_2O_2/Fe^{2+}) as a free radical generator. This protocol was previously successfully tested in our studies of benzimidazole derivatives containing a sterically hindered phenolic fragment [21]. To quantitatively assess the obtained results, the antiradical activity of the compounds was compared with the action of reference antioxidants: ubiquinone (Biologische Heilmittel Hee, Germany) and butylated hydroxytoluene (BHT, 99.0 %, CDH, India)—a representative of the sterically hindered phenol class used in the drug “Dibulin”. The antioxidant activity of ubiquinone under all experimental conditions was taken as the reference (100 %, or 1.0 ubiquinone unit).

General method for determining the *in vitro* antioxidant activity of 2,6-di-tert-butyl-4-[C-alkyl-(aryl)-(*N*-phenyl)-azomethine]-phenols 3a–3h

To study the antioxidant activity (AOA) of compounds (3a–3h), a Fenton system-induced oxidation model was used. Each compound under investigation was pre-dissolved to a concentration of 10 %. The model lipophilic medium was prepared as a water–alcohol–oil emulsion: 800 µL of oil was added to 3 mL of ethanol, vigorously shaken, and brought to the mark in a 100 mL volumetric flask with distilled water. The incubation mixture was formed in centrifuge tubes by combining 2 mL of the prepared emulsion, 100 µL of the test substance solution, 200 µL of a 10 % solution of iron (II) sulfate ($FeSO_4$), and 10 µL of 3 % hydrogen peroxide (H_2O_2) to generate hydroxyl radicals. The reaction was carried out in a thermostat at 37 °C for 60 min. In the control experiment, the test substance was replaced by an equivalent volume of distilled water. After incubation, 1 mL of 28 % trichloroacetic acid (TCA) was added, and the mixture was centrifuged for 10 min at 600 rpm. The content of LPO products was assessed by reaction with thiobarbituric acid. 1 mL of a 1 % TBA solution was added to 2 mL of the supernatant, and the mixture was heated for 15 min in a boiling water bath. After cooling, the optical density was recorded on a SF-46 spectrophotometer (JSC “LOMO”, Russia). For differential assessment of LPO stages, measurements were taken at 450 nm (diene conjugates) and 532 nm (malondialdehyde). The incubation medium without the addition of the test substances served as the control. The percentage of LPO inhibition was calculated by the formula:

$$ING\% = 100 - \left(\frac{I_0}{I_k} \times 100 \right),$$

where I_o is optical density of the test sample; I_k is optical density of the positive control sample.

AOA was expressed in ubiquinone units (Q – ed) by the formula:

$$AOA(Q - ed) = \%\Delta ING_i - \%\Delta ING_Q,$$

where $\%\Delta ING_i$ is a percentage decrease in the formation of TBA-reactive products in the test system in the presence of the tested synthetic sample; $\%\Delta ING_Q$ is a percentage decrease in the formation of TBA-reactive products in the test system in the presence of ubiquinone.

For comparison, the AOA of typical antioxidants—ubiquinone and butylated hydroxytoluene—was studied. A 1 % solution of butylated hydroxytoluene substance was prepared, 100 µL was taken, added to the test system described above, and incubated under standard conditions; for ubiquinone, 100 µL of a working solution of the preparation, prepared by

dissolving 1 capsule of ubiquinone in 10 mL of distilled water, was added to the test system described above and incubated under standard conditions.

Statistical analysis

Statistical analysis of the results was performed using computer software packages: "Microsoft Excel 2010" (Microsoft Office, USA) and "Statistica 10" (Statsoft, USA) using the paired Student's *t*-test. The Pearson method was used to assess correlational relationships between individual parameters studied.

RESULTS AND DISCUSSION

2,6-di-tert-butyl-4-[C-alkyl-(aryl)-(*N*-phenyl)-azomethine]-phenols 3a–3h were synthesized by boiling equimolar amounts of the corresponding substituted 1-(3,5-di-tert-butyl-4-hydroxyphenyl)-alkyl-(aryl)-ketones and primary aromatic amines in anhydrous toluene in the presence of catalytic amounts of p-toluenesulfonic acid for 4 h (Fig. 1).

The structure of 2,6-di-tert-butyl-4-[C-alkyl-(aryl)-(*N*-phenyl)-azomethine]-phenols was confirmed by IR spectroscopy, ^{1}H NMR, and elemental analysis. All compounds exhibit characteristic absorption bands for the valence vibrations of the unassociated (ν 3600 cm^{-1}) and associated (ν 3400 cm^{-1}) O-H groups, bands for the valence vibrations of aromatic ring C-H bonds (ν 3000–3050 cm^{-1}), bands for the asymmetric and symmetric valence vibrations of tert-butyl group C-H bonds (ν 3000–2850 cm^{-1}), and bands for the valence vibrations of the >C=N- bond (ν 1663 cm^{-1}). In the IR spectrum of compound 3h, intense absorption bands in the region of 1520–1526 cm^{-1} and 1330–1360 cm^{-1}, attributed to asymmetric ν^{as}_{NO2} and symmetric ν^{s}_{NO2} valence vibrations of the nitro group, are indicative.

In the ^{1}H NMR spectra of the obtained compounds 3a–3h, there are two singlets for the protons of the tert-butyl groups, which exhibit magnetic inequivalence; the chemical shifts for these protons are observed in the range of 1.34–1.42 ppm and 1.45–1.52 ppm.

A singlet (s) with chemical shifts in the range of 5.72–5.80 ppm corresponds to one proton of the hydroxyl group.

The signal of the aromatic protons of the phenolic fragment of the molecule (positions 3,5) has a chemical shift of 7.61–7.65 ppm and an intensity in the range of 10.01–10.05 ppm, which corresponds to two protons.

The aromatic protons of the aromatic ring (positions 5, 6) give two doublets due to spin-spin coupling with chemical shifts in the range of 7.38–7.40 ppm and 7.43–7.49 ppm.

Information on some physicochemical parameters of compounds **3a–3h** is presented in Table 1.

To assess the prospects of synthesizing new derivatives of 2,6-di-tert-butyl-4-[C-alkyl-(aryl)-(*N*-phenyl)-azomethine]-phenols 3a–3h, an in silico prediction of the spectrum of their probable pharmacological properties was performed using the online service PASS Online. A list of probable biological activities with the probability of their presence (Pa) and absence (Pi) in fractions of a unit is presented in Table 2.

Comparative analysis of predicted data for aromatic Schiff base derivatives containing a shielded phenol fragment indicates their potentially high pharmacological activity. Representatives of this series, according to predictions, may exhibit a cardiotonic effect and the ability to stabilize cell membranes. Furthermore, expected increased expression of the CYP2J2 enzyme may mediate cardioprotection through the activation of mitochondrial ATP-dependent potassium channels (mitoKATP) [22, 23], providing physiological benefits by altering reactive oxygen species production.

The results of computational modeling indicate that a series of studied azomethine derivatives demonstrate potential activity against the GP-4 target. The molecular complex with compound 3h exhibits the greatest stability, with a formation energy of -6.60 kcal/mol, indicating its high affinity (Table 3). Compounds 3f and 3a may also exhibit a pronounced inhibitory effect. A key structural feature common to the active ligands (3a, 3f, 3h) is the presence of strong electron-withdrawing groups—bromine, chlorine, and nitro groups.

Thus, it can be concluded that the introduction of electron-withdrawing substituents into the azomethine structure significantly increases their ability to bind to the active site of GP-4 [24]. Ubiquinone, hydroxybutylated toluene, and mexidol have significantly lower affinity for the GP-4 binding site. It is known [25] that mexidol exhibits pronounced antioxidant activity, including through the activation of endogenous antioxidant enzymes. The performed computer modeling suggests that the binding energy of the studied ligands with the GP-4 enzyme will be comparable in effectiveness to the reference

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compounds: ubiquinone, hydroxybutylated toluene, and mexidol itself. Molecular docking revealed key amino acid residues of the enzyme's active center involved in interaction with virtual ligands: Gln 45, Sec 46, Gly 47, Lys 48, Gln 81, Trp 136, Asn 137, and Phe 138. Analysis of the bond types showed that: compounds 3d and 3g form hydrogen bonds through carbonyl groups with the Asn 137 residue. Ligand 3e forms a similar hydrogen bond with the Gly 47 residue. Compounds 3f and 3h are characterized by the formation of a hydrogen bond between the nitrogen of the azomethine group and the amino acid Trp 136 (see Table 3).

The preliminary prediction stage allows for an assessment of the expediency of both further molecular design and preparative research for the synthesis of highly effective and safe drug substances among derivatives of this series.

Investigation of the antioxidant activity of derivatives (3a–h) in a test system with ultraviolet irradiation

During the first stage of the work, a screening of the AOA of synthesized azomethines containing a sterically hindered phenol fragment was carried out. The study was performed in a model test system based on a complex of saturated and unsaturated fatty acids. LPO was initiated by ultraviolet irradiation (UVI) in the presence of Fe^{2+} ions at an optimal biological concentration (1.0×10^{-3} mol/L). Compounds 3a, 3c, 3e, and 3h demonstrated the greatest antioxidant effect, expressed in ubiquinone units (Q-units). Statistically processed data are presented in Table 4.

Further detailed analysis showed that derivatives 3a, 3c, and 3h inhibit free radical processes by 44–48 %, surpassing the reference compound—hydroxybutylated toluene (39 % inhibition)—in activity. For the remaining compounds in the series (3b, 3d, 3e, 3f, 3g), the inhibition level was 28–37 %. The lowest AOA was characteristic of samples 3d and 3g, which is likely due to steric hindrance created by the bulky acetamide group in the phenyl fragment conjugated with the azomethine bond.

During the final stage, the dependence of AOA on initiator concentration was studied. Comparative analysis with hydroxybutylated toluene confirmed that the maximum antioxidant activity of the studied compounds is observed at a concentration of iron (II) ions (1.0×10^{-3} mol/L).

Investigation of the antioxidant activity of compounds 3a–3h in a test system with hydrogen peroxide

During the study of the AOA of the investigated compounds in a test system using chemical (hydrogen peroxide and iron (II) sulfate—Fenton system H_2O_2/Fe^{2+}) inducers of free radical processes, it was found that the maximum inhibitory effect was also observed in the presence of compounds 3a, 3c, and 3h—42–45 %, exceeding the antioxidant effect in the same system of the reference substance—hydroxybutylated toluene (36.5 %) (Table 5).

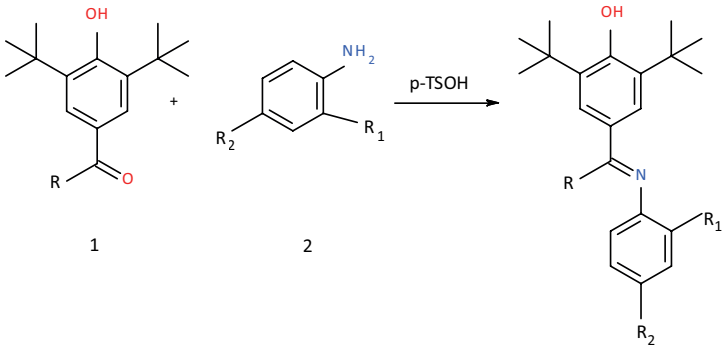
However, it should be noted that compounds **3a, 3c**, and **3h** showed a less pronounced effect in this test system, indicating lower stability of the described compounds towards chemical inducers of free radical processes.

Other tested samples in this series, **3b, 3d, 3e, 3f**, and **3g**, inhibit free radical processes by 29–38 %. Compounds **3b, 3f,** and **3g** exhibited the least pronounced antioxidant properties.

The main structural element determining the polypharmacological profile of this series of hybrid compounds is the 2,6-di-tert-butylphenol fragment. Its incorporation into the structure of *N*-substituted 3-(benzimidazol-2-yl)-chromones and derivatives of 1,3-dimethyl-8-(chromon-3-yl)-xanthine provides not only a pronounced antioxidant effect, comparable to the activity of the standard trolox, but also cytotoxic action against human colorectal cancer cell lines HCT116 and breast cancer MCF7 [10, 26].

The ability of this phenolic fragment to induce cerebroprotective properties is of particular interest. It has been experimentally shown that 4-hydroxy-3,5-di-tert-butylcinnamic acid (at a dose of 100 mg/kg) reduces the degree of neurological deficit in animals, promotes the restoration of mitochondrial membrane potential, normalizes the ratio of aerobic and anaerobic metabolism, and suppresses the activity of caspase-3—a key effector of apoptosis [27].

Furthermore, compounds containing the 4-hydroxy-3,5-di-tert-butylphenyl substituent demonstrate a complex neuroprotective effect. This manifests as the restoration of mitochondrial enzyme activity (aconitase, citrate synthase, and α-ketoglutarate dehydrogenase), as well as a reduction in the pathological accumulation of tau protein in hippocampal tissue [28].



R = Me, R_2 = Br (3a); R = Et, R_1 = NH_2 (3b); R = Me, R_2 = Me (3c); R = Me, R_3 = NHAc (3d); R = Me, R_2 = Ac (3e); R = p-ClPh, R_1 = R_2 = H (3f); R = Et, R_3 = NHAc (3g); R = Me, R_2 = NO_2 (3h).

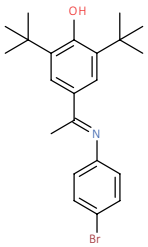
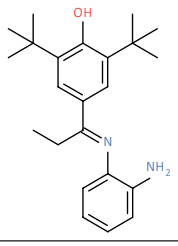
Figure 1 – Synthesis of 2,6-di-tert-butyl-4-[(Z)-C-alkyl-(aryl)-(*N*-phenyl)-azomethine]-phenols (3a–3h).
Note: p-TsOH, p-toluenesulfonic acid.

Table 1 — Physicochemical characteristics of compounds 3a–3h

Compound Code	Yield, %	(THF:DMF)	Mol. mass	Gross formula*
3a	76	145–147	402,34	$C_{22}H_{28}BrNO$
3b	74	132–134	352,48	$C_{23}H_{32}N_2O$
3c	80	147–149	337,47	$C_{23}H_{31}NO$
3d	80	142–144	380.49	$C_{24}H_{32}N_2O_2$
3e	78	100–102	365.48	$C_{23}H_{31}NO_2$
3f	72	185–187	419.95	$C_{27}H_{30}ClNO$
3g	76	128–130	394.52	$C_{25}H_{34}N_2O_2$
3h	80	132–134	383.47	$C_{23}H_{31}N_2O_3$

Note: * according to elemental analysis data (obtained values correspond to calculated values). THF, tetrahydrofuran; DMF, dimethylformamide.

Table 2 — Predicted biological activities of azomethine derivatives of sterically hindered phenol (3a–3h) using the PASS Online Web Resource

Compound Code	Structural Formula of Compound	Biological Activity								
		Ubiquinone cytochrome-c inhibitor	Mucous membrane protector	Aspulvinone dim. inhibitor	Treatment of heart failure	Membrane integrity agonist	Glutathionethiolest. inhibitor	M Inhib. decarbox. dehydrog.	NADP + inhibitor	CYP2J substrate
3a		72	71	65	65	–	55	–	57	–
3b		84	78	79	95	–	64	60	67	71

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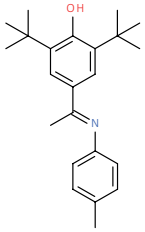
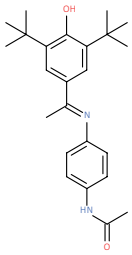
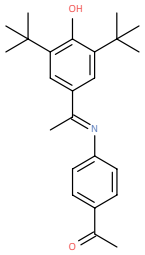
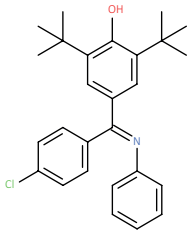
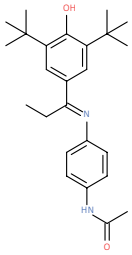
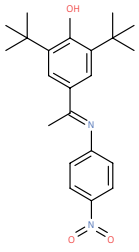
Compound Code	Structural Formula of Compound	Biological Activity								
		Ubiquinone cytochrome-c inhibitor	Mucous membrane protector	Aspulvinone dim. inhibitor	Treatment of heart failure	Membrane integrity agonist	Glutathionethiolest. inhibitor	И Inhib. decarbox. dehydrog.	NADP + inhibitor	CYP2J substrate
3c		73	63	–	59	73	–	–	–	–
3d		71	85	68	69	86	57	58	63	65
3e		83	77	81	78	–	71	73	77	73
3f		73	66	–	69	71	–	–	–	–
3g		75	65	–	89	–	–	–	–	–
3h		89	76	65	56	–	–	–	–	–

Note: the probability of biological activity is characterized by the Pa value in %.

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
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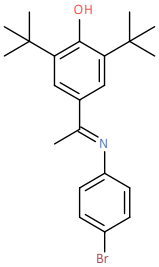
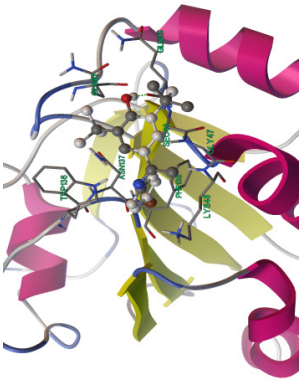
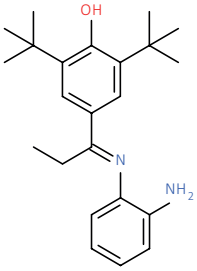
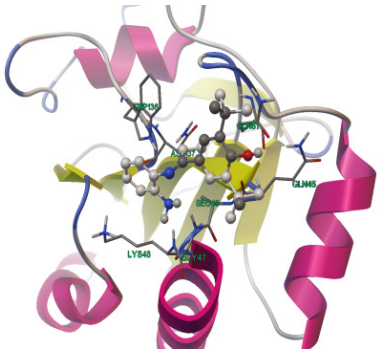
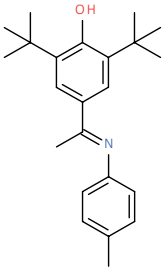
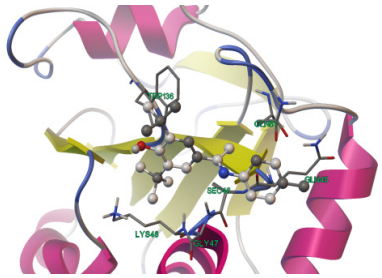
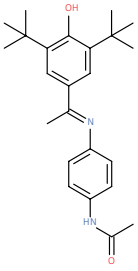
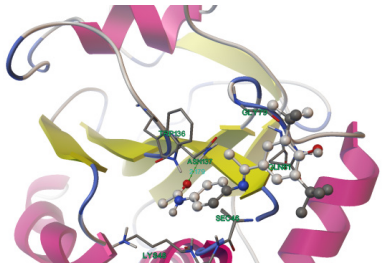
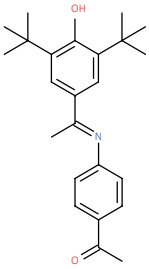
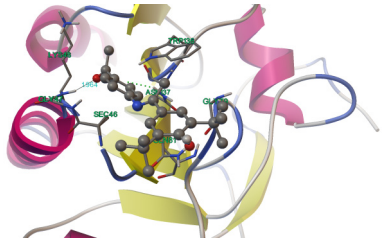
Table 3 – Ligand-enzyme complex formation energies of ligands with glutathione peroxidase-4

Compound Code	Docking Energy, kcal/mol	Ligand Chemical Formula	Ligand Location
3a	-6,02		
3b	-5.51		
3c	-4.49		
3d	-4.54		
3e	-4.67		

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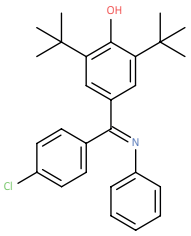
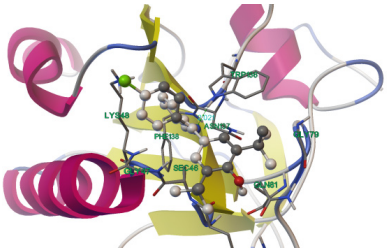
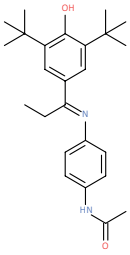
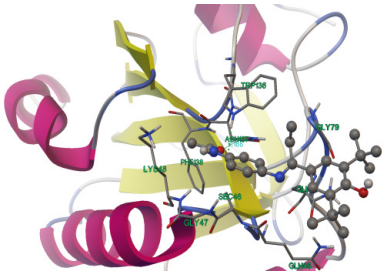
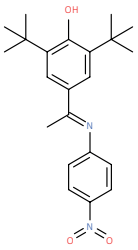
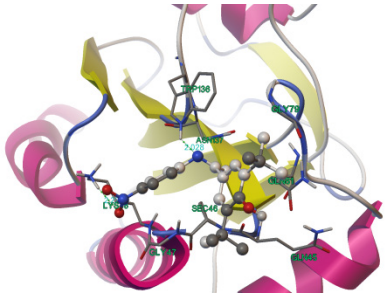
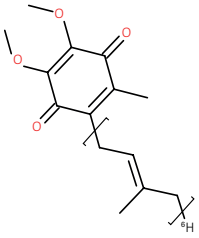
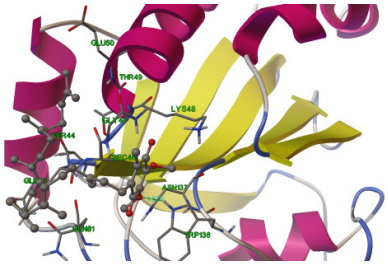
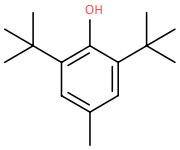
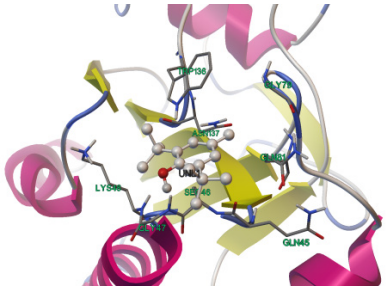
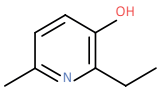
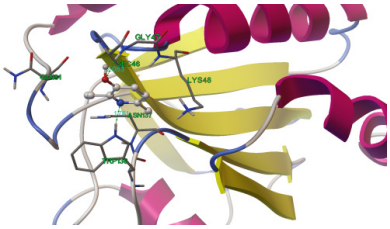
Compound Code	Docking Energy, kcal/mol	Ligand Chemical Formula	Ligand Location
3f	-6.10		
3g	-4.53		
3h	-6.60		
Ubiquinone	-4.23		
Hydroxybutylated toluene	-5.12		
Mexidol	-4.39		

Table 4 — Antioxidant activity of compounds 3a-h and reference substances in the UVI/Fe^{2+} test system

Compound Code	Concentration, mol/L	Optical Density		AOA, Q-ed
		λ= 450 nm	λ=532 nm	
3a	1.0×10^{-3}	0.325 ± 0.004	0.125 ± 0.002	55.0 (5.0)
3b	1.0×10^{-3}	0.352 ± 0.005	0.168 ± 0.003	48.0 (4.4)
3c	1.0×10^{-3}	0.328 ± 0.004	0.152 ± 0.001	52.0 (4.7)
3d	1.0×10^{-3}	0.402 ± 0.006	0.278 ± 0.003	32.0 (2.9)
3e	1.0×10^{-3}	0.431 ± 0.02	0.369 ± 0.01	20.0 (1.8)
3f	1.0×10^{-3}	0.445 ± 0.01	0.375 ± 0.03	18.0 (1.6)
3g	1.0×10^{-3}	0.354 ± 0.005	0.196 ± 0.002	45.0 (4.1)
3h	1.0×10^{-3}	0.454 ± 0.01	0.396 ± 0.02	15.0 (1.4)
Ubiquinone	1.0×10^{-3}	0.354 ± 0.005	0.196 ± 0.002	45.0 (4.1)
Hydroxybutylated toluene	1.0×10^{-3}	0.460 ± 0.003	0.430 ± 0.001	11.0 (1.0)

Note: AOA — antioxidant activity; UVI — ultraviolet irradiation.

Table 5 — Antioxidant activity of compounds 3a-h and reference substances in the Fe^{2+}/H_2O_2 test system

Compound Code	Concentration, mol/L	Optical Density		AOA, Q-ed
		λ= 450 nm	λ=532 nm	
3a	1.0×10^{-3}	0.330 ± 0.004	0.160 ± 0.002	51.0 (3.2)
3b	1.0×10^{-3}	0.355 ± 0.005	0.185 ± 0.003	45.0 (2.8)
3c	1.0×10^{-3}	0.332 ± 0.004	0.168 ± 0.001	50.0 (3.1)
3d	1.0×10^{-3}	0.412 ± 0.006	0.298 ± 0.003	29.0 (1.8)
3e	1.0×10^{-3}	0.448 ± 0.02	0.382 ± 0.01	17.0 (1.1)
3f	1.0×10^{-3}	0.451 ± 0.01	0.389 ± 0.03	16.0 (1.0)
3g	1.0×10^{-3}	0.361 ± 0.005	0.219 ± 0.002	42.0 (2.6)
3h	1.0×10^{-3}	0.460 ± 0.01	0.430 ± 0.02	12.0 (0.7)
Ubiquinone	1.0×10^{-3}	0.450 ± 0.003	0.390 ± 0.001	16.0 (1.0)
Hydroxybutylated toluene	1.0×10^{-3}	0.398 ± 0.005	0.237 ± 0.002	36.5 (2.3)

Note: AOA, antioxidant activity.

Thus, the 2,6-di-tert-butylphenol fragment serves as an effective pharmacophore module, critically important for realizing the potent antioxidant potential of molecules. The obtained experimental data convincingly confirm this role and are in good agreement with known literature findings on the mechanisms of action of sterically hindered phenols.

Study Limitations

Limitations of the characterization method for synthesized azomethine phenols: additional methods (e.g., X-ray diffraction analysis for crystals) might be required to confirm the configuration of the azomethine bond (E/Z isomerism).

Limitations of *in silico* prediction: molecular docking was performed on only one target (HT-4), although biological effects, especially antioxidant effects, may be mediated through other receptors or mechanisms.

The study represents a preliminary screening of a new class of compounds with antioxidant potential. Computer predictions regarding cardiotonic activity and membrane-stabilizing activity require targeted verification on appropriate biological models.

CONCLUSION

An optimized synthesis method allowed for the preparation of 8 derivatives of 2,6-di-tert-butyl-4-[C-alkyl-(aryl)-(*N*-phenyl)-azomethine]-phenols, the structures of which were confirmed by nuclear magnetic resonance, elemental analysis, and IR spectroscopy. According to *in silico* predictions, the studied compounds may possess significant cardiotonic properties, stabilize cell membranes, and increased expression of the CYP2J2 enzyme enhances mitoKATP activation, which is believed to provide physiological benefits by altering reactive oxygen species production. In accordance with the results of molecular docking calculations, it can be assumed that the optimal ligand-receptor interaction energy with HT-4 will be comparable to the values exhibited by ubiquinone, hydroxybutylated toluene, and mexidol.

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DOI: 10.19163/2307-9266-2026-14-2-175-188

During *in vitro* pharmacological screening, the substances under investigation demonstrated pronounced antioxidant activity. The lead compounds are 3a, 3c, and 3h, which surpass the reference compounds – ubiquinone and hydroxybutylated toluene.

FUNDING

This study did not have financial support from third-party organizations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Tamara V. Tsakulova — data curation and formal analysis, investigation, validation, writing—original draft; Ivan P. Kodonidi — conceptualization, validation, data analysis, revision and editing of the manuscript; Alexey S. Chiriapkin — formal analysis; Fatima N. Bidarova — writing—review & editing; Manana T. Kisieva — data curation, validation; Luisa A. Usmanova — data curation. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2026-14-2-189-200

Investigation of toxicological properties and optimal therapeutic doses of compound T1084 with anti-tumor activity

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Received 12 Feb 2026 After peer review 15 Apr 2026 Accepted 20 Apr 2026

Antiangiogenic therapy, despite its effectiveness, is limited by systemic toxicity, the development of organism resistance, and high treatment costs. In this regard, the development of new, safer, and more effective antiangiogenic agents is a relevant task in modern oncology.

The aim. Assessment of toxicological characteristics and experimental substantiation of the optimal range of therapeutic doses of an NOS/PDK inhibitor (compound T1084) for enteral administration.

Materials and methods. The study was conducted on 118 BALB/c mice and 79 F1 hybrids (CBA×C57BL/6j). The acute toxicity of compound T1084 was studied following a single enteral administration. Cumulative effects were assessed using the Lim method with parenteral administration. The optimal range of anti-tumor doses was investigated on a model of Ehrlich's solid carcinoma therapy with subchronic enteral administration of compound T1084 at doses of 200–400 mg/kg.

Results. Parameters of acute toxicity for compound T1084 upon enteral (intragastric) administration were established: LD_{10} — 2031 mg/kg, LD_{16} — 2100 mg/kg, LD_{50} — 2356±15 mg/kg, LD_{84} — 2644 mg/kg. According to toxicological studies, compound T1084, when administered enterally, belongs to hazard class III (moderately hazardous substances) according to GOST 12.1.007–76 and class V according to GOST 32419–2022 for the EAEU. A 5-fold decrease in the toxicity of T1084 was revealed with enteral administration compared to parenteral administration. The absence of cumulative properties in T1084 was established, which allows for prolonged courses of this compound. On the Ehrlich's carcinoma therapy model, a dose-dependent anti-tumor effect was shown: at 200 mg/kg, tumor growth inhibition (TGI) was 15–20%; 300 mg/kg — 28–31%; 400 mg/kg — 30–35%. The absence of significant differences between doses (300 and 400 mg/kg) with more favorable tolerability allowed the selection of 300 mg/kg as the optimal dose.

Conclusion. The obtained data substantiate the promise of preclinical development of an oral dosage form of T1084 for long-term therapy in oncology, including in adjuvant treatment regimens.

Keywords: anti-tumor agents; isothiouronium derivatives; acute toxicity; cumulative effect; optimal doses

Abbreviations: MNs — malignant neoplasms; NO — nitric oxide; NOS — nitric oxide synthase; eNOS — endothelial nitric oxide synthase; iNOS — inducible nitric oxide synthase; DCA — dichloroacetate; PDKs — pyruvate dehydrogenase kinases; ESC — Ehrlich's solid carcinoma; CC — cervical cancer; TGI — tumor growth inhibition.

For citation: A.A. Shitova, M.V. Filimonova, O.V. Soldatova, D.I. Filatova, E.A. Prosovskaya, A.O. Kosachenko, K.A. Nikolaev, A.Yu. Gorbachev, O.S. Izmesteva, V.A. Rybachuk, A.S. Filimonov. Investigation of toxicological properties and optimal therapeutic doses of compound T1084 with anti-tumor activity. *Pharmacy & Pharmacology.* 2026;14(2):189-200. **DOI:** 10.19163/2307-9266-2026-14-2-189-200

Для цитирования: А.А. Шитова, М.В. Филимонова, О.В. Солдатова, Д.И. Филатова, Е.А. Просовская, А.О. Косаченко, К.А. Николаев, А.Ю. Горбачев, О.С. Изместьева, В.А. Рыбачук, А.С. Филимонов. Исследование токсикологических свойств и оптимальных терапевтических доз соединения Т1084 с противоопухолевой активностью. *Фармация и фармакология.* 2026;14(2):189-200. **DOI**: 10.19163/2307-9266-2026-14-2-189-200

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Исследование токсикологических свойств и оптимальных терапевтических доз соединения Т1084 с противоопухолевой активностью

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Получена 12.02.2026 После рецензирования 15.04.2026 Принята к печати 20.04.2026

Антиангиогенная терапия, несмотря на свою эффективность, ограничена системной токсичностью, развитием резистентности организма и высокой стоимостью лечения. В связи с этим разработка новых, более безопасных и эффективных антиангиогенных средств является актуальной задачей современной онкологии.

Цель. Оценка токсикологических характеристик и экспериментальное обоснование оптимального диапазона терапевтических доз NOS/PDK-ингибитора (соединение Т1084) при энтеральном применении.

Материалы и методы. Исследование выполнено на 118 мышах линии BALB/c и 79 гибридах F1 (CBA×C57BL/6j). Острую токсичность соединения Т1084 изучали при однократном энтеральном введении. Кумулятивное действие оценивали по методу Лима при парентеральном введении. Оценку диапазона оптимальных противоопухолевых доз проводили на модели терапии солидной карциномы Эрлиха при субхроническом энтеральном введении соединения Т1084 в дозах 200–400 мг/кг.

Результаты. Установлены параметры острой токсичности для соединения Т1084 при энтеральном (внутрижелудочном) введении: $ЛД_{10}$ — 2031 мг/кг, $ЛД_{16}$ — 2100 мг/кг, $ЛД_{50}$ — 2356±15 мг/кг, $ЛД_{84}$ — 2644 мг/кг. По данным токсикологических исследований соединение Т1084 при энтеральном применении относится к III классу опасности (умеренно опасные вещества) по ГОСТ 12.1.007–76 и V классу по ГОСТ 32419–2022 для ЕАЭС. Выявлено 5-кратное снижение токсичности Т1084 при энтеральном применении по сравнению с парентеральным введением. Установлено отсутствие кумулятивных свойств у Т1084, что позволяет применять это соединение продолжительными курсами. На модели терапии карциномы Эрлиха показан дозозависимый противоопухолевый эффект: при 200 мг/кг торможение роста опухоли (ТРО) составило15–20%; при 300 мг/кг — 28–31%; при 400 мг/кг — 30–35%. Отсутствие значимых различий между дозами (300 и 400 мг/кг) при более благоприятной переносимости позволило выбрать дозу 300 мг/кг как оптимальную.

Заключение. Полученные данные обосновывают перспективность доклинической разработки пероральной лекарственной формы Т1084 для длительной терапии в онкологии, в том числе в схемах адъювантного лечения.

Ключевые слова: противоопухолевые средства; производные изотиуронии; острая токсичность; кумулятивное действие; оптимальные дозы

Список сокращений: ЗНО — злокачественные новообразования; NO — оксид азота; NOS — синтаза оксида азота; eNOS — эндотелиальная синтаза оксида азота; iNOS — индуцибельная синтаза оксида азота; DCA — дихлорацетат; PDK — киназы пируватдегидрогеназы; СКЭ — солидная карцинома Эрлиха; РШМ-5 — рак шейки матки; ТРО — торможение роста опухоли.

INTRODUCTION

Malignant neoplasms (MNs) remain a leading cause of mortality and disability in the Russian Federation, being the second most frequent cause of death after cardiovascular diseases. According to official data, 698,693 cases of MN[1] were newly diagnosed in 2024, with the number of cancer patients continuing to grow steadily each year. Despite the progress made in early diagnosis and treatment methods, the problem of effective therapy for many common and metastatic forms of cancer remains relevant. In modern anti-tumor therapy regimens, anti-angiogenic drugs, which act by suppressing tumor tissue vascularization [1, 2], play an important role. However, the clinical use of these drugs is limited by several factors, including

[1] Kaprin AD, Starinsky VV, Shakhzadova AO. The state of oncological care for the Russian population in 2024. Moscow: Herzen Moscow Medical Research Institute – branch of the NMITS of Radiology; 2025. 275 p. Russian

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DOI: 10.19163/2307-9266-2026-14-2-189-200

insufficient therapeutic efficacy, the development of systemic side effects, and high treatment costs [3, 4]. Furthermore, tumor cells have the ability to adapt to hypoxic conditions by activating alternative metabolic pathways and developing resistance to therapy [5]. These circumstances underscore the importance of searching for and developing new anti-angiogenic agents with good tolerability and the ability to overcome therapeutic resistance.

The discovery in the 1980s–1990s of the role of nitric oxide (NO) as a universal signaling molecule significantly expanded the understanding of tumor angiogenesis mechanisms [6, 7]. NO/eNOS signaling plays a key role in tumor angiogenesis [8]. Under the influence of pro-angiogenic factors (VEGF, bFGF, etc.), endothelial NO synthase (eNOS) expression is activated in endothelial cells, leading to a sustained increase in NO production. The resulting NO acts as a pleiotropic mediator, initiating a cascade of events necessary for new vessel formation. These include: relaxation of vascular smooth muscle cells (vasodilation), increased vascular wall permeability through remodeling of endothelial junctions, and stimulation of endothelial cell migration and proliferation [9]. Collectively, these processes ensure the formation of a functional vascular network that nourishes tumor tissue. A logical consequence of these discoveries was the strategy of pharmacologically suppressing angiogenesis by non-specific inhibition of endogenous NO synthesis with chemical NOS inhibitors. In the laboratory of radiation pharmacology of the Medical Radiological Research Center named after A.F. Tsyb, anti-angiogenic activity was demonstrated for a number of thioamidine NOS inhibitors—N,S-substituted isothiouronium derivatives. In particular, studies have shown that competitive inhibitors of iNOS/eNOS from the isothiouronium class, when administered parenterally chronically at safe doses (1/9–1/5 LD_{16}), suppress the growth and metastasis of solid mouse tumors [10]. However, as with pharmacopoeial anti-angiogenic drugs, prolonged use of NOS inhibitors is accompanied by neoplasm adaptation and the development of therapeutic resistance [11].

A significant number of modern studies are dedicated to finding ways to overcome hypoxic resistance that arises during anti-angiogenic therapy. One of the most studied agents used for these purposes is the structural analog of pyruvate, sodium dichloroacetate (DCA)—the only pyruvate dehydrogenase kinase inhibitor that has completed Phase I and II clinical trials [12, 13]. DCA reactivates the pyruvate dehydrogenase complex, switching tumor cell metabolism from aerobic glycolysis to oxidative phosphorylation, which is accompanied by the accumulation of reactive oxygen species to a level sufficient to cause a toxic effect in the tumor cell [14].

The ability of thioamidine NOS inhibitors (weak bases) to form salts with strong acids, particularly with dichloroacetic acid, has opened up the possibility of creating hybrid molecules. The implementation of this combination led to the synthesis of compound T1084 (1-isobutanoyl-2-isopropylisothiourea dichloroacetate), which combines NOS- and PDK-inhibiting fragments in its structure. In the resulting molecule, NOS inhibition contributes to a vasoconstrictor effect and suppression of angiogenesis [10], while PDK blockade disrupts the metabolic adaptation of tumor cells to hypoxic conditions, inducing their apoptosis [15]. Due to this bifunctional action, compound T1084, when administered parenterally at a safe dose of 70.7 mg/kg (1/4 LD_{10}), not only suppresses the growth of experimental tumors (Ehrlich's carcinoma, cervical cancer (RShM-5), B-16 melanoma) but also prevents the development of hypoxic resistance in neoplasms [16], and enhances the antitumor effects of single and fractionated γ-irradiation [17].

As the compound T1084 has demonstrated pronounced antitumor activity upon parenteral administration (1–2 week course), extrapolating such a regimen to clinical application requires selecting a route and method of administration optimal for long-term therapy. The widespread use of oral dosage forms in standard adjuvant regimens (capecitabine for breast cancer, temozolomide for gliomas, tyrosine kinase inhibitors for various solid tumors, etc.)[2,3,4] confirms that the enteral route is preferred for prolonged treatment. Oral administration allows for outpatient therapy, eliminates the need for invasive procedures, reduces the risk of infectious complications, and lessens the psychological burden on patients [18], which is particularly important for long-term adjuvant treatment courses. In this regard, preclinical studies of T1084 are focused on developing an oral dosage form of this compound.

THE AIM. To investigate the toxicological characteristics and experimentally substantiate the

[2] Clinical Guidelines "Breast Cancer". Ministry of Health of the Russian Federation. Available from: https://cr.minzdrav.gov.ru/preview-cr/379_4. Russian
[3] Clinical Guidelines "Glioblastoma". Ministry of Health of the Russian Federation. Available from: https://cr.minzdrav.gov.ru/recomend/652
[4] Clinical Guidelines "Lung Cancer". Ministry of Health of the Russian Federation. Available from: https://cr.minzdrav.gov.ru/preview-cr/30_5

optimal antitumor therapeutic doses of the NOS/PDK inhibitor T1084 upon enteral administration.

MATERIALS AND METHODS

Object of study

The object of study is an antitumor bifunctional agent based on 1-isobutanoyl-2-isopropylisothiourea dichloroacetate (compound T1084), which was theoretically substantiated and synthesized in the laboratory of radiation pharmacology at the Medical Radiological Research Center (MRRC; Obninsk). The structure of the compounds was confirmed by elemental analysis (C, H, N) on an EA 1108 analyzer (Carlo Erba Instruments, Italy) and 1H, 13C NMR spectroscopy on an AVANCE AV 300 Fourier spectrometer (Bruker, Germany). The purity of T1084 was controlled by thin-layer, high-performance liquid, and gas chromatography, and melting point [16]. Thin-layer chromatography was performed on Silufol UV-254 plates in a benzene–ethanol–triethylamine (9 : 1 : 0.1) system. HPLC studies were conducted on a Hitachi Chromaster HPLC System (Hitachi High-Tech Corp., USA). Melting point was measured on a PTP-M automatic heating system (LOIP, Russia).

Laboratory animals

The studies were performed on 118 male Balb/c mice and 79 female F1 (CBAxC_{57}Bl/6j) mice aged 3–4 months and weighing 22–25 g. The laboratory animals were obtained from the nursery of the Scientific Center for Biomedical Proteins and were housed in the vivarium of the MRRC under conditions compliant with sanitary and epidemiological rules for housing experimental biological clinics (vivariums)[5]. The mice were housed in T-3 cages with sterile wood bedding, 5–10 animals per cage, with free access to water and standard complete feed PK-120-1 (Laborator-Korm LLC, Russia). The cages were located in a room at a temperature of 18–20°C, humidity of 40–70 %, and natural lighting. Animals without signs of health deviations were selected for the studies. All animal work was approved by the Bioethics Committee of the National Medical Research Center of Radiology (protocol No. 1-D-00041, dated October 20, 2023). Upon completion of the studies, experimental animals were euthanized in accordance with bioethical norms using a CO_2 euthanizer (Aw-Tech, Russia).

[5] SP 2.2.1.3218-14. Sanitary and epidemiological requirements for the establishment, equipment and maintenance of experimental biological clinics (vivariums); approved by Resolution No. 51 of the Chief State Sanitary Doctor of the Russian Federation dated August 29, 2014; Moscow: Rospotrebnadzor; 2014. 23 p. Russian

Acute toxicity of T1084 upon enteral administration

The acute toxicity of compound T1084 was assessed after a single enteral (intragastric, ig) administration using a two-stage protocol. In the first stage (preliminary), the approximate LD_{50} was determined in 10 Balb/c mice using the Deichmann and Leblanc method[6]. In the second stage (detailed), 48 Balb/c mice were divided into 7 groups (6–12 animals each) and received doses above and below the approximate LD_{50} (in the dose range of 2000–3000 mg/kg). Compound T1084 was administered intragastrically as a 10 % solution, freshly prepared in water for injection (Dalchimpharm OJSC, Russia). Steel curved gastric tubes (GK Vivarium, Russia) were used for administration. Acute toxicity parameters (LD_{10}, LD_{16}, LD_{50}, and LD_{84}) were calculated using Litchfield and Wilcoxon probit analysis[7]. Animals were observed continuously for the first 4–6 hours after administration, and then daily for 14 days. During these periods, the general condition of the animals was visually assessed based on a combination of clinical signs (behavior, appetite, motor activity, coat condition).

Cumulative action of T1084 upon parenteral administration

The cumulative action of T1084 was assessed upon parenteral administration (intraperitoneal, ip) of the compound according to the method of Lima et al. in accordance with the guidelines for preclinical studies[8]. The method is based on recording mortality rates with stepwise dose increases every four days, starting from 0.1 LD_{50} up to 0.75 LD_{50}. The study design was developed based on previously established parameters of acute toxicity of T1084 after single parenteral administration to mice and is presented in Table 1 [15]. In the experiment, 60 male Balb/c mice were divided into 6 groups (n = 10) according to the number of doses and time points studied. After each administration stage, one group was set aside for mortality assessment, and the remaining animals continued administration according to the study scheme (Table 1). Compound T1084 was administered ip to the animals as 0.4–2.5 % solutions prepared using water for injection.

[6] Deichmann WB, LeBlanc TJ. Determination of the approximate lethal dose with about six animals. J Ind Hyg Toxicol. 1943;25:415–417.
[7] Litchfield JT Jr, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther. 1949;96(2):99–113.
[8] Mironov AN. Guidelines for conducting preclinical studies of medicines. Part 1. Moscow: Grif and K; 2012. 944 p. Russian

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Precise toxicometric parameters (LD_{10}, LD_{16}, LD_{50}, and LD_{84}) were calculated using Litchfield and Wilcoxon probit analysis[9]. The cumulative index (Kk) was determined as the ratio of the cumulative LD_{50} upon repeated administration to the LD_{50} upon single administration.

Experimental evaluation of the optimal antitumor dose range of T1084

The experimental evaluation of the optimal dose range of T1084 was conducted on a model of solid Ehrlich's carcinoma (SEC) therapy in 79 female F1 ($CBAxC_{57}Bl/6j$) mice. The tumor was transplanted by subcutaneous injection into the thigh of 2.5×10^6 cells in 0.2 mL of medium 199 (Pan-Eco, Russia). On the 7 day after transplantation, when the tumor nodules reached reliably measurable sizes (100–150 mm^3), the animals were divided into 5 groups (control and 4 experimental) of 17–21 individuals each. From this day until the 21 day of tumor growth, mice in the experimental groups received compound T1084 daily ig at doses of 200–400 mg/kg (2–4 % solutions), and mice in the control group received a daily ig injection of 0.9 % sodium chloride solution.

The antitumor effect was assessed morphometrically by the dynamics of relative tumor volumes and growth inhibition in accordance with the methodology described in [16]. Standard parameters of variation statistics were calculated for the experimental data, and their values are presented graphically as M ± SD. The significance level of intergroup differences in the evaluated indicators was determined using non-parametric criteria with the Kruskal-Wallis ANOVA test and the post-hoc Mann-Whitney U-test according to the Holm-Bonferroni multiple comparison procedure. Differences were considered significant at $p < 0.05$. Statistical calculations were performed using the Statistica 10.0 software package (StatSoft Inc., USA).

RESULTS

Acute toxicity upon enteral administration

In the acute toxicity test, it was established that the clinical picture of intoxication after a single intragastric administration of compound T1084 was dose-dependent. At doses of 2000–2300 mg/kg, intoxication in animals was weakly expressed and manifested in the first minutes after administration as reduced motor activity, lying down, and weak response to external stimuli. With an increase in dose to 2300–3000 mg/kg, toxic effects were more pronounced and appeared within 10–20 minutes after administration of the compound, including tachypnea, respiratory arrhythmia, ataxia, tremor, and clonic seizures. Lethal outcomes at toxic doses were recorded within the first 20–60 min after administration of compound T1084. In surviving animals, signs of intoxication began to regress after 2 hours, with complete disappearance of neurological symptoms by 4–5 hours of observation. In the long-term period (24 hours and throughout the subsequent 14 days), surviving mice did not differ visually from the control group of intact animals in their general condition, which, overall, indicated the absence of residual toxic effects and restoration of bodily functions.

The data on the lethal effect of compound T1084 after single intragastric administration to BALB/c mice, obtained in the two stages of the study, are presented in Table 2.

Using the Litchfield and Wilcoxon probit analysis method, toxicometric parameters of T1084 upon intragastric administration were established, which are presented in Table 3. The LD_{50} of the studied compound was 2356 ± 15 mg/kg, which corresponds to Hazard Class III (moderately hazardous substances) according to GOST 12.1.007-76 (LD50 upon gastric administration—from 151 mg/kg to 5000 mg/kg) and Class V according to GOST 32419-2022 for EAEU ($2000 < LD_{50} < 5000$ mg/kg, ig).

Cumulative effect of compound T1084 upon parenteral administration

The study of the cumulative effect of T1084 allowed for the assessment of its subchronic application. Upon prolonged parenteral administration of T1084 in increasing doses, the first signs of intoxication and cases of animal death were recorded on the 15 day of the experiment after reaching a cumulative dose of 1296.2 mg/kg. Cumulative doses and mortality rates are presented in Table 4.

Toxicometric parameters of T1084, determined by the Litchfield and Wilcoxon probit analysis method, are presented in Table 5. It was established that the LD_{50} upon repeated parenteral administration of T108 mg/kg was 2227 ± 15 mg/kg. The calculated cumulative coefficients significantly exceeded unity for all lethality levels, indicating the absence of cumulative properties in the multitarget compound T1084.

[9] Litchfield JT Jr, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther. 1949;96(2):99–113.

Table 1 — Scheme for studying the cumulative properties of the multitarget compound T1084 in BALB/c mice

Groups, days of administration	Number of animals		Doses administered	
	Per group	Remaining for subsequent doses	Fraction of LD_{50}	Upon parenteral administration (mg/kg)
1–4	10	60	0.1	44.7
5–8	10	50	0.15	67.1
9–12	10	40	0.22	98.3
13–16	10	30	0.34	152.0
17–20	10	20	0.5	223.5
21–24	10	10	0.75	335.3

Table 2 — Toxicity assessment of compound T1084 after single enteral (ig) administration to Balb/c mice

T1084 doses, mg/kg	Died mice / number of mice in group
First stage of study (preliminary)	
2000	0 / 2
2300	1 / 2
2600	2 / 2
3000	2 / 2
3500	2 / 2
Second stage of study (detailed)	
2000	0 / 6
2125	1 / 6
2375	4 / 8
2500	9 / 12
2750	7 / 8
3000	8 / 8

Note: Increasing the sample size in the region close to LD_{50} improves the accuracy of toxicometric parameter calculation and reduces the standard error.

Table 3 — Acute toxicity parameters of T1084 after single enteral (ig) administration to Balb/c mice

Compound	LD_{10}		LD_{16}		LD_{50}		LD_{84}	
	mg/kg	mM/kg	mg/kg	mM/kg	mg/kg	mM/kg	mg/kg	mM/kg
T1084 i.v.	2031	6.40	2100	6.61	2356 ± 15	7.40	2644	8.33

Table 4 — Cumulative doses and mortality rates of male BALB/c mice receiving parenteral injections of T1084

Groups, days of administration	Test doses, administered over 4 days		Planned maximum cumulative doses		Number of deaths/ number in group	Individual cumulative doses causing death	
	mg/kg	fraction of LD_{50}	mg/kg	fraction of LD_{50}		mg/kg	fraction of LD_{50}
1–4	44.7	0.10	178.8	0.4	0/10	–	–
5–8	67.1	0.15	447.0	1.0	0/10	–	–
9–12	98.34	0.22	840.6	1.88	0/10	–	–
13–16	152.0	0.34	1448.6	3.24	1/10	1296.2	2.90
17–20	223.5	0.50	2342.6	5.24	0/10	–	–
21–24	335.3	0.75	3683.8	8.24	5/10 7/10 10/10	2581.1 2677.5 3012.8	5.78 5.99 6.74

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Table 5 — Toxicity of T1084 after prolonged parenteral administration to male BALB/c mice in increasing doses

Compound	Toxicity indicators, mg/kg			
	LD_{10}	LD_{16}	LD_{50}	LD_{84}
T1084 ig, once*	302	330	448±13	608
T1084 ig, multiple	1410	1562	2227±15	3174
K_{cum}	4.67	4.73	4.97 (3.77 ÷ 6.97)	5.22

Note: * parameters of acute toxicity of T1084 with parenteral administration, obtained earlier [16]; K_{cum}, accumulation coefficient.

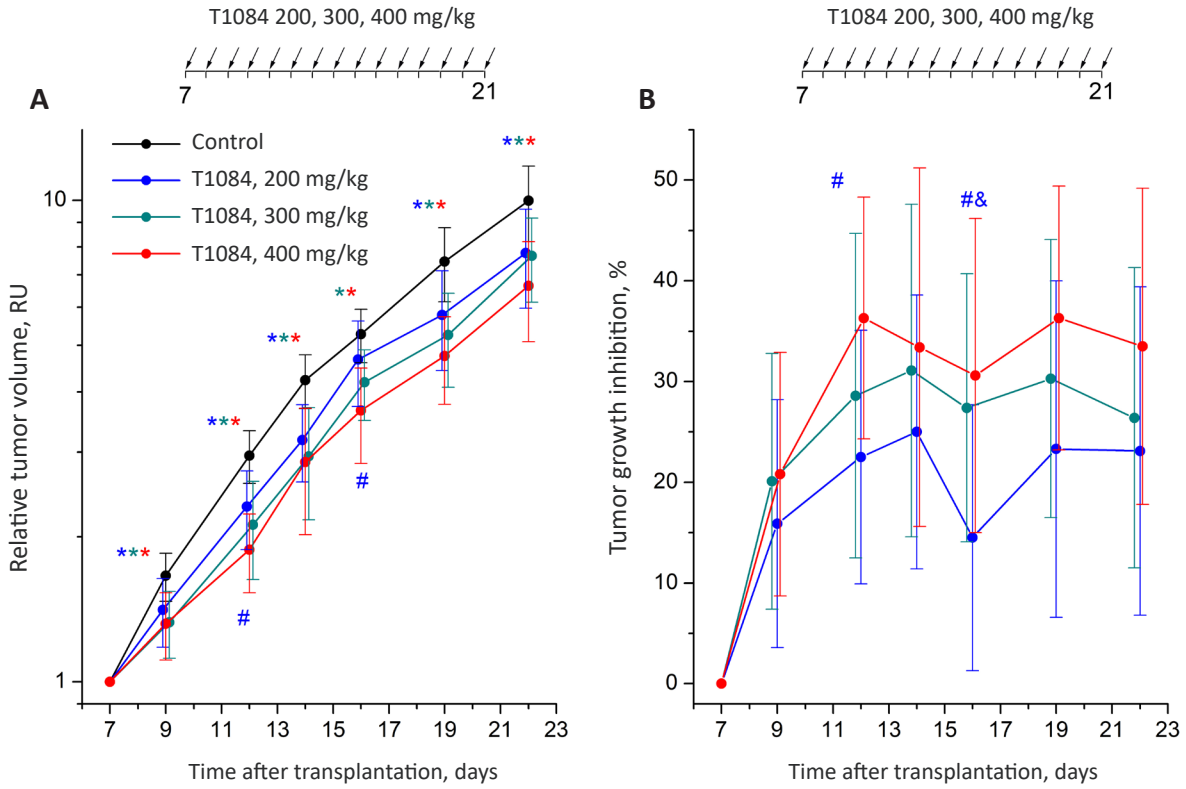

Figure 1 — Effect of T1084 compound administered chronically enterally at doses of 200–400 mg/kg on the growth of solid Ehrlich carcinoma in female F1 (CBA×C57BL6j) mice.

Note: A, tumor growth curves in animal groups. Values for each mouse are normalized to the tumor volume before the start of interventions. Graphical deviations correspond to SD (n = 17–21). * statistically significant differences between experimental groups and control (symbol color corresponds to the compared curve): Control/T1084 200 mg/kg (p = 0.008344, p = 0.000180, p = 0.000042, p = 0.004452, p = 0.004880), Control/T1084 300 mg/kg (p = 0.000330, p = 0.000040, p = 0.000045, p = 0.000290, p = 0.000040, p = 0.001005), Control/T1084 400 mg/kg (p = 0.000156, p < 0.000001, p = 0.000120, p = 0.004456, p = 0.000006, p = 0.000012); # between groups T1084 400 mg/kg and T1084 200 mg/kg (p = 0.002109, p = 0.032400). B, dynamics of tumor growth inhibition (TGI) in animal groups; & statistically significant differences between groups T1084 300 mg/kg and T1084 200 mg/kg (p = 0.046870); # statistically significant differences between groups T1084 400 mg/kg and T1084 200 mg/kg (p = 0.002109, p = 0.003270).

Experimental evaluation of the optimal dose range of T1084 in a mouse model of Ehrlich carcinoma therapy

The conducted study on a model of Ehrlich carcinoma therapy established that the compound T1084, administered subchronically via intragastric instillation (14 injections), exerted a statistically significant antitumor effect at all observation stages (p < 0.05) (Fig. 1 A, B). The intensity of these effects was dose-dependent. At the minimum tested dose (200 mg/kg), a generally weak antitumor effect was observed, accompanied by a 15–20 % inhibition of tumor growth. Increasing the dose to 300 mg/kg enhanced the antitumor activity of T1084: the effect was more pronounced and stable, maintaining a significant level throughout all observation periods (p = 0.0003–0.001). At this dose, T1084 inhibited tumor growth by 28–31 %. The level of antitumor effects of T1084 at this dose was comparable to the efficacy of parenteral administration of this compound at a dose of 70.7 mg/kg, as established in earlier studies [16]. Further increasing the dose of T1084 to 400 mg/kg

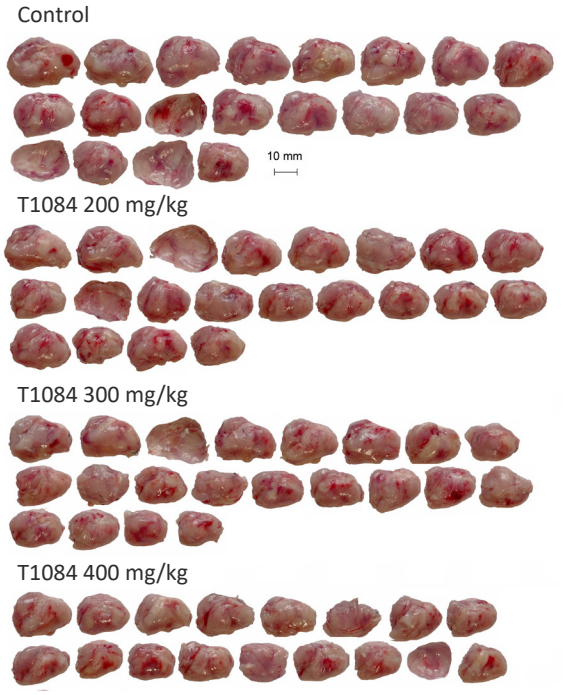

Figure 2 — Macroscopic appearance of solid Ehrlich carcinoma tumor nodules excised from mice in experimental groups on the 22 day of growth.

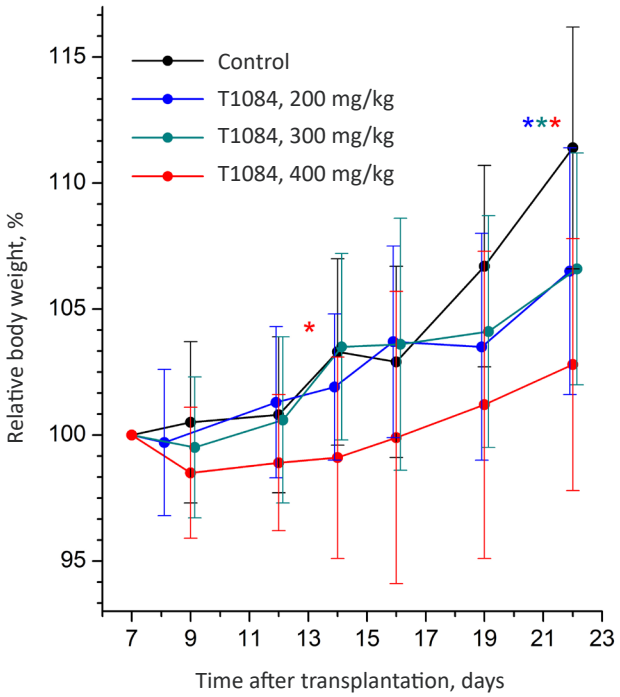

Figure 3 — Effect of T1084 compound administered chronically enterally at doses of 200–400 mg/kg on the dynamics of relative body weight in tumor-bearing mice.
Note: * statistically significant differences between experimental groups and control ($p < 0.05$).

did not result in a significant increase in antitumor efficacy: the maximum tumor growth inhibition was 30–35 %, which did not significantly differ from the rates obtained with T1084 at a dose of 300 mg/kg.

Macroscopic evaluation of tumor nodules excised on the 22 day after transplantation (Fig. 2) confirmed the dose-dependent nature of the compound's antitumor action: in groups of mice receiving T1084 at doses of 300 mg/kg and 400 mg/kg, the tumor nodules were visually smaller than in the control group, but, overall, showed little difference between them.

Evaluation of therapy tolerability based on body weight dynamics showed (Fig. 3) that at doses of 200 mg/kg and 300 mg/kg, a slight (3–5 %) decrease in body weight gain was observed, which was recorded only at later observation stages (days 19–23). At these doses, the observed decrease in body weight gain was not life-threatening for the animals. With an increase in the T1084 dose to 400 mg/kg, changes in this parameter became more noticeable and were recorded earlier. By the end of the experiment, the body weight of mice in this group was approximately 10 % less than that of control and experimental animals, which could most likely be interpreted as an initial sign of T1084 toxicity.

Considering the comparable antitumor efficacy of doses 300 and 400 mg/kg (TGI 26.4–31.5 % and 30–36 % respectively) with a more favorable tolerability profile, the optimal therapeutic dose of T1084 for intragastric administration is 300 mg/kg. Further increasing the dose of T1084 to 400 mg/kg proved to be inexpedient: no significant enhancement of the antitumor effect was observed, but signs of compound toxicity were already evident.

DISCUSSION

More than half a century has passed since J. Folkman's fundamental discovery (1971) of the role of angiogenesis in tumor progression [19] and the emergence of the first anti-angiogenic drug, bevacizumab (2004) [20]. During this time, the arsenal of anti-angiogenic agents has significantly expanded, and their clinical use has become routine practice for oncologists. However, as numerous studies [21–23] indicate, the initial expectations for anti-angiogenic therapy have not been met. The efficacy of monotherapy with anti-angiogenic agents remains moderate, and survival improvements in combination regimens rarely exceed a few months [24]. The main obstacle to achieving a sustained clinical response, according to many researchers [25–27], is the rapid development of tumor resistance to the hypoxic microenvironment induced by anti-angiogenic treatment.

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A possible way to increase the stability and efficacy of anti-angiogenic cancer therapy may be the use of anti-vascular drugs in combination with hypoxia-oriented cytotoxins, which primarily target hypoxic tumor cells. The idea of combining these agents found experimental confirmation in the work of J.H. Baker et al., where the combined use of the NOS inhibitor L-NNA with the bioreductive hypoxic toxin tirapazamine led to an enhanced antitumor effect [28]. In our own studies, the promise of this strategy was confirmed with the use of a NOS inhibitor from the class of N,S-substituted isothiouronium derivatives and sodium dichloroacetate. The combined use of these agents not only enhanced the antitumor effect but also blocked the development of hypoxic resistance in Ehrlich carcinoma (TGI 50–55 % throughout the observation period) [29]. The obtained results formed the basis for creating a new bifunctional molecule, T1084, based on these compounds, combining anti-angiogenic (NOS-inhibiting) and hypoxia-oriented cytotoxic (PDK-inhibiting) activity.

Most modern anti-angiogenic drugs, particularly tyrosine kinase inhibitors, are intended for course-based oral administration in the treatment regimens for many common oncological diseases [30]. The multi-target anti-angiogenic compound T1084 developed by us showed its efficacy with course-based parenteral administration [16]; however, its activity via the enteral route of administration remained unstudied. In this regard, the aim of the present work was to conduct a toxicological evaluation and provide experimental justification for the optimal therapeutic dose of T1084 for enteral administration. The established parameters of acute toxicity of T1084 upon intragastric administration (LD_{50} 2356 ± 15 mg/kg) correspond to Class III (moderately hazardous substances) and Class V hazard[10,11]. Comparison of these parameters with previously obtained data for parenteral administration of T1084 (LD_{50} 447 mg/kg) revealed a significant, 5-fold reduction in the toxic effect of T1084 upon intragastric administration compared to its intraperitoneal administration. This difference may be due to peculiarities of the compound's absorption and metabolism in the gastrointestinal tract [31]. Such a dependence of toxicity on the route of administration is a favorable factor for the development of an oral dosage form of T1084, as it allows for a reduction in the risk of systemic side effects while maintaining therapeutic efficacy. The obtained results are generally consistent with literature data on the more favorable safety profile of oral drug forms [32, 33].

An important result of the work is the experimental confirmation of the absence of cumulative toxicity in T1084. The obtained cumulative value (4.97) significantly exceeds unity and indicates not only the absence of accumulation of toxic effects but also the development of an adaptive response of the organism upon repeated administration of the compound in increasing doses. The absence of cumulative toxicity is an important advantage of T1084 and favorably distinguishes the developed compound from many classical cytostatic and anti-angiogenic agents, the use of which is limited by the development of cumulative dose-dependent toxic effects [34]. For example, cumulative cardiotoxicity is known for tyrosine kinase inhibitors (sunitinib, sorafenib), which limits the duration of therapy and requires careful monitoring of the cardiovascular system function [35, 36]. For bevacizumab and other anti-VEGF agents, cumulative effects such as proteinuria, arterial hypertension, and coagulation disorders are characteristic, which increase with prolonged use [37]. The conducted assessment of the cumulative properties of T1084 indicates a low risk of toxic effect accumulation with repeated use of this agent, and the results obtained in this study open prospects for the use of T1084 in long-term adjuvant therapy regimens.

In the Ehrlich carcinoma model, the optimal therapeutic dose of T1084 for enteral administration was established as 300 mg/kg. At a dose of 200 mg/kg, the antitumor effect of T1084 was insufficiently effective (TGI 15–20 %). Increasing the dose to 300 mg/kg was accompanied by a significant increase in antitumor activity (TGI 28–31 %), whereas further increase to 400 mg/kg did not lead to a substantial enhancement of the effect (TGI 30–35 %) but caused the development of initial signs of toxicity. It is important to note that the tumor growth inhibition (28–31 %) achieved with enteral administration at a dose of 300 mg/kg is practically comparable to the efficacy of parenteral administration of T1084 at a dose of 70.7 mg/kg, which confirms the preservation of T1084's activity upon oral administration. For comparison, when switching from intravenous to oral administration of etoposide, its efficacy can decrease by 20–40 % due to variable drug bioavailability [38]. In the case of T1084, there is practically no loss of efficacy.

When recalculating the optimal therapeutic dose of T1084 for other animal species and humans

[10] GOST 12.1.007-76. A system of occupational safety standards. Harmful substances. Classification and general safety requirements. Introduction. 1977-01-01. Moscow: Standartinform; 2007. Available from: https://docs.cntd.ru/document/5200233. Russian

[11] GOST 32419-2022. Classification of chemical product hazards. General requirements. Introduction. 2023-01-01. Moscow: Russian Institute of Standardization; 2023. Available from: https://www.gostinfo.ru/catalog/ Details/?id=7023565. Russian

(considering body surface area)[12], the equivalent doses were: for rats—150 mg/kg, for rabbits—75 mg/kg, for humans—24 mg/kg (1700 mg/person for a body weight of 70 kg). The obtained data form the necessary foundation for transitioning to the next stages of preclinical development of this agent, intended for adjuvant therapy of oncological diseases (evaluation of specific activity and pharmacokinetics).

Study Limitations

This work has several limitations that should be considered when interpreting the results. The acute toxicity of T1084 was assessed in only one animal species (male BALB/c mice), whereas according to the methodology of preclinical studies, a complete toxicological characterization requires studies in two animal species (mice and rats) of both sexes, with an assessment of possible sex-related differences in sensitivity to the compound. Cumulative effects were studied with parenteral, not enteral, administration; pharmacokinetic parameters (bioavailability, metabolism, tissue distribution) were not evaluated. The indicated limitations define the directions for further research on T1084, but do not diminish the significance of the obtained results.

[12] Deichmann W.B., LeBlanc T.J. Determination of the approximate lethal dose with about six animals.J Ind Hyg Toxicol. 1943;25:415–417.

CONCLUSION

In the conducted toxicological studies, the LD50 value for the multitargeted antitumor agent based on 1-isobutanolyl-2-isopropylisothiourea dichloroacetate (compound T1084) upon enteral administration was determined to be 2356 ± 15 mg/kg. This value corresponds to hazard class III according to GOST 12.1.007-76 (moderately toxic substances) and hazard class V according to GOST 32419-2022 for the EAEU. A significant reduction in toxicity was demonstrated with enteral administration of T1084 compared to parenteral administration of this compound. The absence of cumulative effects for T1084 was experimentally confirmed, which justifies its subchronic use in adjuvant therapy regimens without the risk of toxic effect accumulation. On the model of solid Ehrlich's carcinoma, the optimal enteral therapeutic dose of 300 mg/kg was established, providing a pronounced antitumor effect (tumor growth inhibition of 28–31 %) with satisfactory therapy tolerability.

FUNDING

The study was performed within the framework of the state assignment of the Ministry of Health of the Russian Federation «Pharmaceutical development and preclinical studies of a complex antiangiogenic and hypoxia-oriented antitumor agent based on a bifunctional NOS/PDK inhibitor» CAHN-2026-0003, Registration number is I126011334704-2.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Anna A. Shitova — conceptualization, investigation, data analysis, visualization, writing—original draft, writing—review & editing; Marina V. Filimonova, Alexandr S. Filimonov — conceptualization, data analysis, visualization, writing—original draft, writing—review & editing; Daria I. Filatova, Ekaterina A. Prosovskaya — investigation, writing—original draft; Olga V. Soldatova, Vitaly A. Rybachuk, Aleksandr O. Kosachenko, Kirill A. Nikolaev, Alexander Yu. Gorbachev — investigation, data analysis, validation; Olga S. Izmesteva — data analysis, visualization. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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Научно-практический журнал
ФАРМАЦИЯ И ФАРМАКОЛОГИЯ
(PHARMACY & PHARMACOLOGY)

ОРИГИНАЛЬНАЯ СТАТЬЯ

DOI: 10.19163/2307-9266-2026-14-2-201-213

Development of the Composition and Technology for Obtaining Mini-Tablets of Propranolol Hydrochloride Using the Quality by Design Approach

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Received 12 Jan 2026 | After peer review 16 Apr 2026 | Accepted 24 Apr 2026

Infantile hemangioma (IH) is a benign vascular neoplasm, occurring in 4–10 % of newborns and requiring timely therapy in cases of complicated progression. Currently, propranolol is recognized as the “gold standard” for IH treatment due to its proven efficacy and safety. However, in the Russian Federation, there are no readily available dosage forms (DFs) of propranolol for children, which creates a significant problem for pediatric practice. In this regard, the development of a DFs that ensures accurate dosing and ease of use in children is relevant.

The aim. To develop the composition and technology for obtaining orodispersible mini-tablets (OMT) of propranolol hydrochloride for children using the Quality by Design (QbD) approach.

Materials and methods. The active pharmaceutical substance of propranolol hydrochloride and excipients were used: mannitol, microcrystalline cellulose 102 (MCC 102), crospovidone (CPV), sodium saccharin dihydrate, sodium stearyl fumarate (SSF), and colloidal silicon dioxide. The composition development was carried out using the QbD methodology, with experimental design planned using the Mixture Design (MD) method. The independent variables were the content of MCC 102, CPV, and SSF. OMT with a diameter of 3 mm were obtained by direct compression. The tablet blend and OMT were tested according to the methods presented in the State Pharmacopoeia of the Russian Federation, XV edition: flowability, bulk density and tapped density, crushing strength, friability, disintegration, and mass uniformity. The dose uniformity of the optimized composition was determined by HPLC.

Results. During the first stage, the target quality profile of the OMT was determined. In accordance with this, critical quality attributes (CQAs) were established: for the powder blend — flowability, bulk density, tapped density; for the OMT — crushing strength, disintegration, friability, and dose uniformity. A composition was developed and optimized, which allowed the required values for all CQAs to be achieved. Statistical analysis revealed significant inter-component interactions affecting the crushing strength and disintegration of the OMT.

Conclusion. The composition and technology for obtaining orodispersible mini-tablets of propranolol hydrochloride have been developed.

Keywords: propranolol hydrochloride; mini-tablets; orodispersible forms; Quality by Design; infantile hemangioma

Abbreviations: IH — infantile hemangioma; OMTs — orodispersible mini-tablets; DFs — dosage forms; QbD — Quality by Design; QTPP — Quality Target Product Profile; DoE — Design of Experiments; SSF — sodium stearyl fumarate; MCC — microcrystalline cellulose; CPV — crospovidone; CQA — critical quality attribute.

For citation: Ya.S. Novikov, M.D. Uryasova, S.N. Egorova. Development of the Composition and Technology for Obtaining Mini-Tablets of Propranolol Hydrochloride Using the Quality by Design Approach. *Pharmacy & Pharmacology.* 2026;14(2):201-213. **DOI:** 10.19163/2307-9266-2026-14-2-201-213

Для цитирования: Я.С. Новиков, М.Д. Урясова, С.Н. Егорова. Разработка состава и технологии получения мини-таблеток пропранолола гидрохлорида с применением подхода «качество через проектирование». *Фармация и фармакология.* 2026;14(2):201-213. **DOI**: 10.19163/2307-9266-2026-14-2-201-213

RESEARCH ARTICLE

ISSN 2307-9266 e-ISSN 2413-2241

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

Разработка состава и технологии получения мини-таблеток пропранолола гидрохлорида с применением подхода «качество через проектирование»

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Получена 12.01.2026 После рецензирования 16.04.2026 Принята к печати 24.04.2026

Инфантильная гемангиома (ИГ) представляет собой доброкачественное сосудистое новообразование, встречающееся у 4–10% новорождённых и требующее своевременной терапии при осложнённом течении. На сегодняшний день пропранолол признан «золотым стандартом» лечения ИГ благодаря доказанной эффективности и безопасности. Однако в Российской Федерации отсутствуют доступные лекарственные формы (ЛФ) пропранолола для детей, что создает значительную проблему для педиатрической практики. В связи с этим актуальна разработка ЛФ, обеспечивающей точность дозирования и удобство применения у детей.

Цель. Разработка состава и технологии получения ородиспергируемых мини-таблеток (ОДМТ) пропранолола гидрохлорида для детей с использованием подхода «качество через дизайн» (Quality by Design, QbD).

Материалы и методы. Для достижения цели исследования использовали активную фармацевтическую субстанцию пропранолола гидрохлорида и вспомогательные вещества: маннитол, микрокристаллическая целлюлоза 102 (МКЦ 102), кросповидон (КПВ), натрия сахарината дигидрат, натрия стеарилфумарат (НСФ) и кремния диоксид коллоидный. Разработку состава проводили с использованием методологии QbD, планирование эксперимента методом Mixture Design (MD). Независимыми переменными выступали содержание МКЦ 102, КПВ и НСФ. ОДМТ диаметром 3 мм получали прямым прессованием. Таблеточную смесь и ОДМТ испытывали по методикам, представленным в Государственной фармакопее Российской Федерации XV издания: сыпучесть, насыпная плотность и плотность после уплотнения, прочность на раздавливание, истираемость, распадаемость, однородность массы. Однородность дозирования оптимизированного состава определяли методом ВЭЖХ.

Результаты. На первом этапе определили целевой профиль качества ОДМТ. В соответствии с ним установлены критические показатели качества (КПК): для порошковой смеси — сыпучесть, насыпная плотность, плотность после уплотнения; для ОДМТ — прочность на раздавливание, распадаемость, истираемость и однородность дозирования. Разработан и оптимизирован состав, позволивший достичь требуемых значений всех КПК. В ходе статистического анализа выявлены значимые межкомпонентные взаимодействия, влияющие на прочность и распадаемость ОДМТ.

Заключение. Разработан состав и технология получения ОДМТ пропранолола гидрохлорида.

Ключевые слова: пропранолола гидрохлорид; мини-таблетки; ородиспергируемые формы; качество через проектирование; инфантильная гемангиома

Список сокращений: ИГ — инфантильная гемангиома; ОДМТ — ородиспергируемые мини-таблетки; ЛФ — лекарственные формы; ЛП — лекарственный препарат; QbD — качество через проектирование; QTPP — целевой профиль качества; DoE — планирование эксперимента; НСФ — натрия стеарилфумарат; МКЦ — микрокристаллическая целлюлоза; КПВ — кросповидон; КПК — критические показатели качества.

INTRODUCTION

Infantile hemangioma (IH) is a benign vascular neoplasm, occurring, according to various data, in 4–10 % of newborns [1]. Currently, the pathogenesis of the disease is not absolutely studied [2], however, it is assumed that IH is a consequence of dysregulation of vasculogenesis and angiogenesis [3]. In most cases, IH does not pose a threat and resolves spontaneously, but in 10–15 % of cases, hemangiomas localized in the head and neck area, at the border with mucous membranes, pose a danger[1]. They can lead to complications such as bleeding, ulceration, deformation, and obstruction with functional impairments [1, 4].

Medication therapy is among the main methods

[1] Infantile hemangioma. Classificator of Clinical Guidelines. Available from: https:// cr.minzdrav.gov.ru/preview-cr/769_1. Russian

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DOI: 10.19163/2307-9266-2026-14-2-201-213

for treating IH. Although corticosteroid therapy was considered the most effective approach for a long time, a number of serious side effects due to its prolonged use were subsequently identified [5]. Therapy of IH with the immunosuppressant sirolimus (rapamycin), an mTOR receptor inhibitor, is known. However, due to side effects such as immunosuppression, metabolic disorders, and nephrotoxicity, the drug is not a first-line treatment [4]. Beta-blockers, atenolol and timolol, are used for local and oral administration in the treatment of IH; however, propranolol remains the "gold standard" [3, 6, 7].

Propranolol belongs to non-selective beta-adrenoblockers and has antihypertensive, antianginal, and antiarrhythmic effects. Its use as a treatment for IH has been known since 2008 [8]. The dosage of propranolol according to clinical recommendations[2] depends on the form of IH and ranges from 0.5 to 3 mg per kg of body weight per day, with the dose divided into 2–3 administrations. Overdose can lead to the development of bradyarrhythmia and arterial hypotension. Currently, there are no available dosage forms (DFs) of propranolol for children in the Russian Federation [9]. In this regard, the development of propranolol in a DF that ensures accurate dosing and ease of use in young children is relevant.

Mini-tablets (MTs) are a promising DF for children. According to the definition by P. Lennartz and J.B. Mielck, MTs are tablets with a diameter of 2–3 mm or less [10]. They demonstrate high acceptability in young children, which allows them to be considered a preferred alternative to liquid DFs, particularly syrups [11]. Unlike the latter, MTs do not contain sugar and preservatives, have a stable dosage, do not require the use of a dosing device, and reduce the risk of dose discrepancy.

Interest in MTs has grown rapidly in recent years [11]. MTs have been developed for the treatment of gastrointestinal diseases [12, 13], cardiovascular [14–16], ophthalmological [17], and other pathologies [18–20]. The expediency and safety of using MTs in pediatric practice have been confirmed by regulatory decisions of the European Medicines Agency (EMA). For example, the drug Slenyto (melatonin) [21] is a prolonged-release MT approved for the treatment of insomnia in children from two years of age. The drug Aqumeldi (enalapril maleate) [22], produced in the form of orodispersible mini-tablets (ODMTs), is approved for use in children from the first days of life for the therapy of heart failure. The industrial production of these DFs confirms the technological feasibility and clinical acceptability of MTs even for the youngest age groups.

In accordance with the international standard ICH Q8 (R2), the Quality by Design (QbD) approach is recommended for the pharmaceutical development of medicines. QbD is based on a systematic approach to product design and development, which allows for increased development efficiency, reduced time costs, and optimization of the medicine composition [23]. The development includes the sequential determination of the Quality Target Product Profile (QTPP) of the medicine, identification of Critical Quality Attributes (CQAs), risk assessment, establishment of the design space, development of a control strategy, as well as Product Lifecycle Management and Continual Improvement. One of the key tools ensuring the effective implementation of QbD is Design of Experiments (DoE) [24]. The application of DoE can significantly reduce the time and resources spent on determining the optimal composition and technology for obtaining the developed drug [25]. Among various types of DoE, "mixture design" (MD) deserves special attention. This method allows for the simultaneous determination of the optimal ratio of components in a mixture and the influence of various technological parameters [26].

THE AIM was to develop the composition and technology for obtaining orodispersible mini-tablets of propranolol hydrochloride for children using the QbD approach.

MATERIALS AND METHODS

Materials

During the development of ODMTs, the pharmaceutical substance propranolol hydrochloride (Changzhou Yabang Pharmaceutical Co., Ltd., China) and the following excipients (Excipients) were used: mannitol—Pearlitol 200 SD (Roquette, France), microcrystalline cellulose (MCC) 102, crospovidone—PolyplasdoneTM XL-10 (Huangshan Bonsun Pharmaceuticals Co., Ltd., China), sodium saccharin dihydrate (China Pingmei Shenma Group Kaifeng Xinghua Fine Chemical Ltd., China), sodium stearyl fumarate—PRUV (JRS Pharma, Germany), colloidal silicon dioxide—Aerosil (Madhu Silica Pvt. Ltd., India).

For sample analysis by HPLC, acetonitrile for gradient HPLC (Greenway SPB LLC, Russia), ammonium formate (Thermo Fisher Scientific's, Germany), and formic acid (Scharlab S.L., Spain) were used. Type I ultrapure water was obtained from the Simplicity UV water purification system (Merck, Germany). A standard sample of propranolol hydrochloride (NCSO LLC, Russia) was used to prepare the standard solution. Sample filtration was carried out using a 25 mm polypropylene syringe filter with a pore diameter of 0.45 µm (Filter-Bio, China). Mobile phase filtration

[2] Ibid.

was carried out using 47 mm regenerated cellulose membrane filters with a pore diameter of 0.45 µm (Filter-Bio, China).

All weighings were performed on SHPBG-215i-ION semi-microbalances (Bel Engineering Srl, Italy).

Pharmaceutical development of mini-tablets

Defining the Target Quality Profile of Mini-Tablets

The first step is to define the characteristics of the ODMTs (oral drug delivery tablet) for next obtaining a safe and effective medicine, i.e., the target quality profile of the drug [27].

Defining critical quality attributes and risk assessment

At the initial stage of development, the CQAs of the ODMTs were identified: powder blend flowability, hardness, friability, disintegration time, and absence of adhesion to the tooling. During subsequent risk assessment, factors capable of influencing these CQAs were systematized: process parameters (mixing, compression force); API properties (particle shape and size); type and content of excipients (ratio of filler, disintegrant, and lubricant). Insufficient powder blend flowability and an imbalance in the content of superdisintegrant and lubricant have the greatest impact on achieving the target quality attributes, which was taken into account when planning the experiment. A detailed analysis and ranking of the identified risks are presented in a previously published work [28].

Experimental design and statistical analisys

The development and optimization of the propranolol hydrochloride ODMTs composition were carried out using DoE. The experimental design and statistical processing of the obtained data were performed using Minitab 21 software, USA. The critical significance level for hypothesis testing was taken as $\alpha = 0.05$.

The content of microcrystalline cellulose 102 (MCC 102, X_1, 31.0–36.5 %), crospovidone (CPV, X_2, 1.0–5.0 %) and sodium stearyl fumarate (SSF, X_3, 0.5–2.0 %) as a percentage of the total mass were considered as independent variables. The content of mannitol, sodium saccharin dihydrate, and aerosil was kept constant. The composition of the investigated ODMTs is presented in Table 1. The dependent variables (responses) evaluated were: tablet crushing strength (Y_1), friability (Y_2), disintegration (Y_3), powder blend flowability (Y_4), and Carr's index (Y_5).

An Extreme Vertex design (Table 2) was chosen as the experimental design MD.

Based on the obtained data, the ODMTs composition was optimized. The dependent variables were ranked by degree of significance and priority, with assigned weight and importance coefficients.

Technology for obtaining mini-tablets

The ODMTs were obtained by direct compression in several sequential stages. In the first stage, the active pharmaceutical ingredient and all excipients were sieved through a laboratory sieve with a mesh size of 315 µm. Sieved components, with the exception of SSF and colloidal silicon dioxide, were mixed in a Schatz M10 laboratory mixer (Powteq, China) at a rotation speed of 30 rpm for 30 min, after which the resulting mixture was sieved again. In the final stage, colloidal silicon dioxide and SSF were added and mixed at a speed of 20 rpm for 3 min.

The resulting mixture was loaded into an eccentric tablet press EP-1 (Erweka, Germany). Compression was carried out using a steel press tool, including 3 mm biconcave punches with a single tip and a die.

Powder mixture characteristics

Flowability

Flowability was assessed in accordance with the requirements of the State Pharmacopoeia of the Russian Federation, 15th edition (SPh RF XV ed.) GPhM.1.4.2.0016 "Powder Flowability"[3] using a GTB flowability tester (Erweka, Germany) by measuring the free flow time of 100.0 g of the mixture, placed in a 450 mL funnel with a 10.0 mm opening, in three replicates. The built-in mixing function of the tester was used.

Bulk density and tapped density

Bulk density and tapped density were assessed in accordance with the requirements of SPh RF XV ed. GPhM.1.4.2.0024 "Bulk Density and Tapped Density"[4] using an SVM-223 tester (Erweka, Germany). Bulk density before tapping was calculated by measuring the volume of 40.0 g of the mixture, freely poured into a 100 mL cylinder. Tapped density was calculated by determining the powder volume after 10, 500, and 1250 taps. The Hausner ratio and compressibility index were also calculated.

[3] GPhM.1.4.2.0016 "Powder Flowability". State Pharmacopoeia of the Russian Federation, XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-4/1-4-2/sypuchest-poroshkov/. Russian

[4] GPhM.1.4.2.0024 "Bulk Density and Tapped Density". State Pharmacopoeia of the Russian Federation, XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-4/1-4-2/nasypnaya-plotnost-i-plotnost-posle-uplotneniya/. Russian

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DOI: 10.19163/2307-9266-2026-14-2-201-213

Characteristics of quality parameters of mini-tablets

Mass Uniformity

Mass uniformity was assessed in accordance with SPh RF XV ed. GPhM.1.4.2.0009 "Uniformity of Mass of Dosed Dosage Forms"[5] by individual and collective weighing of 20 ODMTs.

Hardness

The hardness of ODMTs (*n* = 10) was assessed in accordance with SPh RF XV ed. GPhM.1.1.1.0017 "Tablet Crushing Strength"[6] using a TBH-125 tester (Erweka, Germany).

Geometric parameters

The thickness and diameter of ODMTs (*n* = 10) were measured simultaneously with the crushing strength assessment using a TBH-125 tester (Erweka, Germany).

Friability

The friability of ODMTs (*n* = 10) was assessed in accordance with SPh RF XV ed. GPhM.1.1.1.0015 "Tablet Friability"[7], method 2, using a TAR-220 tester (Erweka, Germany). The tablets were pre-weighed, then placed in a drum at 20 revolutions per minute for 5 minutes. At the end, the tablets were dedusted and weighed again.

Disintegration

The disintegration time of ODMTs (*n* = 6) was assessed using a ZT-221 tester (Erweka, Germany) at 37 ± 0.5 °C. Due to the small size of the ODMTs, a modified assembly was used: a steel sieve with 0.25 × 0.25 mm cells was attached to the lower part of the basket (unlike the sieve specified in the GPhM).

Content Uniformity

The content uniformity of the optimized MT composition was assessed in accordance with SPh RF XV ed. GPhM.1.4.2.0008 "Content Uniformity"[8] by direct determination of the active substance content (method 1). The arithmetic mean (Xi), standard deviation (s), relative standard deviation (RSD), and acceptance value (AV) were calculated according to the method specified in the GPhM.

The determination of propranolol hydrochloride was carried out by high-performance liquid chromatography. The method was previously validated for linearity, accuracy, specificity, and repeatability (precision).

Mobile Phase A (MP A)

Approximately 630 mg of ammonium formate was placed in a 1000 mL volumetric flask and dissolved in 900 mL of water for chromatography R, and the pH of the solution was adjusted to 3.0 ± 0.1 with formic acid R. The resulting solution was transferred to a 1000 mL volumetric flask, and the volume was adjusted to the mark with water for chromatography R and mixed.

Mobile Phase B (MP B)

Acetonitrile for chromatography R.

Standard Solution

Approximately 11.4 mg (exact weight) of propranolol hydrochloride reference standard was placed in a 100 mL volumetric flask, 10 mL of water for chromatography R was added, dissolved, and the volume was adjusted to the mark with acetonitrile for chromatography R and mixed.

The concentration of propranolol base is approximately 0.1 mg/mL.

Sample Solution

1 ODMT of propranolol hydrochloride was placed in a 10 mL volumetric flask, 1 mL of water for chromatography R was added, sonicated for 15 min, cooled to room temperature, the volume was adjusted to the mark with acetonitrile for chromatography R, and mixed. The resulting solution was filtered through a syringe filter.

The concentration of propranolol base is approximately 0.1 mg/mL.

Chromatographic conditions

The analysis was performed on a LicArt 62 chromatographic system (Russia) with a gradient quaternary low-pressure pump QP-62d, an autosampler S-42dc, a column thermostat T-85C, and a DAD-62 spectrophotometric detector on an Atlantis HILIC Silica 150 × 4.6 mm column, particle size 5 µm, packed with L3 type sorbent (Waters, Ireland). Propranolol was determined at a wavelength of 290 nm. Elution was carried out in an isocratic mode using mobile phase (A : B) in a ratio of 20 : 80. Flow rate is 1 mL/min,

[5] GPhM.1.4.2.0009 "Uniformity of Mass of Dosage Forms". State Pharmacopoeia of the Russian Federation, XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-4/1-4-2/odnorodnost-massy-dozirovannykh-lekarstvennykh-form/. Russian

[6] GPhM.1.1.1.0017 "Tablet Crushing Strength". State Pharmacopoeia of the Russian Federation, XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-1/1-1-2/prochnost-tabletok-na-razdavlivanie/. Russian

[7] GPhM.1.1.1.0015 "Tablet Friability". State Pharmacopoeia of the Russian Federation, XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-1/1-1-2/istiraemost-tabletok/. Russian

[8] GPhM.1.4.2.0008 "Content Uniformity". State Pharmacopoeia of the Russian Federation, XV edition. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-4/1-4-2/odnorodnost-dozirovaniya/. Russian

column thermostat temperature—30 °C, autosampler temperature—6 °C. Chromatographic run time—6 min, propranolol hydrochloride retention time ~3.8 min.

The content of propranolol hydrochloride in ODMT relative to the nominal content was calculated using the formula:

$$G_{\%} = \frac{s_x \times a_{RS} \times W_x \times P \times 100}{S_{RS} \times W_{RS} \times L \times 100} = \frac{s_x \times a_{RS} \times W_x \times P}{S_{RS} \times W_{RS} \times L},$$

where S_x is the peak area of propranolol hydrochloride in the chromatogram of the sample solution, mAU×min; S_{RS} is the peak area of propranolol hydrochloride in the chromatogram of the reference standard, mAU×min; a_{RS} is the weighed amount of the reference standard, mg; L is the declared content of propranolol hydrochloride in ODMT, mg; W_x is the volume of the volumetric flask used for diluting the sample, W_{RS} is the volume of the volumetric flask used for diluting the reference standard, P is the content of propranolol hydrochloride in the reference standard, %.

RESULTS

The target quality profile of the designed ODMTs with justification of the selected characteristics and their target values is presented in Table 3.

Disintegration

As a result of the experimental evaluation of 10 ODMT compositions, the disintegration times varied from 7.00 to 23.00 s (Table 4). Most compositions showed stable and reproducible results (standard deviation within 1–2 s), with the exception of compositions 6 (15.33 ± 5.30 s) and 7 (10.33 ± 3.82 s), which were characterized by increased disintegration variability. Analysis of the regression model (Table 5) showed that all pairwise interactions between the mixture components had a statistically significant effect on disintegration time ($p < 0.05$). Negative coefficients for these interactions indicate that the actual disintegration time for binary mixtures is less than the calculated time predicted based on the additive contribution of each component. The strongest effect was observed for the pair PVPC and NSF (coefficient -9440).

Hardness

The experimentally determined hardness values for the studied compositions varied from 19.10 ± 1.22 to 33.00 ± 2.84 N (see Table 4). Analysis of the regression model for hardness (see Table 5) showed a heterogeneous pattern of interaction significance between components. Of the three possible pairwise interactions, two were statistically significant: the interaction of MCC 102 × SSF ($p = 0.028$) and CPV × SSF ($p = 0.015$). Negative coefficients for these interactions indicate that the actual hardness of tablets containing NSF with MCC 102 or SSF with CPV simultaneously was lower than the calculated hardness predicted based on the additive contribution of each component. The largest negative effect in magnitude was observed for the CPV × SSF interaction (coefficient -10362). The model showed that the key factor modifying hardness is the interaction of SSF with other mixture components.

Flowability

All studied compositions demonstrated satisfactory flowability: values ranged from 31.77 ± 0.65 s to 38.17 ± 0.94 s, which corresponds to a technologically acceptable range for direct compression (see Table 4). The reproducibility of the results was satisfactory for most compositions; a slight increase in variability was noted only for composition 7 (33.47 ± 3.78 s).

According to the results of the regression analysis (see Table 5), the linear effects of the components did not have a significant impact on flowability ($p > 0.05$). The interaction effects of MCC 102 × SSF ($p = 0.017$) and CPV × SSF ($p = 0.043$) were statistically significant; the interaction of MCC 102 × CPV did not reach the significance level ($p = 0.081$). Negative coefficients for significant interactions indicate improved flowability with the co-presence of the specified pairs. Thus, flowability is mainly determined by inter-component interactions, particularly those involving SSF.

Friability

The obtained data showed no statistically significant influence of interactions on friability ($p > 0.05$) (see Table 5). It should be noted that none of the compositions exhibited friability problems in the form of borderline values. The friability index for all studied compositions did not exceed 3 % (see Table 4).

Mass uniformity and geometric parameters

For all studied compositions, ODMTs were assessed for mass uniformity, thickness, and diameter (see Table 4). The average tablet weight varied from 19.50 ± 0.14 mg to 20.49 ± 0.13 mg, with low standard deviation values (0.07–0.14 mg) indicating high reproducibility of the compression process and uniform die filling. ODMT thickness ranged from 2.60±0.02 mm to 2.84±0.01 mm, and diameter ranged from 2.99±0.01 mm to 3.02±0.03 mm.

Optimization of composition

Based on the experimental data obtained, the composition of propranolol ODMT was optimized using a multifactorial approach. Strength, flowability, disintegration, and adhesion to the

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press tool were selected as responses. For each response, a weight and importance coefficient was assigned in the range of 0.1 to 10, where a higher weight amplified the response's influence on the optimization result. The objective functions included minimization, maximization, or achieving a target value within specified intervals (Table 6). The highest priority was assigned to the absence of adhesion: weight—10, importance—4, goal—minimization (target value 0, acceptable maximum—1). Disintegration, as a critical indicator of orally disintegrating dosage forms (ODDFs), was also subject to minimization: target value—10 s, upper limit—15 s, weight—1, importance—1. For strength, a maximization goal was set: lower limit—21 N, target value—23 N, weight—1, importance—2. Flowability, characterized by the least accurate predictive model, was optimized towards minimization: target value—34 s, upper limit—36 s, weight—1, importance—3. As a result of optimization, a composition satisfying the specified criteria was obtained.

As a result of the optimization performed, the following composition of mini-tablets (wt. %) was determined: MCC 102—31.55; CPV—5.00; SSF—1.45. The desirability function value for all responses was 1.00, indicating full compliance of the achieved indicators with the specified criteria (Table 7). Predicted values of critical quality parameters: strength—23.47 N; flowability—33.98 s; disintegration—7.1 s; adhesion—0.097 (practically complete absence of sticking).

The optimized composition was reproduced and subjected to experimental evaluation. Considering that the strength of the initial composition was insufficient, the authors increased the compression pressure to 1.0–1.1 kN, having confirmed the absence of sticking risk. The obtained mini-tablets were characterized by the following indicators: average mass 20.15 ± 0.11 mg, thickness 2.62 ± 0.01 mm, diameter 3.00 ± 0.01 mm, strength from 30 to 39 N (average 33.60 ± 2.05 N), disintegration 20.67 ± 2.12 s, friability 0.5 %. Thus, increasing the compression pressure allowed achieving strength fully compliant with pharmacopoeial requirements (> 30 N), while maintaining acceptable disintegration parameters and the absence of adhesion.

A test for dosage uniformity was also performed for the obtained mini-tablets (Fig. 1). The average content of propranolol hydrochloride was 99.05 % of nominal, standard deviation — 4.24, relative standard deviation — 4.28, calculated acceptability value — 10.18, which corresponds to established pharmacopoeial requirements (AV < 15).

Table 1 — Composition of propranolol hydrochloride mini-tablets

Ingredient Name	Function	Mass fraction in tablet, %	Quantity per mini-tablet, mg
Propranolol hydrochloride	Active pharmaceutical ingredient	5.70	1.14
Mannitol	Filler	27.15	5.43
Microcrystalline Cellulose 102	Filler	31.00–36.50	6.20–7.30
Crospovidone	Superdisintegrant	1.00–5.00	0.20–1.00
Sodium stearyl fumarate	Hydrophilic Lubricant	0.50–2.00	0.10–0.40
Silicon Dioxide	Glidant	1.00	0.20
Sodium Saccharin	Artificial Sweetener	1.00	0.20
Tablet Mass:		100.00	20

Table 2 — Mixture design experiment matrix

Experiment	Level values		
	MCC 102 content, %	CPV content, %	SSF content, %
1	31.00	5.00	2.00
2	33.00	3.00	2.00
3	31.75	5.00	1.25
4	32.50	5.00	0.50
5	33.75	3.00	1.25
6	35.75	1.00	1.25
7	33.75	3.00	1.25
8	35.00	1.00	2.00
9	36.50	1.00	0.50
10	34.50	3.00	0.50

Note: MCC, microcrystalline cellulose; CPV, crospovidone; SSF, sodium stearyl fumarate.

Table 3 — Target quality profile of mini-tablets

Characteristic	Target Value	Rationale
Dosage Form	Orodispersible mini-tablets	Ensures accurate dosing and ease of use in children under 1 year of age.
Dosage	1 mg of propranolol base in each mini-tablet	In accordance with clinical recommendations, the daily dose of propranolol is 0.5 to 3 mg/kg, divided into 2–3 administrations. The drug is prescribed for infants aged 35 days to 5 weeks, with a therapy duration of 6 to 24 months or more. The child's weight during this period varies from 2.5 to 12 kg; therefore, 1 to 4 mini-tablets will be administered per dose, ensuring ease of use.
Mini-tablets Mass	Not more than 20 mg	Ensures ease of use.
Mini-tablets Size	Mini-tablet diameter not more than 3 mm	Ensures ease of use.
Organoleptic Properties	Neutral taste, absence of bitterness	A neutral taste (not sweet) prevents the perception of the drug as confectionery, minimizing the risk of accidental overdose and the formation of a food-related behavioral habit. The absence of pronounced bitterness is necessary to prevent the gag reflex and refusal to swallow.
Description	White, round, biconvex tablets with a chamfer, without a score line	Splitting of mini-tablets is not permissible; the chamfer will ensure ease of use by smoothing sharp edges.
Authenticity	Compliance with regulatory document quality requirements	Identification of propranolol hydrochloride.
Disintegration	Not more than 30 s in water	A short disintegration time ensures that if a mini-tablet is accidentally chewed or held in the mouth, it will disintegrate instantly, eliminating the risk of aspiration of a solid foreign body, and also making it impossible to subsequently spit out the min-tablet, which ensures the completeness of the administered dose and safety of use.
Crush strength	Not less than 25 N	Ensures the integrity of the mini-tablet during transportation and storage.
Abrasion	Not more than 3 % (Method 1)	Ensures the integrity of the mini-tablet during transportation and storage.
Content uniformity	Acceptable value for 10 tablets not more than 15 %	Ensures the efficacy and safety of each mini-tablet.
Related Substances / Quantitative Determination	Compliance with regulatory document quality requirements	Ensures the efficacy and safety of the mini-tablet.
Microbiological Purity	Category 3A	Efficacy and safety of the medicines.

Table 4 — Parameters of tablet blend and mini-tablets

Parameter	Experiment									
	1	2	3	4	5	6	7	8	9	10
Blend Properties										
Flowability	38.17 ± 0.94	37.33 ± 0.28	33.77 ± 1.78	32.90 ± 1.15	35.13 ± 0.92	31.77 ± 0.65	33.47 ± 3.78	35.70 ± 0.97	34.77 ± 0.76	36.53 ± 1.67
Bulk density	0.49	0.50	0.50	0.50	0.49	0.50	0.49	0.49	0.49	0.49
Tapped density	0.66	0.66	0.67	0.66	0.65	0.65	0.65	0.65	0.64	0.64
Compressibility Index	25.31	24.38	25.46	23.99	24.53	23.15	24.39	23.46	22.84	23.55
Hausner Ratio	1.34	1.32	1.34	1.32	1.33	1.30	1.32	1.31	1.30	1.31
Mini-tablet Properties										
Average mass, mg	20.08 ± 0.10	20.17 ± 0.11	20.01 ± 0.09	20.49 ± 0.13	19.94 ± 0.14	19.91 ± 0.07	19.50 ± 0.14	20.06 ± 0.08	19.84 ± 0.07	19.57 ± 0.11
Average thickness, mm	2.84 ± 0.01	2.79 ± 0.01	2.80 ± 0.09	2.74 ± 0.02	2.75 ± 0.01	2.75 ± 0.00	2.69 ± 0.01	2.76 ± 0.00	2.70 ± 0.01	2.60 ± 0.02
Average diameter, mm	3.02 ± 0.02	2.99 ± 0.01	2.99 ± 0.08	3.01 ± 0.01	3.02 ± 0.03	3.01 ± 0.02	3.02 ± 0.02	3.00 ± 0.01	3.01 ± 0.03	3.02 ± 0.03
Disintegration, s	7.00 ± 1.84	10.33 ± 1.06	9.33 ± 1.06	14.33 ± 1.06	11.00 ± 0.00	15.33 ± 5.30	10.33 ± 3.82	20.00 ± 1.84	23.00 ± 1.84	17.00 ± 1.84
Strength, N	21.80 ± 1.51	21.90 ± 1.26	25.50 ± 2.90	33.00 ± 2.84	20.30 ± 1.94	21.10 ± 1.80	19.10 ± 1.22	20.30 ± 1.52	25.80 ± 2.22	27.50 ± 0.89
Abrasion, %	0.75	0.60	0.50	0.25	0.46	0.45	0.57	0.50	0.41	0.77

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Table 5 — Regression coefficients and significance levels

Independent Variable	Disintegration		Strength		Flowability		Carr's Index	
	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value	Coefficient	*p*-value
MCC 102	33.62	> 0.05	33.09	> 0.05	38.23	> 0.05	21.31	> 0.05
CPV	615	> 0.05	680	> 0.05	-455	> 0.05	1.1	> 0.05
SSF	8219	> 0.05	7730	> 0.05	6271	> 0.05	-1032	> 0.05
MCC 102×CPV	-754	0.103	-665	0.128	555	0.081	37.4	0.553
MCC 102×SSF	-8827	0.026	-8325	0.028	-6696	0.017	1139	0.05
CPV×SSF	-10623	0.015	-10362	0.015	-5097	0.043	1345	0.033

Note: MCC, microcrystalline cellulose; CPV, crospovidone; SSF, sodium stearyl fumarate.

Table 6 — Composition optimization parameters

Response	Aim	Lower	Target	Upper	Weight	Importance
Strength, N	Maximize	21	23	–	1	2
Flowability, s	Minimize	–	34	36	1	3
Disintegration, s	Minimize	–	10	15	1	1
Sticking	Minimize	–	0	1	10	4

Table 7 — Predicted responses

Response	Value	Individual probability of achieving desired results
Strength, N	23.47	1.00
Flowability, s	33.98	1.00
Disintegration, s	7.13	1.00
Sticking	-0.097	1.00
Composite Probability of Achieving Desired Results		1.00

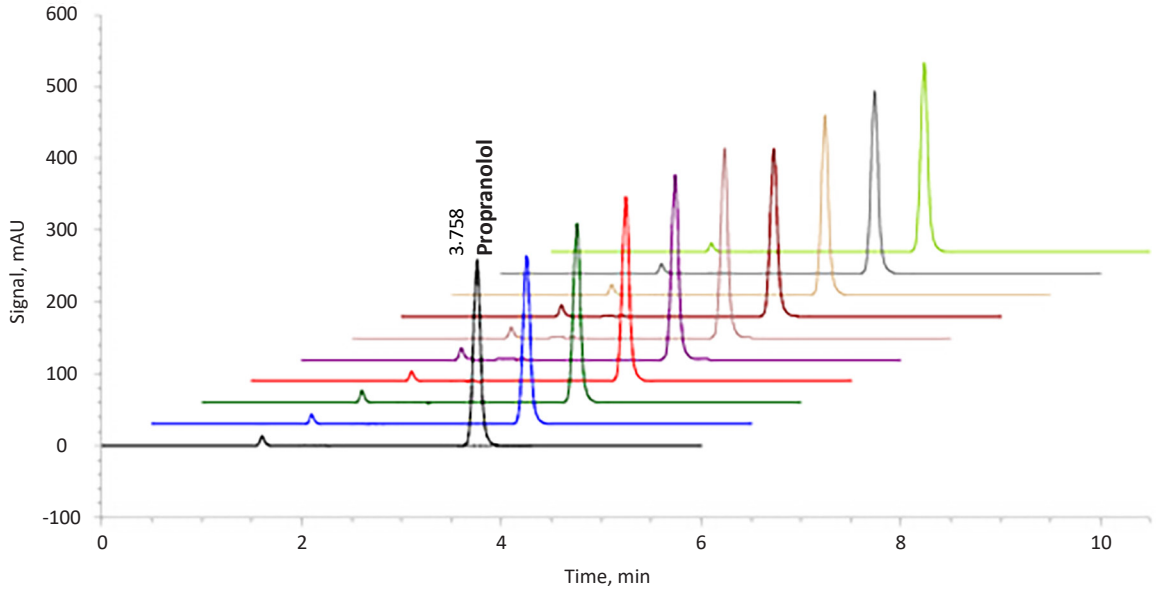

Figure 1 — Chromatograms of test solutions for determining the content uniformity of propranolol mini-tablets.

DISCUSSION

A number of the obtained results, in the opinion of the authors, require more detailed discussion and interpretation in the context of existing literature data.

Justification for the choice of experimental design

The MD method was chosen as the experimental design by the authors. Unlike the Response Surface Methodology, where factors are independent, MD considers components as part of a mixture: the response here is determined not by absolute values, but by the ratio of proportions, the sum of which always equals 100%. There are three types of MD: simplex lattice, simplex centroid, and optimal mixture (Extreme vertex), where "simplex" denotes equal ranges of all components, "lattice"—a grid method of selecting experimental points, and "centroid"—includes only central points. The latter type, extreme vertex, is used when two-sided constraints (lower and upper bounds) are imposed on the components or linear constraints are added for several components[9]. Extreme vertex was chosen as one of the most suitable for the development of a multi-component pharmaceutical composition. The chosen design allowed reducing the number of experimental points from 27 (full three-factor experiment) to 10, including 2 degrees of freedom and one central point, performed in duplicate. In accordance with the specifics of MD, the influence of individual linear variables is not included in the calculation due to the constraint of constant sum of proportions. Their contribution to the response is fully accounted for through a system of significant interactions. The presence of significant negative interactions between all pairs of components allows for targeted optimization of the MT composition to achieve minimal disintegration time.

Justification of the composition and technology for obtaining orally disintegrating mini-tablets

The direct compression method is most commonly used in the production of mini-tablets [11], as its advantages are due to the reduced number of technological stages and, consequently, economic efficiency. The authors of the present study followed this example and also used the direct compression method.

The risk analysis performed showed that the effectiveness of the direct compression method is due to the flowability of the powder mixture. Considering the low content of the active ingredient (propranolol hydrochloride content—5.7 %), flowability is mainly determined by the composition of excipients [14]. To select the optimal composition, the authors analyzed existing ODMT formulations [16, 29–31] obtained by direct compression, as well as reference data on excipients[10].

The main group of excipients in mini-tablets are fillers. The choice of mannitol was necessary—it is one of the few excipients that fully meets the objectives of developing orally disintegrating forms due to its favorable organoleptic properties and low hygroscopicity[11]. However, the use of mannitol in high concentrations led to adhesion of the powder mixture to the punches, due to which its content was limited to 55.3%. At the same time, varying the proportion of mannitol near this value did not significantly affect flowability. To compensate for the filler, MCC 102 was additionally introduced into the composition. Its choice is due to optimal flowability, as well as its ability to perform the function of a binder, ensuring the formation of strong tablets during direct compression. The proportion of MCC 102 was set in the range of 31 % to 36.5 %, which was dictated by the need to comply with the main MD constraint—achieving a total mixture mass equal to 100 %.

However, even with an optimal ratio of fillers, the flowability of the mixture remained insufficient for stable filling of small-sized dies. The die filling process is critical for ensuring the quality of mini-tablets, as their uneven filling leads to variability in mass and, consequently, active ingredient content [32]. The use of a 3 mm diameter die in the present study necessitated a significant increase in the flowability of the tablet mixture. For this purpose, colloidal silicon dioxide was introduced into the composition at a fixed concentration of 1.0%—the upper limit of the recommended range[12]. This measure allowed achieving satisfactory flowability and stabilizing the tableting process.

The second critical quality parameter was disintegration time, which is determined by the content of both disintegrants and superdisintegrants and lubricants. Since the target disintegration time for mini-tablets was less than 30 s, the content of CPV and SSF was chosen as independent variables.

[9] Design of Experiments for Pharmaceutical Product Development: Volume I: Basics and Fundamental Principles; Beg S, editor; Singapore: Springer Singapore; 2021. DOI: 10.1007/978-981-33-4717-5

[10] Rowe RC, Sheskey PJ, Quinn ME. Handbook of pharmaceutical excipients, 6th ed. London: Pharmaceutical Press; 2009

[11] Ibid.

[12] Ibid.

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DOI: 10.19163/2307-9266-2026-14-2-201-213

At the same time, an increase in the proportion of superdisintegrant, on the one hand, can contribute to accelerated disintegration, and on the other hand, reduce the mechanical strength of mini-tablets and worsen the flowability of the mixture. The choice of CPV as a superdisintegrant is due to its ability to ensure rapid disintegration of tablets [33, 34]. The content of the superdisintegrant varied in the range of 1 % to 5 %, which corresponds to the recommended limits for this group of excipients.

The content of the lubricant was also of decisive importance: its deficiency leads to adhesion of the mixture to the punch surfaces, while an excess causes an increase in disintegration time and a decrease in the strength of mini-tablets [30]. The choice of SSF as a lubricant is due to its advantages compared to traditional stearates [35]. It is characterized by lower hydrophobicity and no pronounced retardation of disintegration rate compared to magnesium stearate, while maintaining comparable lubricating ability and not inferior in its effect on tablet strength. It is important to note that its lubricating effectiveness increases with increasing mixing time, and tablet disintegration is not impaired [36].

During the experiment, it was found that compositions containing 0.5 % SSF caused adhesion of the tablet mass to the punch surfaces after pressing only a few units. Despite satisfactory mechanical strength indicators, these compositions could not be considered acceptable. In this regard, an additional response was introduced into the optimization scheme—adhesion of the mixture to the press tool, which was expressed on a binary scale: 0—no sticking, 1—sticking. This indicator had priority in the selection of the composition.

Quality assessment of obtained mini-tablets

When developing mini-tablets, special attention should be paid to the validity of pharmacopoeial testing methods, as standard procedures do not always take into account the geometric features of this dosage form.

The first example is the standardization of the strength indicator. The State Pharmacopoeia of the Russian Federation, XV edition, establishes requirements for the minimum strength of tablets with a diameter of 6 mm and above, equal to 30 N; however, the regulated standards are not applicable to mini-tablets with a diameter of 3 mm. Guided by practical expediency, the authors established a target strength of at least 25 N, which ensures the integrity of mini-tablets during packaging, transportation, and subsequent use.

A similar problem arises when assessing the disintegration of mini-tablets [11]. The main part of the disintegration apparatus is a collecting basket. According to GPhM.1.4.2.0013 “Disintegration of Solid Dosage Forms”, a mesh with holes of 2.0 ± 0.2 mm should be attached to the lower surface of the bottom plate of the basket. At the same time, the size of mini-tablets is 3 mm or less. Often, ODMTs pass through the sieve holes during disintegration testing, leading to unreliable results. One solution has been proposed by Kleinebudde [11]. The ODMT is placed in a cylinder 15 mm high and 10 mm in internal diameter, closed at the top and bottom with a mesh of 710 µm pore diameter. This cylinder was placed in a disintegration testing apparatus. The authors proposed another method: using a sieve with a pore size of 0.25 × 0.25 mm instead of the pharmacopoeial sieve fixed to the lower surface of the basket. This modification proved successful, as it reliably retained the ODMTs in the basket throughout the test and yielded reproducible, objective disintegration time results.

Regarding the results of the abrasion assessment, it should be noted that the reliability of the obtained values may be limited due to the mismatch between the geometric parameters of the ODMTs and the dimensions of the apparatus drum, as stipulated by the SPh RF XV ed. In the authors’ opinion, the pharmacopoeial method requires adaptation for an objective assessment of ODMT abrasion.

Study Limitations

The study is limited to the laboratory stage; further investigation into storage stability, biopharmaceutical studies, and subsequent clinical trials are required.

CONCLUSION

During the study ODMTs of propranolol hydrochloride intended for personalized therapy of IH in children were developed and obtained. The application of the QbD methodology and the Design of Experiments (DoE) method allowed for a scientifically grounded formulation, quantitative assessment of the influence of excipients on CQA, and identification of significant inter-component interactions. The optimized formulation ensures the required technological characteristics of the ODMTs—strength, disintegration, abrasion, and content uniformity—and fully complies with established requirements.

FUNDING

The propranolol hydrochloride and all excipients were provided by JSC Tatchimpharmpreparaty. Solvents for HPLC, a chromatographic column, and a standard sample of propranolol hydrochloride were purchased under the Priority 2030 Program.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

Yaroslav S. Novikov — conceptualization, methodology, investigation, writing—original draft, visualization; Maria D. Uryasova — methodology, investigation; Svetlana N. Egorova — conceptualization, methodology, guidance, writing—original draft, writing—review & editing. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made significant contributions to the development of the concept, research and preparation of the article, read and approved the final version before publication).

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EDITORIAL

ISSN 2307-9266 e-ISSN 2413-2241 *DOI: 10.19163/2307-9266-2026-14-2-214*

Scientific and Practical Journal
PHARMACY & PHARMACOLOGY
(ФАРМАЦИЯ И ФАРМАКОЛОГИЯ)

CORRIGENDUM:
Interim results of the first stage of a multicenter open multi-cohort study of the safety, pharmacokinetics, pharmacodynamics and efficacy of veranafusp alfa in adult patients with mucopolysaccharidosis type II. [Pharmacy & Pharmacology. 2026;14(1):81-96. DOI: 10.19163/2307-9266-2026-14-1-81-96]

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We hereby inform readers that changes have been made to the final version of the article in Russian.

In the published article “Interim results of the first stage of a multicenter open multi-cohort study of the safety, pharmacokinetics, pharmacodynamics and efficacy of veranafusp alfa in adult patients with mucopolysaccharidosis type II”, the authors discovered a technical error: figures 4 and 5 were randomly rearranged in the article relative to the captions to the figures.

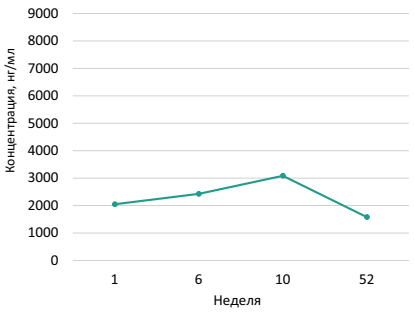


Рисунок 4 — Динамика изменения уровня дерматансульфата в спинномозговой жидкости (медиана) взрослых пациентов с мукополисахаридозом II типа, получавших 3 мг/кг веренафусп альфа.

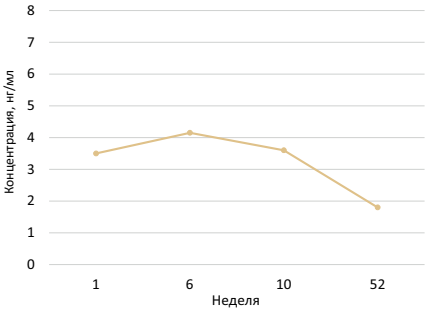


Рисунок 5 — Динамика изменения уровня гепарансульфата в спинномозговой жидкости (медиана) взрослых пациентов с мукополисахаридозом II типа, получавших 3 мг/кг веренафусп альфа.

Correct version:

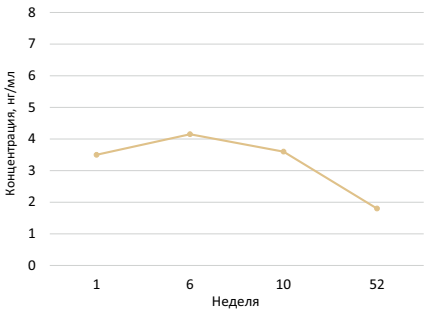


Рисунок 4 — Динамика изменения уровня дерматансульфата в спинномозговой жидкости (медиана) взрослых пациентов с мукополисахаридозом II типа, получавших 3 мг/кг веренафусп альфа.

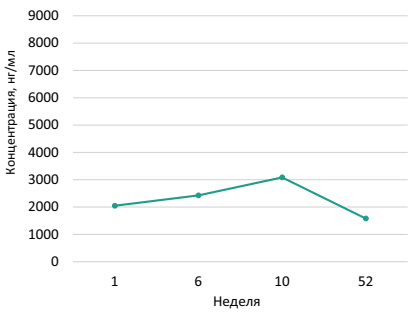
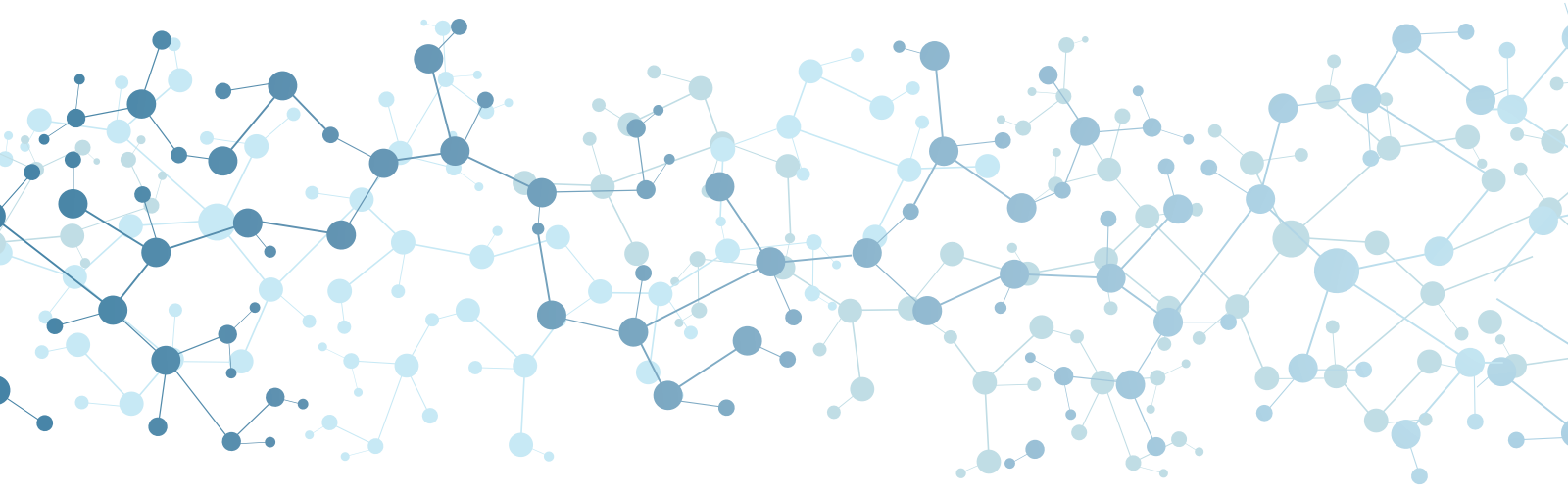


Рисунок 5 — Динамика изменения уровня гепарансульфата в спинномозговой жидкости (медиана) взрослых пациентов с мукополисахаридозом II типа, получавших 3 мг/кг веренафусп альфа.

The authors regret that, probably due to an oversight in the originally published version of this article, Figures 4 and 5 were rearranged relative to the captions to the figures. The error is technical in nature, it does not change the essence of the data presented in the article, but it may affect their correct perception by readers. The English version of the article is correct.

The original article in Russian has been updated in the online version: https://www.pharmpharm.ru/jour/article/view/1814



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