ANNOTATION
of dissertation work
by Khaiitova Malika Davranbekovna
on the topic "Search for potential local anesthetics among the new derivatives of piperidine and piperazine", submitted for the degree of Doctor of Philosophy (PhD) in the specialty
"8D10103 -Medicine"

Relevance of the research topic.

Pain is the most common symptom accompanying various pathological processes, injuries, medical manipulations and surgical interventions. In this regard, the problem of pain relief is very relevant all over the world (Orr PM et al., 2017). At the same time, the use of local anesthetics is most widespread in clinical practice, from minor injuries, biopsies to long-term and complex operations. This group of drugs stops the process of depolarization of nerve fibers, thereby blocking afferent innervation, suppressing pain sensitivity. The use of local anesthetics during medical manipulations, both in outpatient and inpatient settings, is accompanied by a high level of safety for life and their good tolerability. However, drugs of this group in some cases may exhibit systemic toxicity, expressed in the form of neurotoxicity and cardiotoxicity, up to an anaphylactic reaction (Cherobin ACFP et al., 2020), (Kouba DJ et al., 2016). For this reason, the issues of creating new highly effective long-acting and low-toxic local anesthetics and their use for effective anesthesia in various branches of medicine are relevant today. Interest in the development of drugs for anesthesia is also reviving, due to the emergence of new requirements for ensuring optimal depth and duration of action in modern clinical practice of a doctor, which can be satisfied with the help of new drugs (Yu V.K. et al. 2011), (Chitilian HV et al., 2013).

Another important problem today is the creation of new highly effective antiarrhythmic drugs. Cardiac arrhythmias, as one of the leading causes of death, are observed not only in diseases and toxic effects on the heart, electrolyte disorders, but also in practically healthy individuals (M.V. Filyukova, 2018). It is worth noting that, despite high-tech advances in the treatment of arrhythmias, antiarrhythmic drugs remain an integral part of both therapy and prevention (M.L. Stolina et al., 2019), (E.E. Özcan et al., 2018). A sufficient number of antiarrhythmic drugs used, as well as the availability of these drugs in clinical practice, often do not provide the necessary effectiveness. Therapy is accompanied by a high risk of developing various side effects and complications (K.U. Esetova et al., 2014). Antiarrhythmics can independently cause rhythm disturbances, thereby reducing the possibility of long-term therapy to restore rhythm or control heart rate. In addition, modern antiarrhythmic agents interact with various drugs, further increasing the risk of adverse effects (Heijman J. et al., 2017). Thus, there is an obvious need for safer and more effective antiarrhythmic drugs in world medical practice. Therefore, the search and discovery of new compounds for the development of antiarrhythmics with high activity and at the same time low toxicity should become one of the ways to solve this problem (S.A. Grigorieva et al., 2016).

Among organic compounds, piperidine and piperazine derivatives are the most promising for the synthesis of substances with local anesthetic and

antiarrhythmic activity. These substances have a wide range of pharmacological activity, which makes them currently one of the most popular compounds used for the development of new candidate drugs (K.D. Praliev et al., 2020), (K.M. Efendiev et al., 2022).

For a long time, the staff of the Laboratory of Chemistry of synthetic and natural medicinal substances of A.B. Bekturov Institute of Chemical Sciencеы JSC has been conducting systematic research in the field of targeted synthesis of local anesthetics in a number of new modified derivatives. The pharmacological properties of most of them were studied at the Department of Pharmacology of KazNMU. According to the results of previous studies, the compounds are characterized by low toxicity and high activity. The data obtained have been published in foreign and domestic publications (Pichkhadze G.M. et al., 2010, 2014), (Nasyrova S.R. et al., 2008, 2010), including in peer-reviewed journals with impact factor (Pichkhadze, G.M. et al., 2016), (Zhumakova S.S. et al., 2021), and were also reported at international conferences (Pichkhadze G.M. et al., 2011). Over the years of scientific work in this field, the department has received more than 50 innovative patents. During the research, substances were identified, in particular kazkaine, which surpasses in basic characteristics some widely used local anesthetics in practice (Kemelbekov U.S. et al., 2010). This compound has passed all preclinical studies and the first phase of clinical trials (Russia), according to the results of which two patents and author's certificates were obtained (Patent Ru. No.1704415,1996), (Patent RK No. 3137, 1996).

In connection with the data obtained, the search for new highly active substances of this series continued, new compounds were synthesized. The present study is devoted to the study of the local anesthetic and antiarrhythmic activity of 16 new compounds.

The dissertation work was carried out within the framework of projects that received grant funding:

1) According to the project of the Ministry of Education and Science of the Republic of Kazakhstan AP09563106 "Research and pharmacological study of new local anesthetics among piperazine derivatives" (No. 0121RK00621, 2021), studies on the acute toxicity and local anesthetic activity of piperazine derivatives were carried out.

2) According to the intra-university grant of S.D. Asfendiyarov KazNMU "Study of pharmacological properties of new nitrogenous compounds" (No.0122RKI0052, 2022-2023), experimental work was carried out to study the acute toxicity, local anesthetic and antiarrhythmic activity of piperidine derivatives.

The purpose of the dissertation research: To identify among the new derivatives of piperidine and piperazine the low-toxic and most promising compounds with local anesthetic and antiarrhythmic properties.

Research objectives:

1) To identify the least toxic compounds of new piperidine and piperazine derivatives by screening acute toxicity studies for further in-depth study.

2) To conduct screening studies of the local anesthetic activity of new piperidine and piperazine derivatives during infiltration and conduction anesthesia.

3) For the most active derivatives of piperidine and piperazine identified during primary screening, an in-depth study of local anesthetic activity during infiltration and conduction anesthesia should be conducted.

4) To conduct research on screening the antiarrhythmic effects of the most active compounds on various models of cardiac arrhythmias and evaluate the effects on ion channels.

5) To identify the main patterns between the chemical structure and pharmacological activity of new derivatives of piperidine and piperazine.

Research methods: to study new derivatives of piperidine and piperazine according to the tasks, an integrated approach was applied, in accordance with modern regulatory standards. The design of the study is shown in Figure 1.

The design at the initial stage included the use of in silico methods to predict likely targets using the online web tool SwissTargetPrediction and the spectrum of pharmacological activity using the online PASS program on the Way2Drug platform.

The protocol of experiments was built taking into account the international recommendations of the European Convention for the Protection of Vertebrates Used in Experimental and Other Scientific (1986).

In this work, preclinical experimental studies of acute toxicity were conducted with a single subcutaneous injection of solutions of the studied compounds to laboratory mice and intravenous administration to laboratory rats. The toxicity of the compounds was judged by the death of animals and the general picture of intoxication. Based on the results of the study, the LD_{50} was calculated with the determination of its standard error.

Screening studies of local anesthetic activity were performed using the Bulbring and Wajda models for infiltration anesthesia in guinea pigs by intradermal injection of solutions and by the Bianch method in laboratory mice to study activity during conduction anesthesia by subcutaneous injection of solutions into the tail area, below its root.

According to the results of experiments using the Bulbring and Wajda models, the anesthesia index, the duration of complete anesthesia and the total duration of the anesthetic effect were recorded in each series. The results of the Bianch experiment were taken into account in an alternative form in the form of a lack of reaction to a mechanical pain stimulus, which indicated the presence of 100% anesthesia. The latency period and duration of anesthesia were recorded.

For an in-depth study, the method of infiltration anesthesia of the abdominal wall of a rabbit was used by determining the threshold of pain sensitivity when applying minimal irritation with electric current pulses of an electrostimulator before and after intradermal and subcutaneous administration of the studied solutions at the site of electrode fixation. The appearance of a response was recorded in the form of changes in the rhythm and amplitude of the animal's breathing using a veterinary multiparametric monitor. The time of anesthesia development, its depth and duration were estimated by changing the threshold of reaction to electrical irritation of the skin area infiltrated with a solution of the test substance.

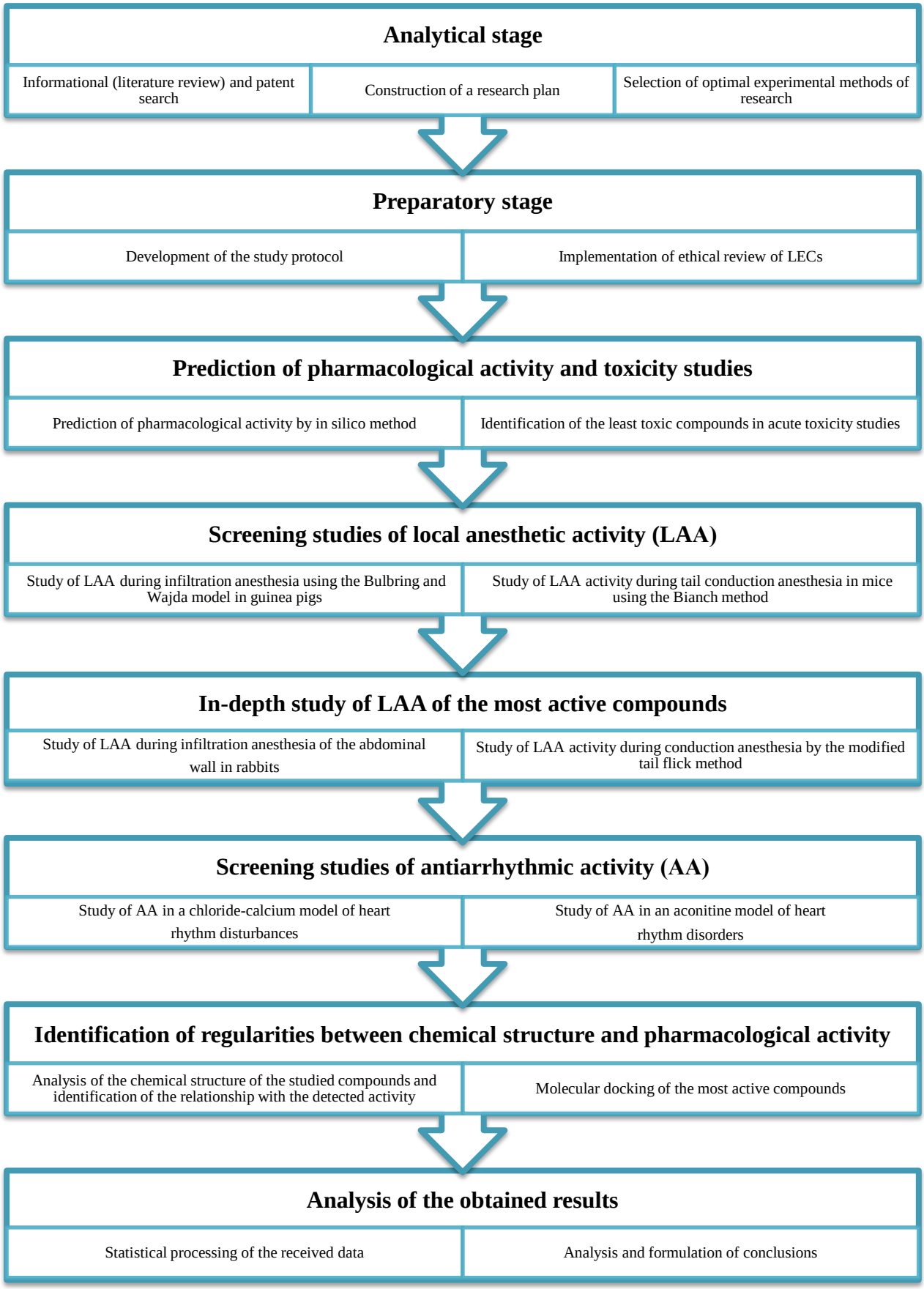

Figure 1. The design of a dissertation study on the search for potential local anesthetics among new derivatives of piperidine and piperazine.

The tail flick method in laboratory rats was used for an in-depth study of conduction anesthesia, where the nociceptive stimulus was a focused beam of light from an optoelectronic analgesimeter lamp, which exerts a thermal effect on the proximal third of the tail. At the same time, the latent period of tail twitching before and after anesthesia was recorded by evenly pricking the root of the rat tail from four sides with the test solution. An increase in the latency period of the tail twitch reflex by 2 times was estimated as complete anesthesia. The compounds and the reference drug were compared according to the duration of complete anesthesia and the total duration of the anesthetic effect.

Novocaine, lidocaine, and trimecaine were used as comparison drugs in the study of local anesthetic activity.

To study antiarrhythmic activity, experimental models of cardiac arrhythmia induced by intravenous administration of aconitine and calcium chloride were selected to study the inhibitory effect on Na^{+} and Ca^{2+} channels, respectively. Experiments on the aconitine arrhythmia model were conducted on anesthetized nonlinear male rats. To induce atrioventricular arrhythmia, aconitine was injected into the lateral caudal vein at a dose of 12 mcg/kg, provided by the Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, where the experiments were conducted. To determine the preventive effect of the studied compound and reference drugs, solutions of the studied substances were administered intravenously 5 minutes before the administration of aconitine solution, after which ECG recording was performed at set intervals. In each experimental group, the number of animals with and without arrhythmia, as well as the antiarrhythmic effect, expressed as a percentage, were recorded. Antiarrhythmic activity was assessed by the ability to prevent the development of cardiac arrhythmias caused by the administration of aconitine, with the determination of the main parameters of the average effective dose and the antiarrhythmic index. Class I antiarrhythmics procainamide and allapinine, which are similar in their intended mechanism of action to the compounds under study, were used as reference drugs.

Studies of the antiarrhythmic activity of compounds on a calcium chloride arrhythmia model were performed on nonlinear male rats under anesthesia and heart rate control using a veterinary electrocardiograph. Arrhythmia was caused by injection into the lateral caudal vein of 10% calcium chloride solution at a dosage of 250 mg / kg, causing severe fatal cardiac arrhythmias. The introduction of the studied solutions, ECG registration, and evaluation of antiarrhythmic activity were performed according to the same criteria and parameters identical to the aconitine model of arrhythmia. The results were compared with the reference antiarrhythmic drug verapamil, class IV.

According to the results of the study, molecular docking was performed to analyze the interaction of molecules of the studied compounds with macromolecules of potential targets – potential-dependent sodium channels using the AutoDock Vina 4 computer program with a completeness parameter equal to 8. After calculating the results of molecular docking, the positions in which the compounds formed the strongest bonds were selected, and at the same time the standard deviation was equal to 0. Lidocaine, which has local anesthetic and antiarrhythmic effects, was chosen as a comparison drug.

Objects of research:

We have studied 16 new synthesized compounds under the laboratory cipher of the developers of LAS (local anesthetic substance):

- 6 piperidine derivatives – LAS-250, LAS-251, LAS-252, LAS-286, LAS-294, LAS-295;

- 10 piperazine derivatives – LAS-242, LAS-253, LAS-267, LAS-268, LAS-269, LAS-270, LAS-271, LAS-276, LAS-277, LAS-278.

These compounds were synthesized in the laboratory of chemistry of synthetic and natural medicinal substances of A.B. Bekturov Institute of Chemical Sciences JSC in the form of substances intended for further preparation of aqueous solutions *ex tempore*.

The subject of the study: indicators of the prognosis of pharmacological activity, acute toxicity, depth and duration of local anesthetic activity during infiltration and conduction anesthesia, electrocardiography data, results of molecular docking.

The main provisions submitted for protection.

1. The studied derivatives of piperidine and piperazine are low-toxic substances. With a single subcutaneous injection, the compounds LAS-286 => LAS-294 => LAS-295 => LAS-267 showed the least toxicity, the indicators of which were lower than the degree of toxicity of the reference drugs.

2. New piperidine derivatives have a local anesthetic effect during infiltration anesthesia. The most active of them, LAS-286 and LAS-294, exceed novocaine and lidocaine in duration of infiltration anesthesia.

3. Derivatives of piperidine and piperazine have, to varying degrees, a local anesthetic effect during conduction anesthesia, inferior to comparison drugs in terms of basic parameters.

4. Piperidine derivative LAS-294 exhibits pronounced antiarrhythmic activity against the background of aconitine arrhythmia in laboratory rats, surpassing novocainamide and allapinine in antiarrhythmic index, which indicates high safety. The new derivatives of piperidine LAS-286 and LAS-294 do not have an antiarrhythmic effect on the calcium chloride model of cardiac arrhythmia.

6. The presence of a fluorine atom in the meta-position in the active compound molecule enhances local anesthetic activity, while the introduction of fluorine into the ortho-position leads to an increase in antiarrhythmic activity. The "weighting" of molecules due to the introduction of various substituents leads to a decrease in activity due to a decrease in solubility and, as a result, bioavailability.

The main results of the study.

A comprehensive analysis of the presented results of computer prediction of piperidine and piperazine derivatives leads to the conclusion that new compounds should be considered as potential substances for the development of new highly effective medicines with a wide range of practical applications. The analysis of the chemical structure in *silico* of the studied compounds revealed a wide range of possible effects.

Two compounds were excluded from the study: LAS-250 - due to poor solubility, LAS-253 – due to pronounced local irritant effect when administered subcutaneously.

Analyzing the results of toxicity, it can be concluded that all the studied derivatives of piperidine and piperazine, in accordance with the LD_{50} indicators, belong to low-toxic substances exceeding that of novocaine. The lowest toxicity during subcutaneous administration was determined in fluorobenzoic analogues of kazkaine, which are derivatives of piperidine LAS-286 (LD_{50} 1447.51±72.25 mg/kg), LAS-294 (LD_{50} 1121.1±157.4 mg/kg) and LAS-295 (LD_{50} 1002.3±111.7 mg/kg). Among the piperazine derivatives, the compound LAS-267 (LD_{50} 792.9±105 mg/kg) showed the least toxicity. LAS-286, when administered intravenously, exceeded the values of LD_{50} of novocainamide and allapinine by 2.5 and 31 times, respectively, while LAS-294 turned out to be less toxic than allapinine by 12.5 times.

An analysis of the experimental screening data indicates that 12 of the 14 compounds studied had varying degrees of local anesthetic activity during infiltration and conduction anesthesia. During infiltration anesthesia, pronounced activity was found among piperidine derivatives LAS-286>LAS-294>LAS-251, the anesthesia index of which reached its maximum value, and 0.5% solutions statistically significantly exceeded similar solutions of novocaine, lidocaine and trimecaine by an average of 1.6 - 4 times in duration of complete anesthesia. Of the piperazine derivatives, the compound LAS-269 showed the longest duration of complete anesthesia, statistically significantly exceeding the reference drugs. At the screening stage of conduction anesthesia, a local anesthetic effect of 75-100% was observed in the study of compounds LAS-286, LAS-294, LAS-277, LAS-278. A local anesthetic effect of 50-63% was observed in the study of other 8 compounds, and therefore this group was also included for further in-depth studies.

In-depth studies of the local anesthetic effect by infiltration of the anterior abdominal wall of the rabbit confirmed the presence of an effect in LAS-251, LAS-286 and LAS-294, but without the onset of complete 100% anesthesia. LAS-286 in 0.5% solution was 3.6 times more active than novocaine and 1.8 times more active than lidocaine in duration of infiltration anesthesia, and was comparable to trimecaine. LAS-294 had a longer latency period and was inferior to trimecaine in duration of action, but at the same time it acted longer than novocaine and lidocaine by 2.5 and 1.3 times, respectively. LAS-251 acted longer than novocaine by 16.5 minutes, inferior to lidocaine and trimecaine.

When studying the local anesthetic activity during conduction anesthesia, all the studied piperazine derivative compounds did not cause complete anesthesia. The total duration of action was inferior to all reference drugs.

A comparative analysis of antiarrhythmic activity with modern antiarrhythmic drugs showed the presence of the most pronounced preventive effect on the development of arrhythmia with the introduction of LAS-294, where the effectiveness of preventing the development of arrhythmia with a low dosage of 0.1 mg /kg was observed in 90% of cases. This compound was superior to novocainamide and allapinine in terms of the conditional breadth of pharmacological action, which confirms its safety in practical use. The maximum value of the antiarrhythmic effect of LAS-286 was 40% at a dosage of 1.0 mg /kg, however, the advantage of this substance is its higher level of safety, as evidenced by a high antiarrhythmic index. During the registration of ECG in the experiment, a significant advantage of these compounds is the effectiveness in low dosage, the

absence of arrhythmogenic effect and stable rhythm control. Thus, the results of experiments using the aconitine model of arrhythmia indicate a significant effect of the above compounds on sodium channels.

Low preventive efficacy was found in the general dosage range from 0.1 mg/kg to 15 mg/kg, accompanied by 100% mortality in laboratory animals, for compounds LAS-286 and LAS-294 in arrhythmia caused by impaired calcium ion flow compared with verapamil. This confirms the lower ability of these compounds to affect calcium channels.

The analysis of the chemical structure and the detected activity allowed us to establish the following relationships. The introduction of a fluorine atom into the meta-position enhances the local anesthetic activity of LAS-286 during infiltration anesthesia. The location of the fluorine atom in the ortho position of LAS-294 retains a sufficiently strong local anesthetic activity, while simultaneously enhancing the antiarrhythmic activity of the compound. More prolonged complete anesthesia was observed in LAS-269, in which fluorine is in the ortho position. In compounds with moderate activity with a long-term effect of LAS-268, the fluorine atom is in the para position, and in LAS-270 it is in the meta position. In a number of piperazine derivatives LAS-276, LAS-277 and LAS-278, groups of meta-phenoxyphenyl and dimethoxysphenyl were introduced, "weighting molecules", which led to a significant decrease in activity, apparently associated with a decrease in solubility and bioavailability.

The results of molecular docking provided important scientific insight into the potential molecular interactions of LAS-251, LAS-286 and LAS-294 with targets Na_v 1.4 and Na_v 1.5. According to the results of LAS-286 and LAS-294, they showed the presence of a large number of types of bonds formed, including stronger ones when docking with macromolecules of sodium channels of both nerve fibers on the periphery and in cardiomyocytes in comparison with lidocaine, which probably provides a pronounced effect. The compound LAS-294 with Na_v 1.5 forms similar types of bonds in the fragments of the molecule in comparison with lidocaine, which is an antiarrhythmic.

The scientific novelty of the work.

For the first time, a preclinical study of the acute toxicity of new synthesized original compounds from the group of azaheterocycles, previously unexplored, was conducted. It was found that the studied substances belong to low-acid compounds, where LD50 values varied within the range of 508.5 - 1447.51 mg/kg with subcutaneous route of administration.

In the course of the work, screening and in-depth studies of the local anesthetic activity of completely new derivatives of piperidine and piperazine during infiltration and conduction anesthesia were conducted for the first time. The presence of compounds under the laboratory codes LAS-251, LAS-286 and LAS-294 among the newly synthesized piperidine derivatives was found to have a pronounced local anesthetic effect during infiltration anesthesia, as evidenced by the indicators of the anesthesia index, the total duration of local anesthesia from 40.3 to 85 minutes. The low efficiency of piperazine derivatives in conducting anesthesia has been determined.

At the screening level, the antiarrhythmic activity of the two most effective piperidine derivatives with high antiarrhythmic indices was shown for the first time

during experimental studies in a wide range of doses with mechanisms of influence on Na^{+} and Ca^{2+} channels. The compound LAS-294 was found to have the most pronounced preventive activity in the development of arrhythmia.

For the first time, studies were conducted to analyze the chemical structure and nature of substituents of compounds and their effect on activity, as well as molecular docking of the most promising compounds, which determined the values of the docking force with the target and confirmed the experimental activity data obtained.

Theoretical and practical significance.

The highly active, long-acting and low-toxic compounds with local anesthetic and antiarrhythmic effects identified among the new derivatives of piperidine and piperazine, as well as a number of advantages over drugs already used in clinical practice, have allowed to expand the scope of study in this direction.

The results obtained will make it possible in the future to develop new drugs based on them that increase the effectiveness and safety of anesthesia in various fields of medicine and are used to treat heart rhythm disorders, which will also lay the foundation for further study of their effectiveness at the level of clinical trials.

The main patterns between the chemical structure and pharmacological activity among the new derivatives of piperidine and piperazine have been revealed, which will allow chemists to further carry out targeted synthesis of chemical compounds with membrane-stabilizing activity.

The results of preclinical studies of acute toxicity and local anesthetic activity of LAS-251, LAS-286, LAS-295 formed the basis for obtaining patents for a utility model of the Republic of Kazakhstan. In accordance with the revealed pronounced antiarrhythmic activity of the compound LAS-294, an application for a patent for an invention was filed for the RSE "National Institute of Intellectual Property" of the Republic of Kazakhstan.

The act of introducing the "Application of the results of research on the study of local anesthetic activity of new piperidine derivatives" into the educational process of the discipline "Preclinical research" for students of the 5th year of the OP "Pharmacy" was obtained.

Personal contribution of the author.

The dissertation work is the author's scientific work of Khayitova Malika Davranbekovna, performed directly by the dissertator under the guidance of scientific consultants. The author has carefully worked out a literary review of the chosen scientific field, developed a research protocol, independently conducted an in silico predictive analysis and all laboratory experiments. The author collected materials and processed them with further analysis, interpretation, as well as a description of the results obtained and the formulation of conclusions. The dissertation prepared and published articles, patent descriptions, as well as the manuscript of this dissertation.

Conclusions:

1. All tested compounds had low LD_{50} values ranging from 508.5mg/kg to 1447.51mg/kg, exceeding that of novocaine as one of the low-toxic local anesthetics. With a single subcutaneous injection, the compounds LAS-286, LAS-294, LAS-295, and LAS-267 showed the least toxicity.

2. During screening, 12 of the 16 compounds studied had varying degrees of local anesthetic activity. Substances of piperidine derivatives LAS-286, LAS-294, and LAS-251 showed high activity during infiltration anesthesia. During conduction anesthesia, high activity was observed in the study of LAS-286, LAS-294, LAS-277, LAS-278.

3. An in-depth study of piperidine derivatives LAS-251, LAS-286 and LAS-294 confirmed the presence of local anesthetic activity during infiltration anesthesia. LAS-286 and LAS-294 act 1.3 – 3.6 times longer than novocaine and lidocaine. During conduction anesthesia, piperidine and piperazine derivatives have a weakly expressed activity, inferior to comparison drugs in all parameters.

4. LAS-294 has pronounced antiarrhythmic activity, surpassing in the breadth of therapeutic action novocainamide and allapinine in the model of aconitine arrhythmia, which indicates the presence of a blocking effect on sodium channels. Experimental studies on the calcium chloride model of arrhythmia indicate that there is no effect on calcium channels.

5. The introduction of a fluorine atom into the meta-position of the LAS-286 molecule enhances local anesthetic activity, while the presence of fluorine in the ortho position in the structure of LAS-294 causes pronounced antiarrhythmic activity. The "weighting" of substituents in the molecule leads to a decrease in local anesthetic activity.

Approbation and implementation of the work.

The materials and provisions of the dissertation were presented at scientific conferences and forums:

- International conference "Science and Education: current issues, achievements and innovations in medicine" (Tashkent – April 16, 2021);
- Republican scientific and practical conference "Actual problems of pharmacology of the Republic of Kazakhstan" (Nur-Sultan - November 12, 2021);
- International scientific and practical conference "Medical Science in the era of digital transformation" (Kursk -December 10, 2021);
- 1st International Asfen Forum - New generation 2023 (Almaty - June 5-6, 2023);
- The 3rd international conference "Scientific Research and Experimental Development" (England, London, June 15-16, 2023).

Publications on the topic of the dissertation.

9 scientific papers have been published on the topic of the dissertation, of which:

- 1 article in the official journal of the Romanian Society of Pharmaceutical Sciences "Farmacia" (Scopus - 61 percentile);
- 3 articles in periodicals recommended by the KOKSNVO of the Ministry of Foreign Affairs of the Republic of Kazakhstan;
- in 2 materials of domestic and international conferences;
- 3 utility model patents of the RSE "National Institute of Intellectual Property" of the Republic of Kazakhstan No. 8253 (07/14/2023), No.8395 (09/01/2023), No.8496 (10/06/2023).
- 1 article was accepted for publication in the Brazilian Journal of Medical and Biological Research (Scopus – 84th percentile).

The volume and structure of the dissertation.

The dissertation is presented on 150 pages, illustrated with 14 tables and 68 figures. The work consists of an introduction, the main part, including a review of the literature (chapter 1), descriptions of materials and research methods (chapter 2), the results of their own research and their discussion (chapter 3), conclusions, a list of sources used, 4 appendices. The bibliography includes 226 literary sources, of which 31 are domestic and 195 are foreign.