FABAD J. Pharm. Sci., 49, 3, 465-480, 2024
Doi: 10.55262/fabadeczacilik.1417891
RESEARCH ARTICLE

The Local Anesthetic Activity of 4-(Naphthalen-1-yloxy)But-2-yn-1-yl)-Containing Piperidine Derivatives in Experimental Animal Models

Malika KHAIITOVA[*°], Yelena SYCHEVA[**°], Vasiliy TRUBACHEV[***], Elmira SATBAYEVA[****], Valentina YU[*****], Talgat NURGOZHIN[******], Edgaras STANKEVIČIUS[*******], Yeskendir GASSANOV[********], Zauresh UTELBAYEVA[*********], Khaidar TASSIBEKOV[**********]

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SUMMARY

Piperidine derivatives are of interest to researchers considering piperidines as an effective scaffold for the synthesis of new compounds. This research aimed to investigate the acute toxicity and local anesthetic activity of new synthesized 4-(Naphthalen-1-yloxy)but-2-yn-1-yl)-containing piperidine derivatives as inclusion complexes with β-cyclodextrin. Moreover, there was hydrogen or 3-methoxyphenyl in position 1 of the substituent at the nitrogen atom of the piperidine ring. The acute toxicity tests were performed on outbred laboratory mice by subcutaneous injection of increasing concentrations of the test solutions. The initial investigation of local anesthetic activity during infiltration anesthesia was performed on guinea pigs using the Bulbring & Wajda animal model. An in-depth study of the efficacy of the most active compound was performed on a model of infiltration anesthesia of the rabbit's abdominal wall by determining the threshold of nociception during electrical stimulation. The new studied piperidine derivatives are low-toxic substances, which are confirmed by the results of an acute toxicity study. At the stage of the primary study of local anesthetic activity during infiltration anesthesia on the experimental Bulbring and Wajda model, the LAS-251 compound showed the greatest activity, surpassing the reference drugs in terms of anesthesia index, duration of full anesthesia and total duration of action. At the stage of in-depth study, despite a longer latency period, LAS-251 has a local anesthetic effect longer than procaine and is slightly inferior to lidocaine. Results of the present study are promising because complex 1-(4-(naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine (LAS-251) with cyclodextrin showed high local anesthetic activity. The new piperidine derivative is future-oriented for prospective studies of other types of anesthesia as a potential medicinal substance for therapeutic use in the future.

Key Words: *Piperidine derivatives, local anesthetic activity, acute toxicity, infiltration anesthesia*

4-(Naftalen-1-iloksi)but-2-in-1-il)-İçeren Piperidin Türevlerinin Deneysel Hayvan Modellerinde Lokal Anestezik Aktivitesi

ÖZ

Piperidin türevleri, piperidinleri yeni bileşiklerin sentezi için etkili bir iskelet olarak gören araştırmacıların ilgisini çekmektedir. Bu araştırma, yeni sentezlenen 4-(Naftalen-1-iloksi)but-2-in-1-il) içeren β-siklodekstrin ile inklüzyon kompleksleri halindeki piperidin türevlerinin akut toksisitesini ve lokal anestezik aktivitesini araştırmayı amaçlamaktadır. Ayrıca piperidin halkasının azot atomundaki sübstitüentin 1. konumunda hidrojen veya 3-metoksifenil bulunmaktadır. Akut toksisite testleri, artan konsantrasyonlarda test solüsyonlarının deri altına uygulanması yoluyla fareler üzerinde gerçekleştirildi. İnfiltrasyon anestezisi sırasında lokal anestezik aktiviteye ilişkin ilk çalışma, Bulbring & Wajda hayvan modeli kullanılarak kobaylar üzerinde gerçekleştirildi. Elektriksel stimülasyon sırasında nosisepsiyon eşiğinin belirlenmesiyle tavşanın karın duvarına uygulanan infiltrasyon anestezisi modelinde en aktif bileşiğin etkinliğine ilişkin derinlemesine bir çalışma gerçekleştirildi. İncelenen yeni piperidin türevlerinin düşük toksisiteli maddeler olduğu, bir akut toksisite çalışmasının sonuçlarıyla da doğrulandı. Deneysel Bulbring ve Wajda modelinde infiltrasyon anestezisi sırasında lokal anestezik aktivitenin birincil çalışması aşamasında, LAS-251 bileşiği, anestezi indeksi, tam anestezi süresi ve toplam anestezi süresi açısından referans ilaçlardan üstün olan en yüksek aktiviteyi gösterdi. Derinlemesine çalışma aşamasında, daha uzun bir gecikme süresine rağmen, LAS-251'in prokainden daha uzun süreli ve lidokainden biraz daha düşük bir lokal anestezik aktiviteye sahip olduğu görüldü. Bu çalışmanın sonuçları umut vericidir, çünkü 1-(4-(naftalen-1-iloksi)but-2-in-1-il)-4-fenilpiperidin (LAS-251) ile siklodekstrin kompleksi yüksek lokal anestezik aktivite gösterdi. Bu yeni piperidin türevi, gelecekte terapötik kullanım için potansiyel bir ilaç olarak diğer anestezi türleriyle ilgili ileriye yönelik çalışmalar yapılması için umut vericidir.

Anahtar Kelimeler: *Piperidin türevleri, lokal anestezik aktivite, akut toksisite, infiltrasyon anestezisi*

Received: 20.01.2024
Revised: 12.07.2024
Accepted: 29.07.2024

[*] ORCID: 0000-0001-6775-4358, Department of Pharmacology, Faculty of General Medicine -1, Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan.
[**] ORCID: 0000-0002-8409-2672, Laboratory of Physiologically Active Compounds chemistry, A.B. Bekturov Institute of Chemical Sciences, Almaty, Republic of Kazakhstan.
[***] ORCID: 0000-0002-8317-7914, Department of Pharmacology, Faculty of General Medicine -1, Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan.
[****] ORCID: 0000-0002-5521-5776, Department of Pharmacology, Faculty of General Medicine -1, Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan.
[*****] ORCID: 0000-0001-6508-707X, Laboratory of Synthetic and Natural Medicinal Compounds Chemistry, A.B. Bekturov Institute of Chemical Sciences, Almaty, Republic of Kazakhstan.
[******] ORCID: 0000-0002-8036-604X, Department of Pharmacology, Faculty of General Medicine -1, Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan.
[*******] ORCID: 0000-0003-2210-708X, Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Kaunas, Lithuania.
[********] Orcid: 0000-0002-4184-4758, Department of Pharmacology, Faculty of General Medicine -1, Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan.
[*********] ORCID: 0000-0003-4312-9093, Faculty of General Medicine -2, Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan.
[**********] ORCID: 0000-0002-1067-5162, Laboratory of Synthetic and Natural Medicinal Compounds Chemistry, A.B. Bekturov Institute of Chemical Sciences, Almaty, Republic of Kazakhstan.

[°] Corresponding Author; Malika Khaiitova
E-mail: khaiitova.m@kaznmu.kz

Yelena Sycheva
E-mail: yelena-sycheva@yandex.kz

INTRODUCTION

Every year, a large number of different surgical interventions and procedures are performed all over the world (Weiser et al., 2008). Thus, surgical biopsies, epidural anesthesia, dental procedures, blockage of major nerves are widespread in clinical practice (Becker et al., 2012). Pathological pain and pain are caused by hypersensitivity, while it is a common concomitant symptom. Therefore, it is necessary to use different methods of anesthesia (Woolf, 2010; Yu et al., 2019).

The methods of infiltration and conduction anesthesia are simple and safe to use, which allows them to be used instead of general anesthesia. The local effect is ensured due to the high concentration of the drug at the injection site of local anesthetics (Raithel et al., 2018). Local anesthesia methods can also be used after various operations to ensure adequate anesthesia in the postoperative period (Ma et al., 2017). The mechanism of action of local anesthetics is associated with a violation of the passage of Na^+ ions through the ionophores of the membrane of neurons, thereby interrupting neural conduction (Xie et al., 2022).

The use of local anesthetics avoids the occurrence of various side effects inherent in other painkillers, such as gastrointestinal bleeding, impaired blood clotting, functional disorders of the liver or kidneys, tolerance, dependence, and others. The use of modern anesthetics also has the advantage of minimising the risk of allergic reactions. However, the development of hypersensitivity is directly related to the frequency of drug use (Grzanka et al., 2016; Ilfeld et al., 2021; Yin et al., 2021).

Another essential problem with the use of local anesthetics is the development of local and systemic toxicity (Tsai et al., 2021). The local toxicity is a significant obstacle in the development of new anesthetic drugs. The majority of local anesthetics have neurotoxicity, chondrotoxicity, and myotoxicity observed after intramuscular and intra-articular injections (Baker et al., 2012; Verlinde et al., 2016). Systemic toxicity develops rarely, but this complication is accompanied by agitation of the central nervous system, cardiovascular disorders, in particular, there is possible arrhythmia and methemoglobinemia. These side effects can be observed with frequent use of prilocaine, articaine, and benzocaine (Wadlund, 2017; Johansson et al., 2020). The anesthetics, which contain para-aminobenzoic acid contribute to the development of allergic reactions, as well as symptoms of numbness and paresthesia (McMahon et al., 2022).

When considering the issue of compliance of local anesthetics with the essential requirements, where rapid blockade of peripheral nerves, long-term effect, and absence of local and systemic reactions are of particular importance, it is worth noting the need to develop new drugs that meet the needs of doctors in modern clinical practice (Chahar et al., 2012; Chitilian et al., 2013).

Considering the above, preclinical studies of new drugs with local anesthetic activity are relevant and practically important. From among the various chemical structures, the piperidine ring is one of the important components in the molecule of local anesthetics such as bupivacaine and ropivacaine. Furthermore, piperidine and its derivatives have different pharmacological properties (Vitaku et al., 2014; Martins et al., 2017).

The studies conducted indicate the possibility of using piperidine molecules in various fields of medicine. Because of the availability of synthesis and ease of embedding into other structural frameworks of various biogenic residues, piperidine derivatives are of interest to researchers considering piperidines as an effective scaffold for the synthesis of new compounds (De et al., 2015).

Based on the description above, two new 4-(Naphthalen-1-yloxy)but-2-yn-1-yl)-containing piperidine derivatives were synthesized in the laboratory. Substituted naphthoxyamine derivatives

belong to the class of biologically active molecules with a broad spectrum of therapeutic effects (Zuffo et al., 2019; Kottapalle, Shinde, 2021). We have previously synthesized β-cyclodextrin (β-CD)complex with 1-[1-(2,5-dimethoxyphenyl)-4-(naphthalene-1-yloxy)but-2-ynyl]-4-methylpiperazine whose structure is presented in Figure 1. New potentially biologically active 4-phenylnaphthoxybutynylpiperidines were synthesized by reacting 1-(prop-2-ynyloxy)naphthalene with 4-phenylpiperidine and aldehydes (benzaldehyde, formaldehyde and 3-methoxybenzaldehyde) in absolute dioxane in the presence of catalytic amounts of copper (I) iodide at temperature 40 °C. The compound had a pronounced promoting effect on the $CD4^+$, $CD8^+$ and myeloid cells during aseptic inflammation, even under the influence of heavy metal salts (Yu et al., 2023).

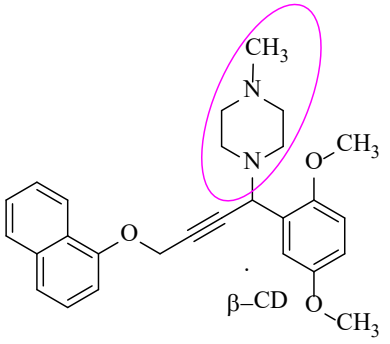


Figure 1. Chemical structure of β-cyclodextrin complex with 1-[1-(2,5-dimethoxyphenyl)-4-(naphthalene-1-yloxy)but-2-ynyl]-4-methylpiperazine.

The molecule contains a fragment of 4-(naphthalen-1-yloxy)but-2-yn-1-yl), however, the piperazine derivative group was decisive in increasing the immune status in previous studies. Therefore, it was exciting to study the type of biological action of substrates "devoid" of a single nitrogen atom. The purpose of present study was to perform experimental animal models to investigate acute toxicity and local anesthetic activity of new compounds, considering that piperidine is an important structure of the molecule of local anesthetics, like the objects of our study.

MATERIAL AND METHODS

Chemical research

The studied compounds that have been assigned the laboratory cipher LAS–251, LAS–252 (local anesthetic substance) were synthesised for the first time in the Laboratory of Chemistry of Synthetic and Natural Medicinal Compounds of Bekturov Institute of Chemical Sciences (Almaty). Total starting reagents used for the synthesis were purchased from Sigma-Aldrich and required no additional purification. The course of the reactions and the purity of the products were monitored by the TLC analysis on "Silufol UV-254" plates with the appearance of substances spots with iodine vapor. The eluent for TLC was a mixture acetone – hexane (1:2). The IR spectra were recorded on a Nicolet 5700 spectrometer in KBr tablets. The ^1H and ^{13}C NMR spectra of the samples were recorded on a JNM-ECA 400 (Jeol) spectrometer with operating frequencies 400 (^1H), 100 MHz (^{13}C) in deuterated chloroform ($CDCl_3$).

Currently, various molecular inclusion complexes with β-CDs are actively used in the pharmaceutical industry. The inclusion of this natural cyclic oligosaccharide makes it possible to convert liquid forms of low molecular weight substances into amorphous and crystalline powders. Also, such compounds become more stable in water, are less susceptible to oxidation in air, dehydration and evaporation. This method, it should be noted, eliminates the odor and taste of the original active substrate. The complexation of the active substance with cyclodextrin can increase bioavailability and reduce toxicity (Uekama, 2002; Barseet al., 2003).

Synthesis 1-(4-(naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine (1)

To a reaction mixture heated to 40° C consisting of 1.5 g (0.0082 mol) 1-(prop-2-iniloxy)naphthalene, 0.24 g (0.0082 mol) paraform, and 0.15 g (0.00078 mol) of copper iodide (I) in 20 ml of dioxane were mixed with 1.32 g (0.0082 mol) of 4-phenylpiperidine

in 10 ml of dioxane. 1-(4-(Naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine (1) was isolated by column chromatography on Al_2O_3 by elution with an acetone mixture: hexane (1:3). The yield is 2.19 g (75%) in the form of oil, R_f 0.71.

Calculated, %: C 84.47; H 7.09; N 3.94 for $C_{25}H_{25}NO$. Found, %: C 84.59; H 7.18; N 4.07

IR (KBr, υ, cm^{-1}): 704, 805, 1319, 1419, 1583, 1681 (Ph), 2121 (C≡C).

^{1}H NMR ($CDCl_3$, δ, ppm): 1.85, 1.83 (dd, 4H, N-$(CH_2)_2$); 2.08-2.12 (m, 4H, N-$(CH_2)_2(CH_2)_2$); 2.56 (m, 1H, CH-piperidin); 3.39 (s 2H, CH_2-N); 4.97 (s, 2H, OCH_2); 6.99 (d, 1H, ArH^2); 7.40 (t, 1H, ArH^4); 7.50-7.53 (m, 3H, ArH^{3,6,7}); 7.82 (d, 1H, ArH^5); 8.29 (s, 1H, ArH^8); 7.25 (t, 2H, Ph-piperidine); 7.33 (t, 2H, Ph-piperidine); 7.38 (d, 1H, Ph-piperidine).

^{13}C NMR ($CDCl_3$, δ, ppm): 33.49 (N-$(CH_2)_2(\underline{C}H_2)_2$); 42.23 (CH-piperidine); 47.69 (N-CH_2); 53.24 (N-$(CH_2)_2$); 56.22 (O-CH_2); 78.73, 80.08 (C≡C); 153.57 (C^1), 105.58 (C^2), 125.46 (C^3), 121.12 (C^4), 126.16 (C^5), 125.71 (C^6), 122.14 (C^7), 121.31 (C^8), 127.58 (C^9), 134.66 (C^{10}) (ArH); 126.98, 127.58, 128.58, 146.31 (Ph-piperidine).

Synthesis of Complex 1-(4-(naphthalen-1-yloxy) but-2-yn-1-yl)-4-phenylpiperidine with β-CD (LAS-251)

Solutions of 1 g 1-(4-(naphthalene-1-iloxy) but-2-inyl]-4-phenylpiperidine (1) in 20 ml of ethyl alcohol and 1 g of β-cyclodextrin in 20 ml of distilled water are mixed at a temperature of 45-50° C for 5 hours. After the end of the reaction, the aqueous ethanol solution is distilled and dried in a drying cabinet. The yield is 1.3 g (65%). The complex appeared as a light yellow powder, melting with decomposition above 240° C.

Calculated, %: C 53.99; H 6.42; N 0.94; O 38.64 for $C_{67}H_{95}NO_{36}$. Found, %: C 53.83; H 6.51; N 1.09.

Synthesis of 1-(1-(3-methoxyphenyl)-4-(naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine (2)

1-(1-(3-Methoxyphenyl)-4-(naphthalen-1-yloxy) but-2-yn-1-yl)-4-phenylpiperidine (2) was synthesized similarly from 1-(prop-2-ynyloxy)naphthalene 2.0 g (0.0109 mol), 3-methoxybenzaldehyde 1.49 g (0.0109 mol) and 4-phenylpiperidine 1.76 g (0.0109 mol) in the presence of copper iodide (I) (0.15 g) in dioxane at 40 °C. The product was isolated by column chromatography on Al_2O_3 by elution with an acetone mixture: hexane (1:3). Yield 2.19 g (44%) oil, R_f (0.69).

Calculated, %: C 83.26; H 6.77; N 3.03 for $C_{32}H_{31}NO_2$. Found, %: C 83.35; H 6.87; N 3.15.

IR (KBr, υ, cm^{-1}): 705, 754, 1493, 1592, 1659 (Ph), 2125 (C≡C).

^{1}H NMR ($CDCl_3$, δ, ppm): 1.78-1.83 (m, 4H, N-$(CH_2)_2$); 2.08-2.15 (m, 4H, N-$(CH_2)_2(CH_2)_2$); 2.55 (m, 1H, CH-piperidin); 3.80 (s, 3H, OCH_3); 4.90 (s, 2H, OCH_2); 4.97 (s, 1H, CH-N); 6.81 (d, 1H, ArH^2); 6.94 (d, 1H, Ph); 7.06(s, 1H, Ph); 7.17-7.20 (m, 2H, Ph); 7.41 (t, 1H, ArH^4); 7.47-7.50 (m, 3H, ArH^{3,6,7}); 7.80(d, 1H, ArH^5); 8.29 (s, 1H, ArH^8); 7.25 (t, 2H, Ph-piperidine); 7.33 (t, 2H, Ph-piperidine); 7.37 (d, 1H, Ph-piperidine).

^{13}C NMR ($CDCl_3$, δ, ppm): 33.90 (N-$(CH_2)_2(\underline{C}H_2)_2$); 42.59 (CH-piperidine); 53.02 (N-$(CH_2)_2$); 55.92 (OCH_3); 56.22 (O-CH_2); 61.60 (N-CH); 82.70, 84.11 (C≡C); 153.64 (C^1), 105.52, (C^2), 125.51 (C^3), 121.28 (C^4), 126.17 (C^5), 125.71 (C^6), 122.22 (C^7), 121.70 (C^8), 127.57 (C^9), 134.65 (C^{10}) (ArH); 126.98, 127.57, 128.47, 146.61 (Ph-piperidine); 113.42, 113.97, 129.16, 122.23, 139.66, 159.55 (Ph).

Synthesis of Complex 1-(1-(3-methoxyphenyl)-4-(naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine with β-CD (LAS-252)

Solutions of 1 g 1-(1-(3-methoxyphenyl)-4-(naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine (2) in 20 ml of ethyl alcohol and 1 β-cyclodextrin in 20 ml of distilled water are mixed at a temperature of 45-50 °C for 5 hours. After the end of the reaction, the aqueous ethanol solution is distilled and dried in a drying cabinet. The yield is 1.4 g (70%). The com-

plex appeared as a light yellow powder, melting with decomposition above 240 °C.

Calculated, %: C 55.67; H 6.38; N 0.88; O 37.08 for $C_{74}H_{101}NO_{37}$. Found, %: C 55.75; H 6.43; N 0.98.

Experimental animals and ethics approval

96 outbred laboratory male and female laboratory mice weighing 20-25 g were used to investigate acute toxicity. The primary study of local anesthetic activity during infiltration anesthesia was conducted on 30 mature male guinea pigs weighing 350-400 g. Mature outbred 24 male rabbits weighing 2500-3000 g were used in experiments to in-depth study the activity of the compounds. The experiments were carried out based on the Life Science laboratory of Asfendiyarov Kazakh National Medical University (KazNMU). Laboratory animals were provided by the KazNMU vivarium.

Laboratory animals were kept in specialized cages with a natural 12-hour day-night light regime with constant free access to clean water and standardized feed. Throughout the period of observation and experiments, the necessary hygienic conditions, a temperature regime of 25 ± 2 °C, a relative humidity of 55-60% and good air circulation were observed. Permanent dyes were used to label the laboratory animals of each group.

The experiments provided for in the protocol using laboratory animals were performed in accordance with the Order of the Minister of Health Care of the Republic of Kazakhstan "On approval of the rules for conducting preclinical (non-clinical) studies and requirements for preclinical bases for assessing the biological effect of medical devices". The care and maintenance of laboratory animals was carried out following the Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee, 2011). All described manipulations and procedures were carried out under the rules of the European Convention for the Protection of Vertebrate Animals and Directive 2010/63/EU. The research protocol was approved by the Local Ethics Committee of Asfendiyarov Kazakh National Medical University (Decision Number: No. 14(120) and date October 28, 2021, with permission to extend the study - Decision Number: No. 1(137) and date January 31, 2023).

Acute toxicity study

Acute toxicity tests were conducted following the guidelines for preclinical studies of medicines (Mironov, 2012). Experiments were performed on healthy mature males and females of outbred laboratory mice. Laboratory animals were randomly divided into experimental and control groups of 6 mice (3 females and 3 males) each. The studied compounds dissolved in sterile water for injection (Novosibkhimpharm Company, Russia) in 3 increasing concentrations (100-1000 mg/kg) were injected once subcutaneously into the lateral surface of the body. Each animal in the group had one injection of the test compound at one concentration only. This route of administration corresponds to using substances as local anesthetics in the future. After the administration of solutions, laboratory animals were under constant supervision on the first day and then for 14 days. The general condition, changes in behavioural reactions, motor activity and metabolism, and toxic reactions from various organs and systems were recorded during observation. At the end of the experiment, the median lethal dose - LD_{50} was calculated for each compound, as well as LD_{16} and LD_{84}, to determine the standard error.

Local anesthetic activity study

The primary study of local anesthetic activity during infiltration anesthesia used the Bulbring and Wajda model (Bulbrıng and Wajda, 1945; Kuzenbayeva et al., 2000). Each experimental group included six male guinea pigs. The day before the experiment, all animals of the studied group had their hair removed in the back area. On the day of the test, 0.25 ml of 0.5% aqueous solutions were injected intradermally into 4 points corresponding to the corners of a square with a side of 3 cm. The method of administration is presented in Figure 2.

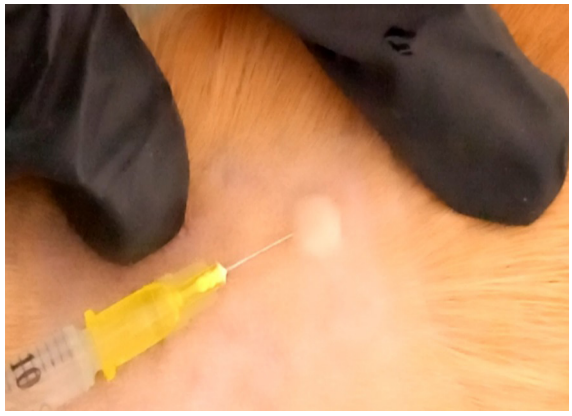


Figure 2. Intradermal administration of a solution (wheal method) on the back of a guinea pig according to the Bulbring and Wajda models (photograph).

Injections of solutions of the compounds were performed at the anterior and posterior points of the square. The solutions of the reference drugs were injected into the remaining parallel points. Following injection, the area of papule formation was marked with ink. The presence or absence of sensitivity at the injection site was assessed every 5 min by applying an irritation when touching the injection needle. The touches were carried out in a series of 6 touches at each point. An interval of 3-4 s was maintained between touches. In each series of experiments, the total number of needle touches that did not cause skin twitching in the area of the studied square on the animal's back for 30 min (anesthesia index), the duration of full anesthesia (absence of any reactions during exposure to an irritant) and the total duration of the anesthetic effect (the time during which the response reached the initial values) were recorded.

An in-depth study of the local anesthetic effect of the most active compound was carried out on a model of infiltration anesthesia of the abdominal wall of rabbits by determining the nociception threshold during electrical stimulation (Kuzenbayeva et al., 2000; Mironov, 2012). The experiments were performed on non-anesthetized male rabbits. The number of animals in each group was six animals. Prior to the start the experiment, the skin of the abdomen on the side surfaces at the level of the middle third was freed from the fur of a rabbit fixed in a position on its back. The electrodes of an electrostimulator (Medistim Co, Russia) with moistened cotton swabs (0.9% NaCl) were applied to the prepared skin areas. First, we determined the threshold of pain sensitivity by applying minimal irritation with electric current pulses (duration 0.3 ms, frequency - 50Hz, amplitude 5-25V). The response to pain irritation was accompanied by a change in the rhythm and amplitude of the animal's breathing. These changes were recorded on the OLV-VM12 veterinary monitor (Zhengzhou Olive Electronic Technology Co, Ltd, China). The next stage of the experiment involved the injection of 0.5% aqueous solutions (solvent - sterile water for injection) of the studied substances intradermally in a volume of 0.5 ml and subcutaneously in a volume of 2 ml at the site of electrode application. Further tests were performed to determine the nociceptive reaction in response to a series of electrical stimulations after 3, 5, 10 min, etc. The process of the experiment is shown in Figure 3.

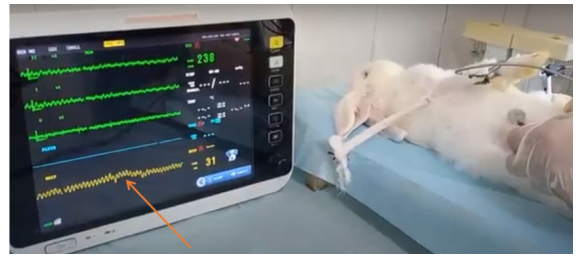


Figure 3. The test for determining the nociceptive reaction of a rabbit in the study of local anesthetic activity during infiltration anesthesia (photograph).

At the end of the experiment, the time of anesthesia development, depth (in percent), and duration were estimated. The assessment was performed by changing the threshold of the reaction to electrical irritation at the site of infiltration with a solution of the compound under study. Elimination of the response to threshold irritation was recorded as 20% anesthesia. An increase in stimulus threshold of 5 V was adopted as 40% anaesthesia. If the sensitivity threshold

was increased by 10V, the depth of anaesthesia was estimated to reach 60% and by 20V the depth of anaesthesia was estimated to reach 100%.

The obtained indicators were compared with local anesthetics widely used in clinical practice for infiltration anesthesia, characterized by varying degrees of efficiency and duration of action: procaine (HIMPHARM JSC, Kazakhstan), lidocaine (BZMP JSC, Belarus), trimecaine (Zhaik-AS LLP, Kazakhstan).

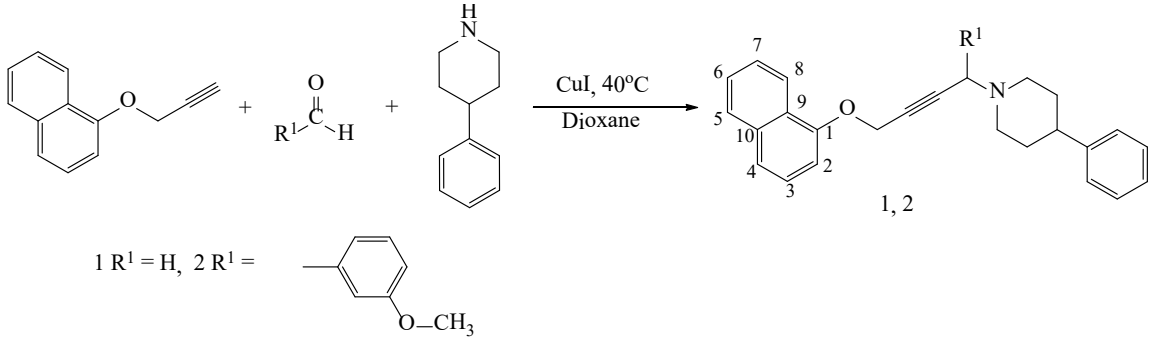
Statistical analysis

The results of the current study are presented as Means±SE or SD. The online software "Quest Graph™ LD_{50} Calculator" was used to calculate the LD_{50}, LD_{16}, and LD_{84} indicators (AAT Bioquest, Inc., 19 Feb. 2023, /https://www.aatbio.com/tools/ld50-calculator/). The standard error was determined for LD_{50} (Randhawa, 2009). All experimental groups were compared with the control group. T-test and ANOVA were used to determine the statistical significance of differences in the compared groups. A value of $p<0.05$ was considered statistically significant. The statistical data analysis was performed using SPSS/27.0 software (IBM, USA) for Windows.

RESULTS AND DISCUSSION

Results of the synthesis of the new piperidine derivatives

Under the conditions of the Mannich reaction, the interaction of 1-(prop-2-iniloxy)naphthalene with heterocyclic amine (4-phenylpiperidine) and aldehydes (formaldehyde and 3-methoxybenzaldehyde) in the presence of catalytic amounts of CuI in an absolute dioxane medium at a temperature of 40 °C for 2 hours, 1-(4-(naphthalene-1-iloxy) but-2-inyl)-4-phenylpiperidine (1) (yield, 75%) and 1-(1-(3-methoxyphenyl)-4-(naphthalene-1-iloxy) but-2-inyl)-4-phenylpiperidine (2) (yield, 46%) were obtainedand presented in Scheme 1.



Scheme 1. Synthesis scheme of novel 4-phenylnaphthoxybutynylamines (1, 2).

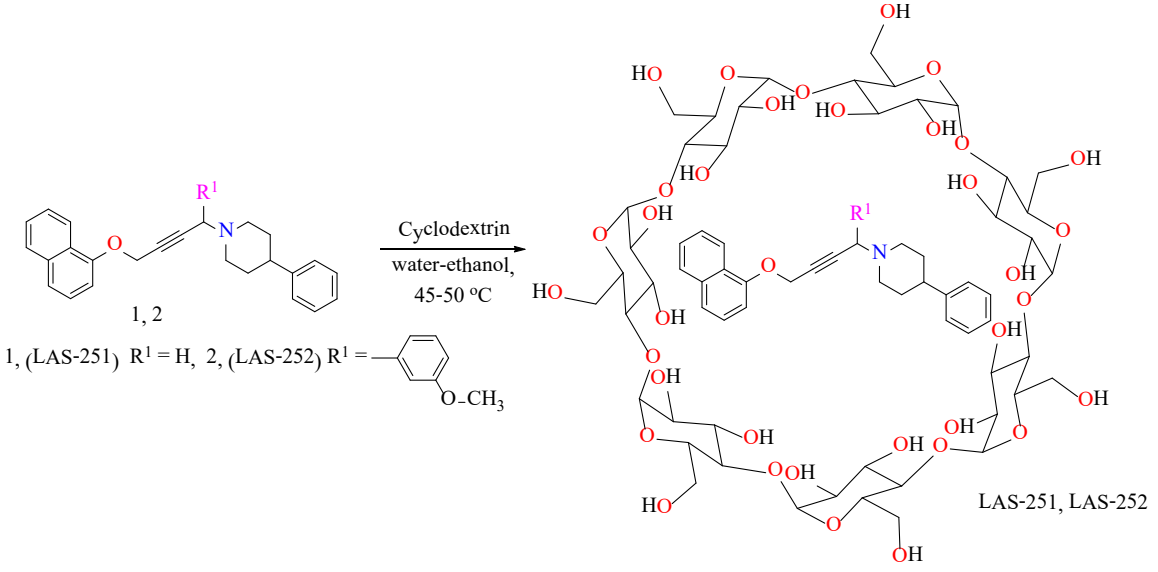
The structure of the synthesized compounds was determined based on the analysis of IR spectra and NMR spectroscopy data of ^{1}H and ^{13}C.

In the IR spectra of compounds 1, 2 there is no absorption band in the 3309 cm^{-1} region, characteristic of the C-H terminal acetylene group of the initial 2-(prop-2-iniloxy)naphthalene, but there is a weak band in the region of 2121 and 2125 cm^{-1}, characteristic of a disubstituted C≡C bond, which confirms the formation of an aminomethylation product.

Signals of protons of the aminomethylene and aminomethine groups were detected in the NMR 1H 4-phenylnaphtoxybutinylamines 1, 2 in the region of 3.39 ppm and 4.97 ppm in the spectra of NMR. The chemical shift in the range δ 4.97 and 4.90 ppm is attributed to protons of the O-methylene group. The signals of the protons of the piperidine methylene groups are at 1.83-2.56 ppm. The strong-field chemical shift (3.80 ppm) is attributed to the protons of the methoxy group at the atom of the benzene cycle. In the weak field area δ 6.81-8.29 ppm, signals of protons of the naphthalene nucleus and Ph rings are detected.

In the NMR ^{13}C spectra of compounds 1, 2 in the range of 47.69 ppm and 61.60 ppm, signals of carbon atoms of the aminomethylene and aminomethine groups were detected. Carbon atoms of the C≡C triple bond resonate at 78.73, 80.08 ppm, and 82.70, 84.11 ppm. The signal with a chemical shift of 56.22 ppm is attributed to oxymethylene carbon. The signals of the atomic carbons of the phenyl rings are in the weak field (105.52-159.55 ppm) of the spectra. The carbon atoms of the piperidine cycle resonate in the range δ 33.49-53.24 ppm, and the signal of the OCH_3 group (55.92 ppm) is present in the spectrum of compound 2.

Hydrophilic natural polymers such as cyclodextrin are used as a matrix to produce water-soluble supramolecular complex systems. The most important property of CD is the ability to selectively bind organic and biological molecules, forming inclusion complexes of the "guest-host" type to obtain water-soluble forms. The complexes are formed by the interaction of ethanol solutions of 4-phenylnaphtoxybutinylamines (1, 2) with an aqueous solution of β-cyclodextrin taken in a mass ratio of 1:1, at a reaction temperature of 45-50 °C and conducting the reaction for 5 hours. After the end of the reaction, the aqueous ethanol solution is distilled. The resulting complexes are washed with 96% ethanol and dried in a drying cabinet. As a result, 1 and 2 inclusion complexes (LAS-251 and LAS-252) were obtained with yields of 65 and 70%, respectively (Scheme 2.). The progress of the reactions was monitored using thin-layer chromatography on silica gel until the disappearance of the starting products (R_f 0.71 and R_f 0.69) in an aqueous-alcohol solution. The products were collected after the slow evaporation of the solution by forming powder products. The complexes were identified through elemental analysis.



Scheme 2. Synthesis of β-cyclodextrin complex with 1-(4-(naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine (LAS-251) and β-cyclodextrin complex with 1-(1-(3-methoxyphenyl)-4-(naphthalen-1-yloxy)but-2-yn-1-yl)-4-phenylpiperidine (LAS-252)

Acute toxicity assessment

Solutions of the studied compounds were administered to laboratory mice in 3 increasing concentrations following body weight, in a total volume of no more than 1 ml. As a result of observations with the administration of toxic doses, the clinical signs of intoxication of both compounds were similar. They differed in the rate of increase in symptoms of intoxication with an increase in dose.

After subcutaneous administration of high doses (500-700 mg/kg) of LAS-251 in the first 15 min, fading, muscle tremor with increased muscle tone, and increased breathing were observed in animals. The motor and research activity decreased in the following first hours of observation. Behavioural responses did not change, while responses to sound stimuli were preserved. From the second day onwards, symptoms of intoxication increased in several animals. Disturbances of motor activity with further developing adynamia and rapid breathing, lack of interest in food and water were revealed in laboratory animals. The animals took a lateral position, after which the death of laboratory animals in experimental groups was noted 2-3 days after administration.

The LAS-252 showed a variable range of symptoms in its poisoning pattern. The first signs of intoxication appeared in 2-4 min at a dose of 700 mg/kg. Almost all animals had decreased motor and exploratory activity and a lack of response to sound stimuli. One animal suffered from seizures, followed by ataxia and subsequent death. The mortality of other animals in the group was recorded after 1-2 days. When the dosage was increased to 1000 mg/kg, this compound showed pronounced neurotoxicity, which was manifested by tonic-clinical seizures with a frequency of 30-40 s. The death of more than half of the mice in the experimental group occurred already in the first 1.5-2 hours of observation.

When studying the acute toxicity of new piperidine derivatives, the median lethal doses were determined. The tested compounds turned out to be less toxic than procaine, lidocaine, and trimecaine by subcutaneous administration. Considering the chemical structure, the LD_{50} values of LAS-251 and LAS-252 differed slightly (Table 1.).

Table 1. Values of LD_{50} piperidine derivatives in the study of acute toxicity.

Compound, reference drug	LD_{16}	LD_{50}, mg/kg	LD_{84}
LAS-251	245.6	531.5±156.3	792.57
LAS-252	352.2	508.5±101.9*	709.2
1. Lidocaine	-	230±35.7	-
2. Procaine		480±1.0	
3. Trimecaine		375±3.1	

Data were reported as means±SE (n=6). *P<0.05 compared to lidocaine (t-test). The differences in the indicators are statistically significant (p<0.05).

The LD_{50} of these compounds significantly exceeded those of lidocaine and trimecaine and were approximately comparable to procaine. However, the difference was statistically significant only in LAS-252 compared to lidocaine ($p < 0.05$).

Assessment of local anesthetic activity

The results of the primary study of local anesthetic activity during infiltration anesthesia (Bulbring and Wajda model) are presented in Figure 4.

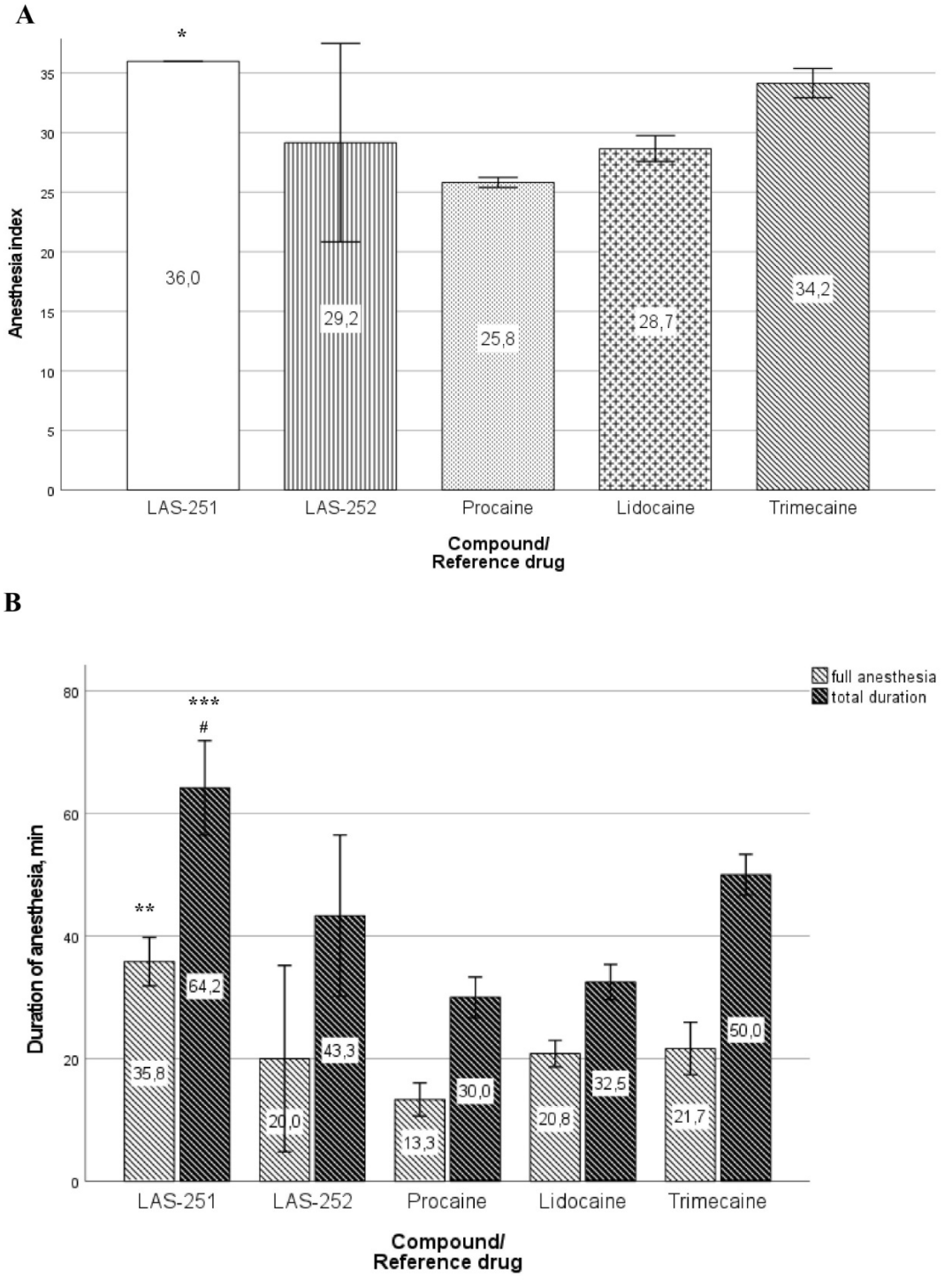


Figure 4. Indicators of the local anesthetic activity (0.5% aqueous solutions). Data are reported as means±SD (n=6), (ANOVA): A - Anesthesia index (max-36). *P<0.001 compared to procaine and lidocaine. B - Duration of anesthesia. **P<0.001 compared to all reference drugs, ***P<0.001 compared to procaine and lidocaine,#P<0.05 compared to trimecaine.

Of the two compounds studied, the piperidine derivative LAS-251 turned out to be the most active. A comparison of the indicators of local anesthetic activity of LAS-251 and reference drugs revealed statistically significant differences. The anesthesia index has reached its maximum value, which indicates the presence of pronounced local anesthetic activity superior to reference drugs, especially in comparison to procaine and lidocaine. According to the duration of full anesthesia, the studied compound was statistically significantly superior to the reference drugs, in particular: procaine - 2.7 times, lidocaine - 1.7 times, and trimecaine - 1.6 times. The total duration of anesthesia with the administration of LAS-251 lasted 64.2 min, which is 2 times longer than with procaine and lidocaine. The tested compound was superior to the most active local anesthetic by 14.2 min.

LAS-252 had advantages over procaine in all parameters determined in the experiment, and in terms of the index and duration of complete anesthesia, it practically corresponded to those of lidocaine. The total duration of the local anesthetic effect of LAS-252 was higher than that of procaine. However, the differences identified with the comparison drugs were not statistically significant ($p>0.05$).

Considering the initial experimental results, an in-depth study of local anaesthetic activity was carried out for compound LAS-251. In the conducted experiments, the rate of anesthesia onset, the duration of full anesthesia and the total duration of effect were determined (Table 2.).

Table 2. The local anesthetic activity of LAS-251 during the infiltration anesthesia of the abdominal wall in a rabbit (0.5% aqueous solutions).

Compound/ Reference drug	Anesthesia onset rate, min	Duration of full anesthesia, min	Total duration of anesthesia, min
LAS-251	9.7±2.0*	0	40.3±2.5**#
Procaine	3.0±0	0	23.8±1.5
Lidocaine	3.0±0	0	47.5±1.1
Trimecaine	3.0±0	5.0±0	86.6±2.1

Data reported as means±SE (n=6).*P<0.05 compared to all reference drugs, **P<0.001 compared to procaine and trimecaine, #P<0.05 compared to lidocaine (t-test). The differences in the indicators are statistically significant (P<0.05).

The local anesthetic effect of LAS-251 developed much slower, and the latency period was more than 3 times higher than the corresponding values of the reference drugs. Full anesthesia was not achieved, which was also typical for procaine and lidocaine. The experiments found that LAS-251 was significantly inferior to trimecaine and to a small extent to lidocaine in terms of total duration of infiltration anaesthesia, but exceeded this indicator of procaine by 16.5 min. Thus, the study confirmed the presence of local anesthetic activity with the achievement of anesthesia depth in laboratory animals by an average of 26.7%, with some advantage compared to procaine.

Currently, the piperidine cycle is quite a sought-after framework in pharmaceutics for developing new drugs. The chemical structure of piperidine can be found in more than twenty pharmacological groups and alkaloids (Frolov et al., 2023). A review of scientific research showed that several thousand different piperidine derivatives have been reported from preclinical and clinical studies over the last decade (Källström et al., 2008). There are not many studies on searching for compounds with local anesthetic activity among them, even though the piperidine ring is one of the components of the chemical structure of local anesthetics (Martins et al., 2017).

The results of our study revealed local anesthetic activity of previously unstudied new piperidine derivatives during infiltration anesthesia, expressed to varying degrees. The primary toxicity study demonstrated the safety of the substances in a further series of experiments to determine their efficiency, which also compares favorably with other studies. Another distinctive feature is the use of an in-depth research model, the conditions of which are as close as possible to clinical practice, which made it possible to obtain more reliable and expanded results. The local anesthetic indices presented in this article demonstrate the presence of local anesthetic activity of the piperidine derivative LAS-251, which correlates with the data of world studies. The lower activity of LAS-252 is probably due to the presence of a methoxyphenyl fragment, which affected solubility and, consequently, efficiency. The presence of a free hydrogen atom in the molecule LAS-251 apparently improved the solubility and increased its efficiency.

Earlier studies revealed local anesthetic activity of several new phenylpiperidine derivatives exceeding procaine, but the substances caused necrotic changes and exhibited higher toxicity in the cocaine range (Fellows et al., 1944). The findings of this review confirm the results of previous studies of different series of substances from the group of piperidines with local anesthetic activity in various types of anesthesia (Khaiitova et al., 2022). As in our study, the experimental drug kazcaine [1-(2-ethoxyethyl)-4-ethynyl-benzoyloxypiperidine hydrochloride], has high activity in infiltration anesthesia even in 0.1% solution (Kemelbekov U et al., 2010). In contrast to kazcaine, LAS-251 was effective in 0.5% and inferior in activity to lidocaine and trimecaine in a series of in-depth tests.

Another similar study [4-(benzoyloxy)-3-butoxypiperidinium chloride] also showed marked activity not only for infiltration but also for conduction anesthesia with the effect enhanced by combination with epinephrine (Pichkhadze et al., 2016). The study of potentiation of the efficacy of the compounds of the present research in interaction with vasoconstrictors is of interest in further tests. The activity of piperidine derivatives is also interesting in terminal anesthesia. In one study, the S-isomer of 2-{2-[N-(2-indanyl)-N-phenylamino]ethyl} piperdine was shown to be effective in local anesthetic action when applied to the surface of skin and mucous membranes (Gunnar et al., 2010).

The emergence of a new class of safer, highly active opioid analgesics and anesthetics is associated with the creation of several compounds containing 4-phenylpiperidine, found in morphine, in their structure (Kudzma et al., 1989). Verification of the analgesic activity of the compounds studied in this article may be a target for future studies. Considering that analgesic effects are also present in other piperidines with proven efficacy, such as promedol and fentanyl (Vasilyuk et al., 2021).

A comprehensive analysis of the results presented in this article allows us to conclude that the new piperidine derivative LAS-251 should be considered as a potential drug substance for the development of new highly effective drugs based on it. Preclinical studies of efficacy in other types of anesthesia, other pharmacological effects may become the subject of future studies.

CONCLUSION

In conclusion, it should be noted that the new 4-(Naphthalen-1-yloxy)but-2-yn-1-yl)-containing piperidine derivatives synthesized quite simply under the conditions of the Mannich reaction by the interaction of 1-(prop-2-ynyloxy) naphthalene with 4-phenylpiperidine and aromatic aldehydes in the presence of CuI are low-toxic substances, which are confirmed by the results of an acute toxicity tests. LAS-251 compound showed the greatest activity in the initial study of local anaesthetic activity during infiltration anaesthesia in the Bulbring and Wajda experimental model, surpassing the reference drugs in terms of anesthesia index, duration of full anesthesia and total duration

of action. The stage of in-depth study showed that, despite a longer latency period, LAS-251 has a local anesthetic effect longer than procaine and is slightly inferior to lidocaine. The results of the research suggest that the novel piperidine derivative LAS-251 is promising for further study in other types of anesthesia as a potential medicinal substance for therapeutic applications in the future.

ACKNOWLEDGEMENTS

The pharmacological part of Research was financially supported by Asfendiyarov Kazakh National Medical University of the Republic of Kazakhstan (grant 0122РКИ0052).

The synthetic (chemical) part of Research was provided by the Committee of Science of the Ministry of Science and High Education of the Republic of Kazakhstan (grants AP09057956, AP09057500).

AUTHOR CONTRIBUTION STATEMENT

Conduction of experiments, analysis of data and writing of original draft (MK), synthesis and structural analysis of compounds (YS), assistance with modelling of experiments and writing the manuscript (VT), design and direction of the project, editing of the pharmacological part (ES), writing and editing of chemical part (VY), review and supervision of the pharmacological part (TN), study conception and supervision of the pharmacological part (ES), data recording and assistance with experiments (YG), visualization and software (ZU), review and supervision of chemical part (KT)

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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