https://doi.org/10.31925/farmacia.2023.1.18 **ORIGINAL ARTICLE**

SYNTHESIS AND EXPERIMENTAL STUDY OF THE LOCAL ANESTHETIC ACTIVITY OF NEW MODIFIED PIPERAZINE DERIVATIVES

MALIKA KHAIITOVA [1]*, AIDA SEITALIYEVA [2], GULMIRA SMAGULOVA [1], ASSEL TEN [3], VALENTINA YU [3], ELMIRA SATBAYEVA [1]

[1]*Department of Pharmacology, Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan*
[2]*Department of Fundamental Medicine, Al-Farabi Kazakh National University, Almaty, Republic of Kazakhstan*
[3]*Laboratory of Synthetic and Natural Medicinal Compounds Chemistry, Bekturov Institute of Chemical Sciences, Almaty, Republic of Kazakhstan*

**corresponding author: khaiitova.m@kaznmu.kz*

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Abstract

N-substituted piperazines, an important class of compounds with different pharmacological activities were taken as the basic starting object for the synthesis and study of α-aminophosphonates. The objective of the study was the synthesis, analysis of biological effects (toxicity and local anaesthetic activity) of some new piperazine derivatives, and the evaluation of their potential pharmacological effects. The new piperazine derivatives were coded as LAS-276, LAS-277, LAS-278, where LAS is a local anaesthetic substance. The compounds were synthesized under the conditions of the Kabachnik-Fields reaction. Their acute toxicity in mice, the local anaesthetic activity in a regional and infiltration anaesthesia models were studied, and their estimated pharmacological activity was analysed using the online PASS (Prediction of Activity Spectra for Substances) program. In a series of experiments the administration of high doses of the studied compounds has not caused death of laboratory animals. The study of local anaesthetic activity in the infiltration anaesthesia model showed varying degrees of activity. The compounds showed moderate activity on the regional anaesthesia model. The PASS analysis revealed a very high probability of thrombolytic, anticoagulant, antisclerotic effects and stimulation of endothelin receptors. The implemented studies revealed the low toxicity, moderate local anaesthetic activity of the studied piperazine compounds. There is also a high probability of their thrombolytic activity, which requires further research.

Rezumat

Piperazinele N-substituite sunt o clasă importantă de compuși cu activitate farmacologică ce stau la baza sintezei și studiul α-aminofosfonaților. Obiectivele studiului au fost sinteza, analiza efectelor biologice (toxicitate și activitate anestezică locală) și evaluarea efectelor farmacologice ale noilor derivați de piperazină. Compușii au fost sintetizați în condițiile reacției Kabachnik-Fields. Apoi s-a studiat toxicitatea lor după administrarea în doză unică la șoareci, activitatea anestezică locală într-o anestezie regională și modele de infiltrație. Activitatea lor farmacologică a fost evaluată folosind programul online PASS (Predicția Spectrelor de Activitate pentru Substanțe). Într-o serie de experimente, administrarea de doze mari din compușii studiați nu a provocat moartea animalelor de laborator. Studiul activității anestezice locale în modelul de anestezie prin infiltrare a arătat grade variate de activitate. Compușii au prezentat activitate moderată în modelul de anestezie regională. Analiza PASS a relevat o probabilitate foarte mare de efecte trombolitice, anticoagulante, antisclerotice și de stimulare a receptorilor de endotelină. Studiile implementate au evidențiat toxicitatea scăzută, precum și activitatea anestezică locală moderată a compușilor piperazinici studiați. Există, de asemenea, o probabilitate mare a activității lor trombolitice, ceea ce necesită cercetări suplimentare.

Keywords: piperazines, toxicity, local anesthetic activity, computer screening

Introduction

Many medical manipulations are accompanied by persistent pain. As known, pain syndrome reduces the ability to work, can cause disability and even death. Local anaesthetics are widely used to alleviate it in various fields of medicine and have been used for more than a century. However, the modern understanding of the mechanisms of their action, interaction with biological structures continues to surprise not only researchers, but also clinicians. The constant use of drugs of this pharmacological group in everyday practice is accompanied by the risk of developing various kinds of adverse reactions and toxic manifestations of a systemic and local nature. The intensity of adverse reactions varies from clinically insignificant to life-threatening [18, 22, 39, 43].

Thus, in modern clinical practice there is an obvious need for safer and more effective medicines with local anaesthetic activity. This leads to a righteous research for ways to reduce toxicity by developing micro- or nano-encapsulated forms to ensure controlled

release of the drug substance and the development of safe long-acting local anaesthetics with sufficient depth of anaesthesia [9, 20].

Piperazine is a nitrogen-containing heterocycle with a unique structure. It is possible to provide selective ligands for various biological targets due to the universal binding property of the piperazine matrix. This fact is confirmed by the presence of many piperazine derivatives with different pharmacological activity in the literature. Currently, piperazine derivatives are one of the most popular heterocyclic compounds used for the development of new candidate drugs. In particular, literary sources reveal the analgesic potential of piperazine derivatives [26, 58, 66].

In recent years, studies have revealed the presence of antinociceptive, locally anaesthetic, analgesic types of activity in modified piperazine derivatives. Salat K *et al*. were successful in the synthesis of 3-[4-(3-trifluoromethylphenyl)piperazine-1-yl]-dihydrofuran-2-one, which is an effective antinociceptive and local anaesthetic in rodents. The extended study of pharmacological activity showed the presence of analgesic, anticonvulsant effects and antioxidant properties *in vitro* [55]. The compound (1-cyclo-hexyl-4-(1,2-diphenylethyl)-piperazine), originally developed as a potential analgesic substitute for morphine, is currently a new synthetic opioid [7]. Antinociceptive and anti-inflammatory effects of piperazine derivative 4-[(1-phenyl-1H-pyrazole-4-yl)-methyl]-1-piperazine carboxylic acid ethyl ether and participation in the serotonergic pathway were presented in the study by Silva DP *et al.* [60]. The effect of piperazine-1-yl-dihydrofuran-2-one on nociceptive receptors has been studied, which have shown good results in models of neuropathic pain and are able to relieve neurogenic pain [56]. Piperazines have different biological activity of the nuclei, which is due to easy modification, solubility in water, the ability to form hydrogen bonds and the ability to regulate the molecular physical and chemical properties of the compound. Among piperazine derivatives, Pudukulatham Z *et al*. identified a new T-type calcium channel blocker with a pronounced analgesic effect in an inflammatory pain model [50].

In the course of domestic studies of local anaesthetic activity during infiltration anaesthesia, a number of organophosphorus, thio-containing and naphthyl derivatives of piperazine demonstrated a pronounced effect, apparently associated with the content of phenyl-propionic and phenoxyethyl radicals in the sulphur atom [57]. Based on scientific data and the revealed patterns of the relationship between the chemical structure and the presence of local anaesthetic activity of piperazine compounds, they were used for further targeted synthesis of new modified piperazine derivatives and formed the basis for this study.

The prospect of searching for new substances with useful properties, primarily those with biological activity, remains the main driving force behind the development of chemistry of a particular class of organic compounds, therefore it is obvious that the final stage of any synthetic research to find new effective safe medicines is to identify substances with valuable pharmacological properties among synthesized compounds and establish the relationship between their structure and biological activity.

Important class of compounds with a wide range of biological activity, α-aminophosphonates, are of particular interest among the piperazine derivatives. α-aminophosphonates are a typeof bioisostere analogues of natural α-amino acids, which largely determines their physiological activity. Various aminophosphonates exhibit antitumour, antibiotic, antifungal, herbicidal and fungicidal activity and have growth-stimulating activity [1, 10, 29, 34, 44, 53]. Thus, they are crucial for medical chemistry.

Various N-substituted piperazines, an important class of compounds that have shown various pharmacological activity [2, 14, 31, 38], including antibacterial, anti-histamine, anticancer, analgesic and especially widely used in medicine as an anthelmintic agent, were taken as the basic starting object for the study and synthesis of α-aminophosphonates.

At the present stage of development of the pharmaceutical industry, molecular inclusion complexes based on cyclodextrins (natural cyclic oligosaccharides consisting of 6 (α-CD), 7 (β-CD) and 8 (γ-CD) glucose residues) are widely used, which make it easy to convert liquid low-molecular substances into solid ones [8, 61].

As a rule, inclusion complexes with cyclodextrins are amorphous and crystalline powders. They are more stable in water, less susceptible to air oxidation, dehydration and evaporation, in addition, the smell and taste of the original substance are eliminated, their bioavailability increases, and the complexing of the active substance with CD reduces its toxicity, for example, obtaining CD complexes of many well-known drugs has reduced their ulcerogenic effect.

At the beginning of the study, a classical approach was applied to obtain aminophosphonates, namely, the three-component "*one-pot*" Kabachnik-Fields reaction, which is a universal general method for the synthesis of phosphorus-containing organic compounds and takes place in the amine-carbonyl compound (aldehyde or ketone)-hydrophosphoryl compound [17].

The amine component used in our study was phenyl-piperazine and pyrimidinylpiperazine Phenylpiperazines are known as a class of antidepressants with a defined targeted action [5]. Pyrimidinylpiperazines are also used in medicine, for example 1-(2-pyrimidinyl)-piperazine is an active metabolite of buspiron - anxiolytic medicine (tranquilizer), which also has an antidepressant effect [28, 30]. In addition, many other drugs have a pyrimidine fragment in their structure: acyclovir (an antiherpetic drug), methyluracil (a

regeneration stimulant), trimethoprim (a dihydrofolate reductase inhibitor of bacteria), chloridine (an antimalarial agent), risperidone (an antipsychotic agent), vitamin B1; cytosine, thymine, uracil, which are widely known and are part of nucleotides as well they have a pyrimidine nucleus [46].

The objective of this work was the synthesis of new piperazine derivatives and the study of their general toxicity, local anaesthetic activity and the relationship with the chemical structure.

Materials and Methods

Chemical research

Synthesis of compounds

Synthesis of compounds was carried out in the laboratory of chemistry of synthetic and natural medicinal substances of A.B. Bekturov Institute of Chemical Sciences (Almaty).

Interaction of 4-phenyl-, 4-(2-pyrimidinyl)-piperazines with 3-phenoxy, 2,5-dimethoxy-benzaldehydes and dimethylphosphite occur under the conditions of classical Kabachnik-Fields reaction (boiling in benzene using a Dean-Stark nozzle to drain the resulting water from the reaction medium) and leads to the formation of target α-aminophosphonates 5 - 7 (Figure 1).

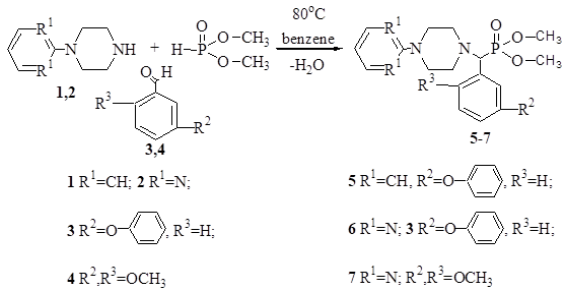


Figure 1.
Synthesis of new compounds

Synthesis of LAS-276 – dimethyl((3-phenoxyphenyl)-(4-phenylpiperazine-1-yl)methyl)phosphonate (5, LAS-276). 1.88 mL (0.0123 mol) of 1-phenylpiperazine in 150 mL of abs. benzene is placed in a flat-bottomed three-necked conical flask equipped with a Dean-Stark nozzle with backflow condenser. Then 2.13 mL (0.0123 mol) of 3-phenoxybenzaldehyde and 1.14 mL (0.0123 mol) of dimethylphosphite are added sequentially. The reaction mixture is stirred for 20 minutes at room temperature. Then, with constant stirring, the mixture is heated at the boiling point of benzene for 48 hours. After distillation of the solvent, the product is isolated by column chromatography on Al_2O_3 by elution with a mixture of chloroform: hexane (1:1), collecting the second fraction and obtaining 3.0 g (52% of the theoretical) (dimethyl-((3-phenoxyphenyl)(4-phenylpiperazine-1-yl)methyl)-phosphonate) (5) in the form of yellow oil, with R_f = 0.38 (hexane:chloroform = 1:3). Found, %: C 66.26; H 6.49. $C_{25}H_{29}O_4N_2P$. Calculated, %: C 66.36; H 6.46. IRS (infrared spectrum), cm^{-1}: 760 (P-C); 1165 (P=O); 671, 754, 1488, 1559, 3058 (Ph).

Synthesis of LAS-278 – dimethyl((3-phenoxyphenyl)-(4-(pyrimidine-2-yl)piperazine-1-yl)methyl)phosphonate (6, LAS-278). 1.73 mL (0.0122 mol) of pyrimidine piperazine in 150 mL of abs. benzene is placed in a flat-bottomed three-necked conical flask equipped with a Dean-Stark nozzle with backflow condenser. Then 2.09 mL (0.0122 mol) of 3-phenoxybenzaldehyde and 1.12 mL (0.0122 mol) of dimethylphosphite are added sequentially. The reaction mixture is stirred for 20 minutes at room temperature. Then, with constant stirring, the mixture is heated at the boiling point of benzene for 32 hours. After distillation of the solvent, the product is isolated by column chromatography on Al_2O_3 by elution with a mixture of chloroform:hexane (1:1), collecting the second fraction and obtaining 3.65 g (66% of the theoretical) (dimethyl((3-phenoxy-phenyl)(4-(pyrimidine-2-yl)piperazine-1-yl)methyl)-phosphonate) (6) in the form of yellow oil, with R_f = 0.32 (hexane:chloroform = 1:3). Found, %: C 60.83; H 5.92. $C_{23}H_{27}O_4N_4P$. Calculated, %: C 60.79; H 5.99. IRS, cm^{-1}: 756 (P-C); 1165 (P=O); 696, 760, 1496, 1600, 2944 (Ph).

Synthesis of LAS-277 – dimethyl((2,5-dimethoxyphenyl)-(4-(pyrimidine-2-yl)piperazine-1-yl)methyl)-phosphonate (7, LAS-277). 1.73 mL (0.0122 mol) of pyrimidine piperazine in 150 mL of abs. benzene is placed in a flat-bottomed three-necked conical flask equipped with a Dean-Stark nozzle with backflow condenser. Then 2.03 g (0.0122 mol) of 2.5-dimethoxy-benzaldehyde and 1.12 mL (0.0122 mol) of dimethyl-phosphite are added sequentially. The reaction mixture is stirred for 20 minutes at room temperature. Then, with constant stirring, the mixture is heated at the boiling point of benzene for 36 hours. After distillation of the solvent, the product is isolated by column chromatography on Al_2O_3 by elution with a mixture of chloroform:hexane (1:1), collecting the second fraction and obtaining 4.09 g (80% of the theoretical) (dimethyl-((2,5-dimethoxyphenyl)(4-(pyrimidine-2-yl)piperazine-1-yl)methyl)phosphonate) (7) in the form of yellow oil, with R_f = 0.27 (hexane:chloroform = 1:3). Found, %: C 52.16; H 6.27. $C_{19}H_{27}O_5N_4P$. Calculated, %: C 52.04; H 6.44. IRS, cm^{-1}: 765 (P-C); 1167 (P=O); 673, 756, 1490, 1553, 3059 (Ph).

General technique for obtaining complexes of inclusion of substances 5-7 with β-cyclodextrin

Solutions of 0.01 mol of the compound (substances 5 - 7) are mixed in 30 mL of ethyl alcohol and 0.01 mol β-cyclodextrin in 90 mL of distilled water. The mixture is placed in a drying cabinet, ethanol and water are evaporated at 50 - 55°C. Inclusion complexes are obtained in the form of a white powder.

The composition and structure of the synthesized derivatives 5,6,7 (LAS-276, LAS-278, LAS-277) are

confirmed by the results of elemental analysis and spectral data. The structure of the synthesized compounds was also confirmed by the method of nuclear magnetic resonance spectroscopy (NMR 1H, 13C).

Pharmacological research

Methods of experimental research

The models recommended by the manual for the experimental (preclinical) study of new pharmacological substances Mironov AN were used [41]. Experimental work was performed with outbred white mice, guinea pigs and rats. All laboratory animals were previously quarantined for 2 weeks and kept in the same standard conditions. All animals were kept with complete, certified feeds produced by SSNIFF (Germany) with free access to water and food under natural light mode "day-night". All types of experiments were conducted in accordance with the Order of the Minister of Healthcare of the Republic of Kazakhstan dated December 11, 2020, No. ҚР ДСМ-255/2020 "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 provision of the European Convention for the Protection of Vertebrate Animals and Directive 210/63/EU. All studies were pre-approved by the Local Ethics Committee of Asfendiyarov Kazakh National Medical University.

Acute toxicity study

Toxicity experiments were performed with healthy mature outbred mice, both sexes of the same age, with a range of the initial mass not exceeding ± 10%. Each study group included 6 laboratory animals. The distribution into groups was random. Aqueous solutions (solvent - water for injection) of the studied compounds in 3 concentrations were injected once into the lateral surface of the body, subcutaneously. This type of administration was chosen as the intended route for future use as a local anaesthetic. A single injection of a solution in only one concentration (800 mg/kg, 1200 mg/kg) was provided for each group.

After administration, continuous monitoring was carried out on the first day, and then daily for 14 days. During the observation period, the general condition of the animals was recorded, in particular, behavioural characteristics, intensity and nature of motor activity, the presence and nature of seizures, coordination of movements, skeletal muscle tone, reaction to tactile, painful, sound and light stimuli, the condition of the coat and skin, the colour of the mucous membranes, the position of the tail, the amount and consistency of faecal masses, feed and water consumption, body weight changes.

Study of local anaesthetic activity in infiltration anaesthesia

The study of local anaesthetic activity in infiltration anaesthesia was carried out using the Bulbring and Wajda method [41] with male guinea pigs. Each compound was tested on six animals weighing 200 - 250 g. Freshly prepared 0.5% solutions of the compound under study in a volume of 0.25 mL were intradermally injected into the back area of each animal, at 4 points (A, B, C, D) at the corners of the square with a side of 3 cm. The solutions were administered in such a way that the test compound in each concentration was injected into one anterior (A) and one posterior (D) points, and the reference preparation in the corresponding concentrations was injected into points B and C. Local anaesthetic activity was evaluated 6 - 8 times for each of the selected concentrations. Sensitivity at the injection site was determined by touching with an injection needle in a series of 6 touches at intervals of 3 - 4 seconds, every 5 minutes, for 30 minutes. The total number of needle touches that did not cause an animal reaction (skin twitching) for 30 minutes was regarded as an index of infiltration anaesthesia for this solution of the compound. The intensity of anaesthesia in the "anaesthesia indexes", the duration of complete anaesthesia and the total duration of the anaesthetic effect were also determined. The activity of the compounds was compared with the reference drug (lidocaine and trimecaine).

Study of local anaesthetic activity in regional anaesthesia

Modified method "tail flick", developed by the Department of Pharmacology of St. Petersburg Medical University [37], was used for the experimental study of local anaesthetic activity in regional anaesthesia. The study was performed with the use of outbred white male rats. The principle of the method is to record the latent period of tail retraction during thermal exposure of its middle part to a focused light beam from an optoelectronic algesimeter TF-003 (Russia, 2009) before and after anaesthesia. The intensity of the thermal nociceptive effect was adjusted so that the initial tail-pulling reactions occurred with a latent period in the interval of 3 - 6 seconds. Initially, the threshold of pain sensitivity was determined. Then, a solution of the tested compounds and reference preparations in a volume of 1 mL were evenly administered from four sides of the rat tail root. After the administration of the tested substance and reference preparations, repeated testing was carried out at a certain time interval. An increase in the latent period of the tail twitch reflex by 2 times was estimated as complete anaesthesia. The compounds and the reference preparation were compared by the time of the onset of anaesthesia, the duration of complete anaesthesia and the total duration of the anaesthetic effect of the preparation.

Study of chemical structure using PASS online program

PASS online program was used for the study of the chemical structure of new piperazine derivatives. This program allows to assess of a wide range of expected pharmacological properties of substances based on their structure [21]. The results were obtained in the form of indexes P_a - reflecting the

probability of an effect and P_i - indicating the probability of no effect. Accordingly, the greatest difference between these indicators shows a high probability of determined pharmacological effect.

Statistical analysis

Statistical processing of the results obtained during the study was carried out using the software Statistica, version 13.3 for Windows 10. The research results are presented as mean(M) ± standard error (m). The results were evaluated using the ANOVA test.

Results and Discussion

Results of the synthesis of new compounds

The following compounds were obtained: LAS-276, LAS-277, LAS-278. The chemical structure of the compounds is shown in Table I.

Table I

Chemical structure of piperazine derivatives

Code	Chemical structure
LAS-276	Complex dimethyl-((3-phenoxyphenyl)(4-phenylpiperazine-1-il)methyl)phosphonate with β-CD
LAS-277	Complex dimethyl-((2,5-dimethoxyphenyl)(4-(pyrimidine-2-yl)piperazine-1-yl)methyl)phosphonate with β-CD
LAS-278	Complex dimethyl-((3-phenoxyphenyl)(4-(pyrimidine-2-yl)piperazine-1-yl)methyl)phosphonate with β-CD

Table II

Outputs and physico-chemical characteristics of 4-phenyl- and 4-benzhydrylpiperazine aminophosphonates

Compound	Output, %	Reaction time, h	*R_f,	Found %, Calculated, %		Molecular formula
				C	H	
5 (LAS-276)	52	48	0.38	66.26 66.36	6.49 6.46	$C_{25}H_{29}O_4N_2P$
6 (LAS-278)	66	32	0.31	60.83 60.79	5.92 5.99	$C_{23}H_{27}N_4O_4P$
7 (LAS 277)	80	36	0.27	52.16 52.04	6.27 6.44	$C_{19}H_{27}O_5N_4P$
* - Eluent: hexane:chloroform = 1:3						

The results of elemental analysis and spectral data are presented in Table II.

The formation of new aminophosphonates 5 - 7 is indicated primarily by the disappearance of the absorption band of the N-H group of the initial secondary amine in the IR spectrum at 3500 - 3300 cm^{-1}. The most characteristic absorption bands of P=O and P-C bonds are observed at 1165 - 1167 cm^{-1} and 756 - 765 cm^{-1}, respectively. Absorption bands of phenyl substituents in compounds 5 - 7 are observed in the high-frequency region – 3060 - 2944 cm^{-1}, as well as at 780 - 656 cm^{-1}, the latter corresponds to out-of-plane deformation vibrations of the benzene ring.

In the PMR (proton magnetic resonance) spectra of compounds 5 - 7 (LAS-276, LAS-278, LAS-277) (Table III) protons of methylene groups piperazine cycle provide two multi-blade block signal in the region of strong fields at 1.50 - 2.77 ppm and 2.49 - 3.14 ppm, and that evidences the magnetic non-equivalence of the methylene protons of the cycle and their interaction with each other, and the first multiplet correlated with the signals of protons in the axial orientation, the second - in the equatorial. Protons of the CH(P) group resonate in the range of 3.82 - 3.90 ppm Widened singlets with an intensity of 6 hydrogen atoms at 3.58 - 3.62 ppm belong to protons of two methoxygroups of the phosphoryl fragment, and at 3.82 ppm – protons of two methoxy groups of the dimethoxyphenyl fragment of amino-phosphonate 7. The protons of pyrimidine cycle of compounds 6 - 7 give signals in the downfield at 6.46 - 6.92 ppm and 8.29 - 8.45 ppm. Protons of the phenyl substituent of piperazine cycle connection 5 give a triplet signal at 6.79 ppm and two multiplet signals with the intensity of two hydrogen atoms 6.93 ppm and 7.27 ppm. Also, the most downfield signals at 6.95 - 7.58 ppm belong to the aromatic protons of 3-phenoxyphenyl and 2.5 – dimethoxy-aniline substituents of compounds 5 - 7. The carbon composition (Table IV) fully corresponds to the estimated composition of the obtained amino-phosphonates (5 - 7).

For the compounds 5 - 7, upfield signals of double intensity at 48.5 - 55.0 ppm belong to the carbon atoms of the piperazine (C-2, C-6 and C-3, C-5) cycle. Signals of carbon atoms of the dimethoxy-phosphoryl fragment are observed at the same 52.1 - 56.2 ppm. The downfield region is "populated" by aromatic carbon atoms. Thus, in the far part of the spectrum, signals of the phenyl substituent of the piperazine fragment of compound 5 are observed at 115.0 - 149.3 ppm, as well as signals of the pyrimidine ring at 115.3 - 161.3 ppm, and the most downfield signal belongs to the carbon of the pyrimidine ring located between two nitrogen atoms. Also, downfield signals at 124.5 - 159.5 ppm belong to aromatic carbon atoms of 3-phenoxyphenyl- and 2,5-dimethoxyphenyl substituents. Methine carbon CH(P) resonates at 66.6 - 67.3 ppm. The signals of carbon atoms of the methoxyl groups of derivative 7 are observed at 55.1 and 55.6 ppm.

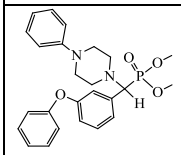
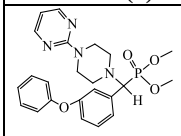
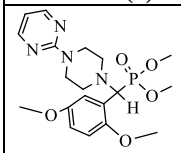
Table III

NMR spectra data of ^{1}H compounds

	NMR spectrum ^{1}H (δ, ppm, J (Hz)		
	5	**6**	**7**
CH_2(piperazine)	2.77a (2H) 3.14e (2H)	2.11a (2H) 2.84e (2H)	1.50a (2H) 2.49e (2H)
CH(pyrimidine)	-	6.92m 8.45d (2H)	6.46t 8.29d (2H)
CH(P)	3.90	3.82	3.90
$2CH_3$(P)	3.62	3.66	3.58
$2CH_3$(O)	-	-	3.82
C_6H_4 C_6H_3	6.95 m (2H) 7.16 m 7.29 m	6.95 m (2H) 7.16 m 7.29 t	7.24 s 7.56 d 7.58 d
NC_6H_5	6.79 t 6.93 m (2H) 7.27 m (2H)	-	-
OC_6H_5	7.14 m (2H) 7.17 m 7.41t(2H)	7.14d (2H) 7.17m 7.41t(2H)	-

Table IV

Values of chemical shifts of carbon atoms in the NMR spectrum of ^{13}C α-aminophosphonates

Compound	**Chemical shifts ($CDCl_3$), δ, ppm.**								
	<u>C</u>H(P)	C-2, C-6	C-3, C-5	O<u>C</u>H_3 (C)	O<u>C</u>H_3(P)	<u>C</u>$_6H_4$(O)	<u>C</u>$_6H_3$ $(OMe)_2$	4-Ph(N) 4-Pyr	<u>Ph</u> (O)
 LAS-276(5)	67.3	48.5	51.2	-	52.1 55.2	130.0 - 159.5	-	115.0 - 149.3	115.0 - 149.3
 LAS-278(6)	66.3	48.4	50.2	-	52.3 56.2	129.1 - 158.7	-	115.3 - 161.3	116.0 - 152.2
 LAS-277(7)	66.6	52.9	55.0	55.6 55.1	52.9 53.0	-	124.5 - 151.3	115.6 - 160.8	-

Acute toxicity results

The administration of high doses (800 mg/kg, 1200 mg/kg) of solutions of the studied compounds LAS-276, LAS-277, LAS-278 did not lead to the death of laboratory animals in a series of experiments. Due to poor solubility, a further increase in the dose was impractical. After administration and during the observation period any toxic effect (behavioural changes, weight loss, breathing or cardiovascular problems, seizures, etc.) of the substance were not revealed.

An experimental study of the acute toxicity of compounds with subcutaneous administration showed that all modified piperazine derivatives belong to low-toxic substances.

Thus, the results of the experiments showed that all the studied compounds are less toxic than lidocaine and trimecaine, and they are low-toxic substances. The difference in chemical structure did not significantly affect the difference in toxicity of the studied compounds.

Results of a study of the local anaesthetic activity of piperazine derivatives in infiltration anaesthesia model

The results of the study of the local anaesthetic activity of piperazine compounds during infiltration anaesthesia are shown in Table V.

Table V shows the mean indexes in the 6 groups with a standard deviation. An analysis of the experimental data of this series of experiments indicates that all tested compounds have an effect to varying degrees during infiltration anaesthesia.

Table V

Indexes of piperazine derivatives and reference preparations during infiltration anaesthesia in 0.5% solutions

Compound, preparation	0.5% solutions					
	Anaesthesia index M ± m		Duration of full anaesthesia min.		Duration of action min.	
LAS-276	6 ± 1.4	$p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$	0	$p_1 > 0.05$ $p_2 > 0.001$ $p_3 < 0.001$	10±0	$p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$
LAS-277	9 ± 1.6	$p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$	12.5 ± 1.1	$p_1 > 0.05$ $p_2 < 0.001$ $p_3 < 0.001$	22±1.6	$p_1 > 0.05$ $p_2 < 0.001$ $p_3 < 0.001$
LAS-278	20 ± 0.3	$p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$	12.5 ± 1.7	$p_1 > 0.05$ $p_2 < 0.001$ $p_3 < 0.001$	32±1.06	$p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.001$
Procaine	30.0 ± 0.2		10.0 ± 0		22.0 ± 0.1	
Lidocaine	32.3 ± 2.3		25.8 ± 0.8		54.5 ± 2.3	
Trimecain	34.1 ± 0.5		30.0 ± 1.7		44.1 ± 1.7	

p_1 – correlation coefficient compared to procaine; p_2 – compared to lidocaine; p_3 – compared to trimecaine

The most active compound is LAS-278, the anaesthesia index of which is 55% of the maximum, exceeding that of LAS-277 and LAS-276 by 2.2 and 3.3 times, respectively; LAS-278 is less active in comparing with reference preparations. The anaesthesia index LAS-278 is 1.5 times lower than that of procaine, 1.6 times lower than that of lidocaine and 1.7 times lower than that of trimecaine. Compound LAS-277 has a moderate activity, and LAS-276 is the least active. Anaesthesia indices LAS-277 and LAS-276 are significantly inferior to those of reference preparations. The duration of complete anaesthesia is of great importance for determination of activity of a compound with a particular type of anaesthesia. The complete insensitivity of the skin of guinea pigs at the administration of LAS-278 lasted 12.5 minutes, which corresponds significantly to LAS-277. LAS-276 did not cause complete anaesthesia at all. The indicator of complete anaesthesia of LAS-278 and LAS-277 is 1.25 times higher than the corresponding indexes of procaine. The duration of complete anaesthesia of LAS-278 and LAS-277 is shorter than that of lidocaine and trimecaine by 2 and 2.4 times.

The total duration of action of LAS-278 is 32 minutes, which exceeds the corresponding parameter of LAS-277 and LAS-276 by 1.4 and 3.2 times, respectively. According to the duration of general anaesthesia, LAS-278 statistically significantly exceeds the corresponding parameter of procaine, is inferior to lidocaine and does not differ significantly from trimecaine.

Summarizing the results of the experiments, it can be concluded that modified derivatives of piperazine have varying degrees of pronounced activity during infiltration anaesthesia. LAS-278 has a significant effect on the duration of complete anaesthesia and infiltration anaesthesia. At infiltration anaesthesia, LAS-278 is superior to procaine, but inferior to trimecaine and lidocaine.

Results of a study of the local anaesthetic activity of piperazine derivatives in regional anaesthesia model

In this series of experiments, the compounds LAS-276 and LAS-277 were the most active. However, all the studied compounds did not cause complete anaesthesia. Table VI presents the study results.

The total duration of anaesthesia induced by LAS-276 and LAS-277 was 33 and 30 minutes and approximately corresponds to procaine ($p > 0.05$).

Table VI

Indexes of piperazine derivatives and reference preparations in regional anaesthesia in 1% solutions

Compound, preparation	1 % solution			
	Duration of full anaesthesia min.		Total duration of action min.	
LAS-278	0	-	28 ± 4.0	$p_1 < 0.01$ $p_2 < 0.05$ $p_3 > 0.05$
LAS-277	0	-	30 ± 1.8	$p_1 < 0.01$ $p_2 < 0.05$ $p_3 > 0.05$
LAS-276	0	-	33 ± 3.8	$p_1 < 0.01$ $p_2 > 0.05$ $p_3 > 0.05$
Lidocaine	65.0 ± 18.1	-	90.0 ± 18.6	
Trimecain	47.3 ± 8.8	-	56.9 ± 12.8	
Procaine	35.0 ± 7.0	-	42.5 ± 13.8	

p_1 – correlation coefficient compared to trimecaine; p_2 – compared to lidocaine; p_3 – compared to procaine

By duration of action (total duration of action) these compounds are inferior to lidocaine and trimecaine and do not differ significantly from procaine ($p > 0.05$). LAS-276 and LAS-277 act 2.7 and 3.3 times shorter than lidocaine and trimecaine, respectively. 1% solutions by the total duration of action are similar to procaine. LAS-278 has the weakest local anaesthetic effect, which was inferior to LAS-277 compounds.

Study of the pharmacological properties of piperazine derivatives using the PASS program

The analysis of LAS-276 revealed a very high probability of thrombolytic, anticoagulant, antisclerotic effects, stimulation of endothelin receptors, as well as the possibility of application for strokes. At the same time, the probability of local anaesthetic activity of this compound has not been identified. The analysis of LAS-277 showed the same effects as that of LAS-276, but also revealed a slight probability of general anaesthesia. Local anaesthetic activity was not detected. LAS-278 was almost identical in its properties to LAS-277, with very little activity as a general anaesthetic.

Thus, the analysis of the chemical structure revealed a very high probability of thrombolytic, anticoagulant and antisclerotic effects for compounds LAS-276, LAS-277, LAS-278.

In general, it seems promising to further study the pharmacological properties of compounds LAS-276, LAS-277 and LAS-278 in the prevention and treatment of thrombosis. Table VII presents the numerical values of the results of this research method.

Table VII

Pharmacological effects of piperazines

Compound	Pharmacological effect	Pa	Pi	(Pa - Pi)
LAS-276	Thrombolytic	0.990	0.000	0.990
	Anticoagulant	0.944	0.003	0.941
	Antisclerotic	0.939	0.003	0.936
	Stimulation of endothelin receptors	0.926	0.000	0.926
	Stroke treatment	0.729	0.005	0.724
LAS-277	Thrombolytic	0.990	0.000	0.990
	Anticoagulant	0.944	0.003	0.941
	Antisclerotic	0.939	0.003	0.936
	Stimulation of endothelin receptors	0.926	0.000	0.926
	Stroke treatment	0.538	0.009	0.529
	General anaesthesia	0.367	0.049	0.318
LAS-278	Thrombolytic	0.993	0.000	0.993
	Anticoagulant	0.977	0.003	0.974
	Antisclerotic	0.966	0.003	0.963
	Stimulation of endothelin receptors	0.944	0.000	0.944
	Stroke treatment	0.660	0.005	0.655
	General anaesthesia	0.261	0.087	0.174

Pa – value of probability of belonging to the class of "actives"; Pi - value of probability of belonging to the class of "inactives"; (Pa - Pi) – relative value of probability of biological activity

Piperazine compounds are known for their high biological activity, for example, there is a lot of data on their antimicrobial properties [15, 35, 47, 59], including their antifungal [27], antituberculous [48] and anti-protozoal [25] activity. There are many works about the antitumour activity of such compounds [12, 23, 24, 54].

A large group of piperazine derivatives has a central effect [6, 13], for example, it acts on serotonin [42, 64, 65], dopamine [16, 49] and even on cannabinoid [40] receptors, affects the activity of MAO (monoamine oxidase) [49, 62], therefore represents a potential class of drugs that have antipsychotic [11], antidepressant [36, 63], in some cases anxiolytic activity [13, 19]. In some cases, their analgesic and anti-inflammatory effects are described [26]. There are works describing their antiarrhythmic properties [3, 4, 33, 51, 52].

There is information about their moderate local anaesthetic effect in earlier works [45]. Therefore, these data are quite correlated with our data since we also detected a certain local anaesthetic activity of compounds during infiltration anaesthesia.

In general, the compounds of the newly studied piperazine compounds had relatively low toxicity, which is most likely due to the inclusion of beta-cyclodextran in their molecule, but this modification also led to a decrease in their biological activity.

Information on the presence of antiplatelet activity in new piperazine derivatives was reflected in separate papers [32], therefore, the potentially high antiplatelet activity revealed by computer analysis confirms the literature data and suggests their prospects in this direction.

Conclusions

As a result of this work, new piperazine derivatives were synthesized, which was confirmed by elemental analysis and spectral data. Experimental studies have shown that all modified piperazine derivatives are

low-toxic substances. The difference in chemical structure did not significantly affect the difference in toxicity of the studied compounds.

The low toxicity of the presented compounds can be explained by the inclusion of cyclodextran compounds in the molecule, which leads to changes in bio-pharmaceutical and physical properties. This in turn leads to increased bioavailability, pharmacological activity, prolongation of action and reduction of side effects, and hence toxicity.

The analysis of experimental data shows that all the studied compounds are effective in infiltration anaesthesia to a certain extent. Also, moderate activity during infiltration anaesthesia is shown by LAS-278 compounds, causing approximately the same strength of anaesthesia. In the series of experiments, the compounds LAS-276 and LAS-277 were the most active in regional anaesthesia. However, all the studied compounds did not cause complete anaesthesia.

Low probability of the presence of local anaesthetic properties in all new piperazine derivatives was revealed in study of compounds with PASS program. There are prospects for further study of the pharmacological properties of compounds LAS-276, LAS-277 and LAS-278 in the prevention and treatment of thrombosis.

In general, the study showed low toxicity of the compounds. Despite the absence of the probability of local anaesthetic activity, according to the results of the study using the PASS program, LAS 278 demonstrated moderate activity in the infiltration anaesthesia model in infiltration anaesthesia, while the activity of the compounds LAS 276 and LAS 277 was moderate in the regional anaesthesia model. There was also a very high probability of the presence of thrombolytic activity in all the studied compounds, in this regard, they may be promising.

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Conflict of interest

The authors declare no conflict of interest.

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