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STUDY OF THE PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF A NEW BISPIDINE WITH A MORPHOLINE FRAGMENT

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Abstract. *Introduction.* The experimental results obtained during the work enrich modern medicinal chemistry with new information about the development and local anesthetic activity of a promising class of chemical compounds - O-benzoyloxime of bispidine. The discovered new properties of the molecule can serve as the basis for the creation of a new domestic safe local anesthetic drug, which is effective in the models of infiltration and conduction anesthesia and has low toxicity. *The aim of this work* is to synthesize and study the physicochemical and biological properties of a new morpholino-substituted bispidine derivative. *Results and discussion.* 3-(3-(Ethan-1-yloxy)propyl)-7-[3-(morpholine-4-yl)propyl]-3,7-diazabicyclo[3.3.1]nonan-9-one has been constructed in the 56.9% yield. Oximation under the influence of a powerful oximylating agent has led to the oxime. O-Benzoyloxime has been synthesized by benzoylation of the oxime. The complex of O-benzoyloxime with β-cyclodextrin - LA-180 has been studied for the local anesthetic activity and acute toxicity. *Conclusion.* It has been established that LA-180 has manifested itself as a highly active compound in a series of experiments, studying infiltration and conduction anesthesia. The results of a study of acute toxicity after a single subcutaneous administration to white outbred mice have shown that LA-180 (925 mg/kg) is a low-toxic compound in comparison with standard drugs such as trimecaine, lidocaine and novocaine.

Keywords: morpholine fragment, bispidine, acylation, complexation, local anesthetic activity, acute toxicity.

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1. Introduction

An anesthesia plays an important role in modern medicine, allowing various medical procedures to be performed without pain or discomfort for the patient.

Existing painkillers do not always fully satisfy the needs of the patients, especially during complex and lengthy surgical procedures. Over time and frequent use of the same drugs, resistance to anesthetics may occur. This increases the need to develop new agents, which can be effective where old ones no longer work. Thus, the continuous search and development of new anesthetics is necessary to improve the quality of medical care, provide greater safety and effectiveness of pain relief, and to meet the specific needs of various medical and veterinary fields.

The search for biologically active substances among azaheterocyclic compounds is an urgent task in the domestic medicinal chemistry and organic synthesis. The azaheterocyclic pharmacophore fragment morpholine serves as a template for a number of clinically used anti-inflammatory, antifungal, antileukemic and neuroprotective agents. The introduction of a morpholine fragment as an effective building block imparts to the organic molecule antimicrobial, antileukemic, antiviral and myelostimulating properties [1-6].

The aim of this work is to develop a method of synthesis and study the physicochemical and biological properties of a new bispidine, containing a morpholine fragment.

2. Experimental part

2.1 Experimental chemical part

The reaction progress and purity of the compounds were monitored by thin layer chromatography on alumina, and the components were visualized by iodine vapor. The spectroscopic data were recorded, using a Nicolet 5700 IR spectrometer and a JNM-ECA 400 spectrometer (Jeol). All reactions sensitive to air and/or H_2O were carried out under an inert gas atmosphere using dry, purified solvents.

*Starting synthon - 1-(3-(ethan-1-yloxy)propyl)piperidin-4-one (**1**).*

Stage 1. To 40.6 g (0.4360 M) of methyl acrylate dissolved in 50 ml of methyl alcohol was added a mixture of 20 g (0.1938 M) of 3-ethoxypropylamine and 13 ml of methyl alcohol within 15-20 min. The reaction mixture was stirred at 60-65^{o}C for 5 hours. After the completion of the reaction, methanol and excess methyl acrylate were distilled off at 140^{o}C. 35.77 g (68.48%) diester (R_f 0.41, Al_2O_3, eluent benzene: isopropanol - 7:1) was obtained in the form of a red liquid.

Stage 2. To 122 ml of absolute toluene 3 g (0.1301 M) of metallic Na was added. The reaction mixture was heated up to 110°C until the sodium dissolves. Then the reaction mixture was cooled down to 75-80^{o}C and 40 ml of methyl alcohol was added dropwise.

While the azeotropic mixture of toluene and methanol was simultaneously distilled off a mixture of 35.77 g (0.1301 M) of diester with 16 ml of methyl

alcohol was added dropwise to the reaction mixture. The heating was stopped when the temperature reached 110°C.

A solution of 90.5 ml of concentrated hydrochloric acid and 90.5 ml of distilled water were gradually added to the reaction mixture. The resulting organic and aqueous layers were separated. The lower aqueous-acidic layer was boiled at 100°C for 7 hours. The progress of the reaction was monitored using a 1% solution of $FeCl_3$. After the reaction was completed, the solution was alkalinized with NaOH to pH 10-11. Then the extraction followed with benzene and chloroform, dried over anhydrous $MgSO_4$. Purification by the distillation at 1 mm Hg pressure gave ***1*** (13.43 g, 46.4%) as light yellow oil, b.p. 100-110 °C, R_f 0.47.

*3-(3-(Ethan-1-yloxy)propyl)-7-[3-(morpholin-4-yl)propyl]-3,7-diazabicyclo[3.3.1]nonan-9-one (**2**) and bispidine derivatives* were synthesized according to the methods described in the our previous paper *[7]*. Purification by column chromatography gave ***2*** (16.27 g, 56.9%), R_f 0.44.

Calculated, %: C 61.93; H 9.85; N 15.20. $C_{19}H_{36}N_4O_3$.

Found, %: C 61.68; H 9.86.

The IR spectra (KBr), ν, cm^{-1}:1730 (C=O), 1119 (C-O-C).

The NMR ^{13}C spectra (100 MHz, $CDCI_3$), δ, ppm: 46.5 (C-1,5); 58.4 (C-2,4); 56.8 (C-6,8); 214.0 (C-9); 58.5 (C-10); 27.7 (C-11); 66.7 (C-12); 68.5 (C-13); 15.2 (C-14); 53.7 (C-15); 24.3 (C-16); 53.4 (C-17); 53.7 (C-18); 66.9 (C-19).

*Oxime of 3-(3-(ethan-1-yloxy)propyl)-7-[3-(morpholin-4-yl)propyl]-3,7-diazabicyclo[3.3.1]nonan-9-one (**3**).* Purification by column chromatography gave ***3*** (3.75 g, 50.7%) as light yellow oil, R_f 0.17.

Calculated, %: C 61.96; H 9.78; N 15.21. $C_{19}H_{36}N_4O_3$.

Found, %: C 61.89; H 9.56.

The IR spectra (KBr), ν, cm^{-1}: 3346 (OH), 1678 (C=N).

The NMR ^{13}C spectra (100 MHz, $CDCI_3$), δ, ppm: 30.7; 32.1 (C-1,5); 56.4, 56.3, 58.6, 58.1 (C-2,4,6,8); 161.2 (C-9); 58.7 (C-10), 27.5 (C-11); 66.4 (C-12); 68.1 (C-13), 15.2 (C-14); 53.5 (C-15); 24.6 (C-16); 53.7, 58.6, 66.9 (C-17,18,19).

*O-Benzoyloxime of 3-(3-(ethan-1-yloxy)propyl)-7-[3-(morpholin-4-yl)propyl]-3,7-diazabicyclo[3.3.1]nonan-9-one (**4**).* Purification by column chromatography gave ***4*** (0.36 g, 13.4%) as a light yellow oil, R_f 0.47.

Calculated, %: C 66.08; H 8.53; N 11.86. $C_{26}H_{40}N_4O_4$.

Found, %: C 66.88; H 8.38.

The IR spectra (KBr), ν, cm^{-1}: 1643 (C=O), 1677 (C=N).

*The inclusion complex of O-benzoyloxime of 3-(3-(ethan-1-yloxy)propyl)-7-[3-(morpholin-4-yl)propyl]-3,7-diazabicyclo[3.3.1]nonan-9-one with ß-cyclodextrin (**5**).* 1.11 g of ***5*** was obtained as an amorphous cream-colored powder, which chars when heated.

Calculated, %: C 50.80; H 6.22; N 3.48. $C_{68}H_{110}N_4O_{39}$.

Found, %: C 50.17; H 6.71.

2.2 Biological assay

For the experimental study of the local anesthetic activity, primary screening methods were used, recommended by the guidelines for conducting preclinical studies of drugs [8] and the basic document on compliance with the rules of laboratory experiments "Rules for the preclinical assessment of the safety of pharmacological agents (GLP)" [9].

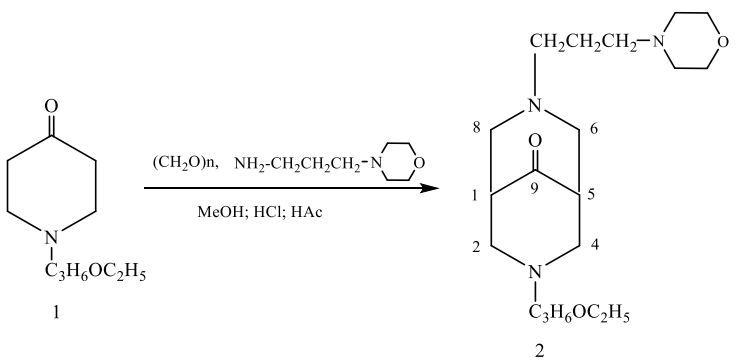
The acute toxicity was studied by a single subcutaneous injection of the compound ***5*** and reference preparations - trimecaine, lidocaine, and novocaine to white outbred mice of both sexes, weighing 17-22 g.

The infiltration anesthetic activity was studied by the Bulbring-Wade method on male guinea pigs weighing 200-250 g. The local anesthetic activity was assessed 6-8 times for each of the selected concentrations. The depth of anesthesia, expressed in "anesthesia indices" (average of 6 experiments, maximum index - 36), the duration of complete anesthesia and the total duration of the anesthetic effect were determined. The activity of the compound was compared with reference drugs - trimecaine, lidocaine and novocaine in appropriate concentrations. The research results were processed statistically.

The model of conduction anesthesia was studied by the injection of a solution of ***5*** and reference preparations - trimecaine, lidocaine, and novocaine in a volume of 1.0 ml under the skin of the tail into the area where the thermal effect was applied. Animals in the control group were injected with physiological solution in the same way and in the same volume. The first testing was carried out 5 minutes after the injection, the subsequent tests were carried out every 10 minutes until the threshold values were completely restored. Doubling of the latent period was taken as complete anesthesia.

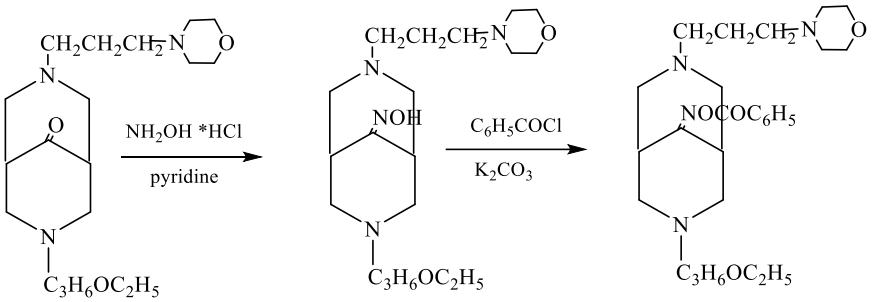
3. Results and discussion

N-(3-(Ethan-1-yloxy)propyl)-4-oxopiperidine (***1***) has been synthesized under the Dieckmann reaction conditions in the presence of sodium methoxide in toluene. The simultaneous condensation of ***1*** with paraform and 3-morpholinopropylamine in an acetic-methanol medium has given 7-[3-(morpholin-4-yl)propyl]-3,7-diazabicyclo[3.3.1]nonan-9-one (***2***).



1 2

Taking into account the low reactivity of the carbonyl group of heteroanalogs of bicyclo[3.3.1]nonan-9-one, the oximylation of compound ***2*** has been carried out in the alcoholic medium under the influence of a powerful oxymylation agent: hydroxylamine hydrochloride in the presence of pyridine. The prolonged boiling (for 12 hours) has led to the target oxime (***3***). O-Benzoyloxime (***4***) has been obtained by the acylation of compound ***3*** with benzoyl chloride in benzene, followed by the treatment of the resulting hydrochloride with potassium carbonate.



2 3 4

The composition and structure of all newly synthesized compounds (***2-4***) have been elucidated by the elemental analysis and spectral data. In the IR spectrum of ***2*** the characteristic bands of stretching vibrations of the carbonyl group and of the ether bond have been observed at about 1736 cm^{-1} and 1119 cm^{-1} respectively. In the ^{13}C NMR spectrum of ***2*** a signal of C_9 has been observed at 214.0 ppm. The evidence of the formation of a bicyclic product has been doublet signals of $C_{1,5}$ with an intensity of two carbon atoms at 46.5 ppm, as well as triplet signals of $C_{2,4}$ and $C_{6,8}$ with an intensity of two carbon atoms at 56.8 and 58.4 ppm.

In the IR spectra of ***3*** the absorption bands of the C=N bond (1678 cm^{-1}) and OH group (3346 cm^{-1}) have been observed. The structure of the esterification product are consistent with the data of IR spectroscopy. In the IR spectra of ***4*** the absorption band of the OH group has disappeared, and the intense absorption bands of the C=O bond of the ester group (1743 cm^{-1}) and aryl radical have appeared.

Compound ***5 (LA-180)*** has been synthesized as an amorphous powder, which melts above 240°C with the decomposition to study the local anesthetic activity and acute toxicity. All data have been compared with the indicators of lidocaine, novocaine and trimecaine (Tables 1-3).

In the result ***LA-180*** (925) has shown low toxicity. The LD_{50} indicator of the drugs has been: for trimecaine - 378.2+19.4 mg/kg, lidocaine - 248.6+18.4 mg/kg, novocaine - 480.0+9.8 mg/kg, dicaine - 41.5+1.9 mg/kg. In terms of toxicity none of the reference drugs can be compared with ***LA-180*** (Table 1).

Table 1 – The acute toxicity of LA-180, trimecaine, lidocaine, novocaine and dicaine

Compound, drugs	LD_{50}, mg/kg	P – reliability of results
LA-180	925	P_1<0.001 P_2<0.001 P_3<0.001 P_4<0.001
Trimecaine	378.2+19.4	
Lidocaine	248.6+18.4	
Novocaine	480.0+9.8	
Dicaine	41.5±1.9	
Notes: P_1 – the correlation coefficient compared to trimecaine; P_2 - compared to lidocaine; P_3 – compared to novocaine		

Compound ***5*** and reference drugs have been tested in the 0.25% solutions for the infiltration anesthetic activity. As can be seen from the data in Table 2, ***LA-180*** (34.5) has a fairly pronounced effect.

Table 2 – The anesthetic activity of LA-180 during infiltration anesthesia, according to the Bulbring and Uyedu method

Compound, drugs	0.25%					
	Anesthesia index, M±m		Duration of complete anesthesia, min		Total duration of anesthesia, min	
LA-180	34.5+0.42	p_1>0.05 p_2>0.05 p_3<0.001	27.0±2.57	p_1<0.02 p_2<0.001 p_3<0.001	40.0±1.44	p_1>0.05 p_2<0.001 p_3<0.001
Trimecaine	32.1±1.5		20.0±1.7		38.3±1.05	
Lidocaine	23.1±0.9		14.2±0.8		30.8+0.8	
Novocaine	25.0±1.0		10.0±1.2		29.1+1.5	
Notes: p_1 – correlation coefficient compared to trimecaine; p_2 - compared to lidocaine; p_3 – compared to novocaine						

The anesthesia index of ***LA-180*** compared to the anesthesia index of trimecaine (32.1), lidocaine (23.1), and novocaine (25.0) has been 1.1, 1.5 and 1.4 times higher respectively. The duration of complete anesthesia compared to trimecaine (20.0), lidocaine (14.2) and novocaine (10.0) has exceeded 1.4, 1.9 and 2.7 times, respectively. When comparing the duration of action of ***LA-180*** with

trimecaine (38.3), lidocaine (30.8), and novocaine (29.1), it has been 1.04, 1.3, and 1.4 times higher respectively.

LA-180 (15.0) has caused complete anesthesia lasting 55.0±3.1 min. In terms of the total duration of action, the compound has exceeded novocaine (42.3) by 1.3 times (Table 3).

Table 3 – The indicators of conduction anesthesia, using the "tail flick" model

Compound, drugs	1% solution			
	Duration of complete anesthesia, min		Total duration of action, min	
LA-180	15.0±5.14	p_1<0.01 p_2<0.05 p_3>0.05	55.0±3.1	p_1>0.05 p_2>0.05 p_3>0.05
Trimecaine	47.3±8.4		56.9±12.8	
Lidocaine	65.0±18.4		90.0±18.4	
Novocaine	35.2±7.1		42.3±13.6	
Notes: p_1 – the correlation coefficient compared to trimecaine; p_2 - compared to lidocaine; p_3 – compared to novocaine				

Conclusion. Benzoyl derived 3-(3-(ethan-1-yloxy)propyl)-7-[3-(morpholin-4-yl)propyl]-3,7-diazabicyclo[3.3.1]nonan-9-one (LA-180) in a series of experiments, studying the infiltration and conduction anesthesia has manifested itself as a highly active compound, exceeding the activity of the reference drugs by a number of indicators. In addition, the compound is low toxic in comparison with the reference drugs. LA-180 is of interest for the further in-depth study of its biological activity.

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МОРФОЛИН ФРАГМЕНТТІ ЖАҢА БИСПИДИННІҢ ФИЗИКА-ХИМИЯЛЫҚ ЖӘНЕ БИОЛОГИЯЛЫҚ ҚАСИЕТТЕРІН ЗЕРТТЕУ

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Түйіндеме. *Кіріспе*. Жұмыс барысында алынған тәжірибелік нәтижелер қазіргі медициналық химияны перспективалы химиялық қосылыс класы биспидин О-бензоилоксим әзірленуі мен

жергілікті анестетикалық белсенділігі туралы жаңа ақпаратпен байытады. Молекуланың ашылған жаңа қасиеттері инфильтрациялық және өткізгіштік анестезия үлгілерінде тиімді және уыттылығы төмен жаңа отандық қауіпсіз жергілікті анестетикалық препарат дайындау үшін негіз бола алады. *Бұл жұмыстың мақсаты* – жаңа морфолинорынбасқан биспидин туындыларын синтездеу, физика-химиялық және биологиялық қасиеттерін зерттеу. *Нәтижелер және талқылау*. 3-(3-(Этан-1-илокси)пропил)-7-[3-(морфолин-4-ил)пропил]-3,7-диазабицикло[3.3.1]нонан-9-он 56,9% шығыммен конструирленді. Күшті оксимдеуші агент әсерімен оксимирлеу оксимге әкелді. О-Бензоилоксим оксимді бензоилдеу арқылы синтезделді. 3-(3-(Этан-1-илокси)пропил)-7-[3-(морфолин-4-ил)пропил]-3,7-диазабицикло[3.3.1]нонан-9-он О-бензоилоксимінің β-циклодекстринмен кешені - LA-180 жергілікті анестетикалық белсенділікке және өткір уыттылыққа зерттелді. *Қорытынды*. Зерттеулер нәтижесінде инфильтрациялық және өткізгіштік анестезияны зерттейтін эксперименттер сериясында LA-180-ның жоғары белсенді қосылыс екендігі анықталды. Ақ тұқымды тышқандарға бір рет тері астына енгізгеннен кейін өткір уыттылықты зерттеу нәтижелері LA-180 тримекаин, лидокаин және новокаин сияқты стандартты препараттармен салыстырғанда уыттылығы төмен қосылыс екенін көрсетті.

Түйін сөздер: морфолин фрагменті, биспидин, ацилдеу, кешен түзу, жергілікті анестетикалық белсенділік, өткір уыттылық.

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ИЗУЧЕНИЕ ФИЗИКО-ХИМИЧЕСКИХ И БИОЛОГИЧЕСКИХ СВОЙСТВ НОВОГО БИСПИДИНА С МОРФОЛИНОВЫМ ФРАГМЕНТОМ

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Резюме. *Введение*. Экспериментальные результаты, полученные при проведении работы, обогащают современную медицинскую химию новыми сведениями о разработке и местноанестезирующей активности перспективного класса химического соединения – О-бензоилоксиме биспидина. Вскрытые новые свойства молекулы могут послужить основой создания нового отечественного безопасного местноанестезирующего лекарственного средства, эффективного на моделях инфильтрационной и проводниковой анестезии и обладающего низкой токсичностью. *Целью настоящей работы* является синтез, изучение физико-химических и биологических свойств нового морфолинозамещенного производного биспидина. *Результаты и обсуждение.* Сконструирован 3-(3-(этан-1-илокси)пропил)-7-[3-(морфолин-4-ил)пропил]-3,7-диазабицикло[3.3.1]нонан-9-он с выходом 56.9%. Оксимирование под действием мощного оксимилирующего агента привело к оксиму. О-Бензоилоксим синтезирован бензоилированием оксима. Комплекс О-бензоилоксима с β-циклодекстрином - LA-180 изучали на местноанестезирующую активность и острую токсичность. *Заключение.* Установлено, что LA-180 в сериях опытов по изучению инфильтрационной и проводниковой анестезии проявляет себя как соединение с высокой активностью. Результаты исследования острой токсичности при однократном подкожном введении белым беспородным мышам показали, что LA-180 малотоксичное соединение в сравнении с эталонными препаратами как тримекаин, лидокаин и новокаин.

Ключевые слова: морфолиновый фрагмент, биспидин, ацилирование, комплексообразование, местноанестезирующая активность, острая токсичность.

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