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Analgesic action of hexaazaisowurtzitane derivative in somatic pain models caused by TRPA1 and TRPV1 Ion channels activation

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ABSTRACT

The aim of this study was to assess the analgesic action of thiowurtzine in somatogenic nociception models by activation of TRPA1 and TRPV1 ion channels.

Materials and methods. The object of the study is the compound 4-(3,4-dibromothiophenecarbonyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo [5.5.0.^{03,11}.0^{5,9}]dodecane (thiowurtzine). The analgesic activity of thiowurtzine was studied under the conditions of a chemogenic activation model of TRPA1 channels (by the formalin test), and by a selective test with an agonist of TRPV1 channels (the capsaicin test). The compound was administered once *per os* in a dose range of 50–200 mg/kg (water-tween solvent) an hour before the experimental manipulations. The reference drugs were diclofenac sodium in a preventive single *per os* dose of 10 mg/kg in 1% starch gel in a volume of 0.2 ml/mouse, and ketorolac in a dose of 6 mg/kg in the same solvent, volume and route of administration.

Results. Thiowurtzine, when administered in *per os* doses of 100 and 200 mg/kg, was found to effectively block nociceptive reactions caused by activation of TRPA1 and TRPV1 ion channels. At the same time, the analgesic activity of thiowurtzine turned out to be comparable and/(or) superior to the ketorolac and diclofenac action, depending on the model situation. In addition, it was found that thiowurtzine (200 mg/kg *per os*) corresponds to diclofenac sodium (10 mg/kg *per os*) and is superior to ketorolac (6 mg/kg *per os*) in terms of anti-inflammatory severity in the formalin test.

Conclusion. The biphasicity of behavioral reactions in the prognostic formalin test do not allow for an unambiguous conclusion about the direction of the action mechanism of thiowurtzine, which confirms the polymodality hypothesis. The data obtained in the two models of somatogenic nociception do not exclude the fact that the modulation of the TRPA1 and TRPV1 activity is one of the mechanisms of the thiowurtzine analgesic action. By the key analgesic characteristics found herein, thiowurtzine proves to be a unique compound with a high therapeutic and innovation potential.

Key words: hexaazaisowurtzitane, thiowurtzine, analgesic activity, somatogenic nociception, TRP ion channels, formalin test, capsaicin, ketorolac, diclofenac, anti-inflammatory activity.

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Анальгетическое действие производного гексаазаизовюрцитана на моделях соматической боли, вызванной активацией TRPA1- и TRPV1-ионных каналов

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РЕЗЮМЕ

Цель. Оценка анальгетического действия тиовюрцина в условиях активации TRPA1- и TRPV1-ионных каналов на моделях соматогенной ноцицепции.

Материалы и методы. Объектом исследования является соединение 4-(3,4-дибромтиофенкарбонил)-2,6,8,12-тетраацетил-2,4,6,8,10,12-гексаазатетрацикло [5,5,0,$0^{3,11}$,$0^{5,9}$]додекан (тиовюрцин). Исследование анальгетической активности тиовюрцина проводили в условиях хемогенной модели активации TRPA1-каналов (формалиновый тест) и в селективном (капсаициновом тесте) с агонистом TRPV1-каналов – капсаицином. Соединение вводили однократно *per os* в диапазоне доз 50–200 мг/кг (водно-твиновый растворитель) за 1 ч до экспериментальных воздействий. В качестве референс-препаратов использовали диклофенак натрия превентивно однократно *per os* в дозе 10 мг/кг на 1%-м растворе крахмальной слизи в объеме 0,2 мл/мышь, кеторолак – в дозе 6 мг/кг в аналогичном растворителе, объеме и пути введения.

Результаты. Установлено, что тиовюрцин при превентивном однократном *per os* введении в дозах 100 и 200 мг/кг эффективно блокирует ноцицептивные реакции, обусловленные активацией TRPA1- и TRPV1-ионных каналов. При этом анальгетическая активность тиовюрцина оказалась сравнимой и (или) превосходящей действие кеторолака и диклофенака в зависимости от модельной ситуации. Кроме того, выявлено, что тиовюрцин (200 мг/кг *per os*) соответствует диклофенаку натрия (10 мг/кг *per os*) и превосходит кеторолак (6 мг/кг *per os*) по выраженности противовоспалительного действия в формалиновом тесте.

Заключение. Бифазность поведенческих реакций в прогностическом «Формалиновом тесте» не позволяет однозначно сделать вывод о направленности механизма действия тиовюрцина, что подтверждает гипотезу о полимодальности. Данные, полученные на двух моделях соматогенной ноцицепции, не исключают того, что модуляция активности рецепторов TRPA1- и TRPV1-ионных каналов является одним из механизмов его анальгетического действия. По сочетанию выявленных ключевых для анальгетика характеристик тиовюрцин является уникальным веществом с высоким терапевтическим и инновационным потенциалом.

Ключевые слова: гексаазаизовюрцитан, тиовюрцин, анальгетическая активность, соматогенная ноцицепция, TRP-ионные каналы, кеторолак, диклофенак, капсаицин, формалиновый тест, противовоспалительная активность.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Nowadays, one of the new trends in pharmaceutics is the synthesis of candidate molecules for use in designing non-narcotic analgesics to alleviate severe and moderate pain [1]. Three types of pain killers are principally used to treat pain of various etiologies in patients in inpatient and outpatient settings: opiate- and cannabinoid-based drugs with different analgesic activity, nonsteroidal anti-inflammatory drugs (NSAIDs), and different combinations thereof in multimodal treatment regimens [1, 2, 3]. For management of severe and moderate pain, non-narcotic analgesics could serve as opioid alternatives that act on the central and peripheral nervous systems in humans and offer a range of advantages. However, the number of possible candidate molecules of non-opioid analgesics is extremely limited. One of the up-to-date approaches to making innovative molecules is developing methods for streamlined construction and synthesis of original chemical entities.

The interdisciplinary partnership between the IPCET SB RAS and the GRIP&RM of NRMC resulted in 4-(3,4-dibromothiophenecarbonyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo [5.5.0.$0^{3,11}$.$0^{5,9}$] dodecane (thiowurtzine), the world's first molecule in a new class of compounds for medical use (RU Patent No. 2565766 as of 23.09.2015). This innovative compound was created by the streamlined synthesis technique using the PASS software prediction data, and it is the ever-first in the class of hexaazaisowurtzitanes. Since the 80-ies of the last century, numerous studies focused on the synthesis of this class of compounds have primarily been of defense nature. And only in recent years, hexaazaisowurtzitane has been in use as a pharmacophore for the design of original pharmaceutical agents [4].

The previous studies revealed that this innovative compound demonstrated a pronounced analgesic activity comparable and/or superior to diclofenac sodium, ketorolac and tramadol in the "Hot plate" test and Acetic acid-induced writhing test, the model of acute visceral and deep somatic pain. Because thiowurtzine had no impact on the secretion of basic inflammation mediators (histamine, serotonin, and prostaglandin) and had no ulcerogenic activity in a dose range of 25-200 mg/kg, there was a conclusion made that it has no COX-dependent action [5]. With that, its prominent anti-inflammatory action in the arachidonic acid-induced test and its moderate action in the bradykinin- and carrageenan-induced inflammation models were validated [6]. This compound can be referred to hazard category III (lethal dose (LD_{50}) is in the range of 150–5000 mg/kg), GOST 12.1.007-76. The maximum possible single dose of thiowurtzine did not reach LD_{50} (or animal lethality). Thiowurtzine did not evoke respiratory depression nor did changes in gastrointestinal reflexes. The study into the thiowurtzine action mechanism revealed naloxone-sensitive analgesia, and no affinity to peripheral opioid receptors was proven using naloxone methiodide [5].

The above data altogether give evidence of the polymodal mechanism of the thiowurtzine action, which necessitates further research in this aspect.

The present study aimed to evaluate the analgesic action of thiowurtzine by activation of TRPA1 and TRPV1 ion channels in somatogenic nociception models such as formalin test and capsaicin test.

MATERIALS AND METHODS

The experiments were done on 50 outbred sexually mature male mouse of CD1 stock and 48 male CBA strain mice (aged 7–8 weeks) of the first category, conventional. The animals were provided by the Department of Experimental Biological Models at the GRIP&RM of Tomsk NRMC (animal health certificate available). The animal husbandry and experimental design were approved by the Bioethics Committee of the GRIP&RM (JACUC protocol No. 96092015 of 16.09.2015) and were in compliance with Directive 2010/63/EU "On the Protection of Animals Used for Scientific Purposes" of the European Parliament and

the Council of the European Union and with Order No. 199n as of 01.08.2016, of the Ministry of Health of the Russian Federation.

The experimental design, sample size, experimental regimen, and choice of statistical analysis were determined in the optimal way for this kind of research to acquire robust data for interpretation of results without expanding the number of animals. Before the experiment, each animal inside the group was assigned an individual number labeled with carbol-fuchsin marks. The animals were divided into groups randomly by body weight criterion so that the individual weight value would not deviate from the average within one group by no more than ±10%. After the experiments were completed, the mice were euthanized by the cervical dislocation method.

The innovative molecule under study represents a polynitrogen polycyclic cage compound, 4-(3,4-dibromothiophene carbonyl)-2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo[5.5.0.$0^{3,11}$.$0^{5,9}$] dodecane. This is a newly synthesized compound obtained by acylation of commercially available 2,6,8,12-tetraacetyl-2,4,6,8,10,12-hexaazatetracyclo[5.5.0.$0^{3,11}$.$0^{5,9}$]dodecane with 3,4-dibromothiophene carboxylic chloroanhydride. Thiowurtzine, a colorless crystalline product with an API content of 99.0% and a single impurity content of below 0.2% (as per HPLC), Mp = 328–330°C), was characterized in full and validated by physicochemical analytical methods such as IR (ν/cm^{-1}), ^{1}H nuclear magnetic resonance (NMR) (DMSO-d6, δ, ppm) and ^{13}C NMR (DMSO-d6, δ, ppm) spectroscopies.

The therapeutic analgesic dose of thiowurtzine was estimated to be *per os* 100 mg/kg as determined in the previous studies on thiowurtzine analgesic activity [5, 6]. Here, thiowurtzine was administered in a single dose at 50–200 mg/kg (water-tween solvent) through an oral gavage one hour before experimental manipulations. The reference drugs were diclofenac sodium (Hemoharm, Russia) administered through gavage in a preventive single dose of 10 mg/kg dissolved in 1% starch mucilage in a volume of 0.2 mL/mouse, and ketorolac (Dr. Reddy's Laboratories Ltd., India) in a dose of 6 mg/kg with the same solvent, administration volume and route. The used doses of the reference drugs were equivalent to the mean therapeutic human dose. The animals of the negative control group received equivolumetric infusions of the water-tween solvent in the same regiment.

Formalin test. Formalin test, a chemogenic model of acute pain response, mimics pain reactions of different genesis (somatic traumas, burns, cuts, chemical injuries, surgical skin incisions) [7]. One of the mechanisms of the formalin nocigenic action is the activation of TRPA1 channels responding normally to cold and stimulating the development of thermal and mechanical hyperalgesia [8].

The animals were divided into 5 groups: I. Control, no treatment; II. Ketorolac, 6 mg/kg; III. Diclofenac, 10 mg/kg; IV. Thiowurtzine, 100 mg/kg; V. Thiowurtzine, 200 mg/kg. The test drug and the reference drugs were administered one hour before starting the tests. Hyperalgesia was simulated by subcutaneous injection of 20 μL/kg 2% aqueous formalin solution injected intraplantarily into the right hind paw pad after one hour. The pain response intensity in the first and second test phases were documented every second for the number and duration of behavioral patterns (flinches) of pain responses (licks, shakes) of the affected hind paw for 60 minutes. The pattern times were summated for each animal. The analgesic activity of the drugs was evaluated against the decreasing number of pain responses relative to the negative control, separately for phase I (initial 10 min after formalin injection) and phase II (from the 10th to the 60th minute following formalin injection) of the nociceptive response.

Capsaicin test. Examination of a new molecule for analgesic action in the selective test with a TRPV1 channel agonist is an essential step in exploring the specific pharmacological activity. This test helps evaluate how a compound influences the sensitivity of TRPV1 channels found basically in nociceptive neurons of the central and peripheral nervous systems [9].

The animals were divided into 5 groups: I. Control, no treatment; II. Ketorolac – 6 mg/kg; III. Thiowurtzine – 50 mg/kg; IV. Thiowurtzine – 100 mg/kg; V. Thiowurtzine – 200 mg/kg. The test drug and the reference drugs were administered one hour before starting the experiment. In an hour, a 10 μL capsaicin solution (3 μg/10 μL of 10% ethanol dissolved in 0.9% NaCl) was injected intraplantarily into the left hind paw. After injection, the paw was rubbed with ethanol to prevent capsaicin-induced skin irritation. Then, the time (latency period) to pass until response initiation (the mouse began violently shaking and licking the paw) was recorded. Afterwards, paw-lick patterns and total licking time were counted every second for 15 minutes for each mouse. The basic criteria of the drug efficacy were the total licking time and the pain reaction time.

Anti-inflammatory effect assessment. After the two tests were completed, the mice were euthanized, whereupon the both hind paws (inflamed and intact) were excised along the bone prominence below the junction of the splint bone and shin bone and above the talocalcaneal joint, and were weighed on an electronic analytical balance. The anti-inflammatory effect evaluated against the edema weight change was expressed as percentage to the control and was estimated by the appropriate formulae [10].

Statistical processing of the obtained results was performed by the variation statistics method using Statistica 6.0 software. For all the data, the mean value (X) and the standard error of the mean ($M \pm m$) were estimated, which are given in summary Tables 1, 2 together with the quantity n (number of variants in a group). Differences in the quantities under comparison were considered significant if the probability of their identity was below 5% ($p < 0.05$). Using sample coefficients of asymmetry and excess, the approximation degree of the distribution law of the test characteristic to the normal was evaluated. The non-parametric Mann – Whitney U-test was used for independent samples in the case of deviations in distributions of a characteristic from the normal. To reveal the reliability of differences in these qualitative characteristics, the Fisher transformation was used [10].

RESULTS

The modern experimental system including the nociceptive tests using electrical, thermal, mechanical, and chemical stimuli for pain modulation at different sensitivity levels can provide a range of anti-nociceptive characteristics of novel chemical entities and, in some ways, predict the nature of their analgesic action in humans [9, 11, 12].

The subcutaneous injection of formalin into the dorsal surface of the hind paw evoked a typical, biphasic, nociceptive behavioral response (Fig. 1), as evidenced by the score of pain reactions in the control mice: (30.8 ± 1.6) flinches in nociceptive phase I and (13.2 ± 2.6) in nociceptive phase II.

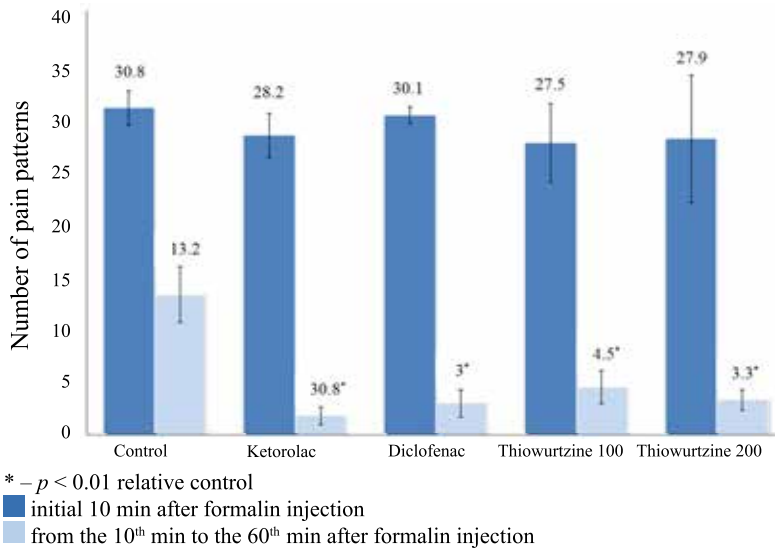

Fig. 1. Pain response in mice within 40–50 min after formalin injection

In the formalin test, which simulates a clinical model of both acute (phase I) and tonic pain (phase II), thiowurtzine exhibited an analgesic activity in the both phases [8]. In test phase I, thiowurtzine when administered as preventive single doses of 100 and 200 mg/kg via an intragastric gavage caused a statistically significant decrease in the number of initial pain reactions in the form of paw lifting, as compared to the control group; with that, no activity of the reference drugs diclofenac and ketorolac was noticed. Phase I of the formalin test characterizes acute pain in response to an injected chemical, and is mainly attributed to the direct activation of the thin unmyelinated C-fibers, most of which transmit impulses from the nociceptors;in our case, from the TRPA1 channels [7, 8, 9]. At this point of observation, thiowurtzine moderately limited the acute pain caused by the formalin-induced activation of the TRPA1 channels (Fig. 1).

In phase II of formalin-induced inflammation, thiowurtzine in doses of 100 and 200 mg/kg diminished the number of paw-lick behavioral responses typical for this test by 3.4 ($p < 0.05$) times and by 3.2 times

($p < 0.05$) as compared to the control group (Fig. 1). The analysis of the number of paw-shake patterns revealed that the test drug and the reference drugs had a similar analgesic activity. This reaction was not detected in animals which received 200 mg/kg thiowurtzine, while a 100 mg/kg dose resulted in a 5.3-fold decrease in shakes compared to the negative control, indicating a pronounced inhibition of nociceptive manifestations. The scores of pain reactions when thiowurtzine was administered in doses of 100 mg/kg (4.5 ± 1.6, $p < 0.01$) and 200 mg/kg (3.3 ± 1.0, $p < 0.01$) appeared to be similar to those of the reference drugs ketorolac (1.8 ± 0.8, $p < 0.01$) and diclofenac (3.0 ± 1.3, $p < 0.01$) in terms of formalin hyperalgesia severity.

By examining the anti-inflammatory action of thiowurtzine in phase II of nociceptive behavior, its statistically significant effect at a dose of 100 mg/kg was detected. By the reduced paw inflammatory swelling, a 200 mg/kg thiowurtzine dose (26%) was commensurable with diclofenac sodium (32%) and superior to ketorolac (14%) (Table 1).

Table 1

Indicators of anti-inflammatory action of thiowurtzine in formalin test in outbred male CD1 stock mice

Test group, dose (mg/kg), (number of animals)	Paw swelling, $M \pm m$, %	Paw swelling inhibition, %
Control, water-tween solvent ($n = 10$)	48.0 ± 2.5	–
Ketorolac, 6 ($n = 10$)	41.2 ± 4.8*	14
Diclofenac, 10 ($n = 10$)	32.8 ± 1.5*	32
Thiowurtzine, 100 ($n = 10$)	41.2 ± 2.2*	14
Thiowurtzine, 200 ($n = 10$)	35.4 ± 3.2*	26

* $p_U < 0.05$.

In test phase II, thiowurtzine diminished the tonic pain intensity due to the reduced inflammation (Table 1) in the peripheral tissues, and due to a possible change in the neuronal function of the dorsal horns of the spinal cord where the neurons of ascending pain pathways are located. The formalin test has a quite high prognostic significance when the action mechanism of novel potential analgesics are examined, as far as the opioid analgesics are known to block the both nociceptive phases, while non-steroid anti-inflammatory agents inhibit only the second phase, while local anesthetics suppress only the first phase [7, 8, 10].

The biphasic behavioral responses of the mice groups, which received thiowurtzine, do not allow for the unambiguous conclusion about the direction of the thiowurtzine action mechanism. Even though thiowurtzine exhibited a moderate anti-nociceptive effect in phase I wherein the reference drugs of the nonsteroidal anti-inflammatory group showed no activity, it effectively blocked the pain response in animals in phase II. That being said, the pain response level in phase II, judging from the shortened licking time in the thiowurtzine groups, turned out to be comparable with anti-nociceptive activities of ketorolac and diclofenac. Besides, the earlier detected naloxone-sensitive analgesia of thiowurtzine does not preclude possible involvement of opioidergic system in its antinociceptive activity in the both test phases [5]. The obtained results altogether necessitate further research in other nociceptive models.

The intraplantarily injected capsaicin solution evoked a marked nociceptive behavioral response manifested as paw licking and shaking in the control mice group. With that, all the indicators of pain reaction development and behavioral patterns were in agreement with the literature data (Table 2) and suggest that the model situation was reproduced (Table 2).

Table 2

Indicators of antinociceptive activity of thiowurtzine administered in a preventive single dose in the capsaicin test in male CBA mouse stock, $M \pm m$

Animal group, drug dose, (number of animals)	Pain reaction latency time, sec	Counts		Total licking time, sec	Pain reaction time, sec
		Licks	Shakes		
Control, purified water, ($n = 8$)	8.8 ± 1.8	9.4 ± 1.6	5.6 ± 1.3	86.6 ± 20.6	820.0 ± 31.1
Ketorolac, 6 mg/kg ($n = 10$)	25.1 ± 7.1*	5.0 ± 0.9*	1.6 ± 0.5*	38.4 ± 13.0	347.6 ± 79.6*
Thiowurtzine, 50 mg/kg ($n = 10$)	62.8 ± 21.6*	8.7 ± 2.0	2.6 ± 1.0	82.0 ± 35.1	579.4 ± 57.9*
Thiowurtzine, 100 mg/kg ($n = 9$)	18.7 ± 5.0*	6.3 ± 0.9	1.4 ± 0.4**	30.4 ± 10.2**	421.4 ± 75.0*
Thiowurtzine, 200 mg/kg ($n = 10$)	61.2 ± 20.1**	5,2 ± 1.2*	1.6 ± 0.7*	20.6 ± 5.0**	326.6 ± 86.4*

* $p_U < 0.05$; ** $p_U < 0.01$ relative to the negative control.

As one should expect, ketorolac evoked a significant decrease in pain reaction severity in male CBA mice: pain response latency period increased by 2.9 times ($p < 0.05$), paw licks declined by 1.9 times ($p < 0.05$) and paw shakes declined by 3.5 times ($p < 0.05$) compared to the control group (Table 2). It should be noted that the pain reaction time shortened by 2.4 times ($p < 0.01$). The results given above are consisted with the literature data on expressiveness of ketorolac analgesic effect.

The analysis of the results for the thiowurtzine groups allows for the conclusion of its prominent dose-dependent analgesic effect. With that, no statistically significant differences from the ketorolac group were detected. For instance, when thiowurtzine was administered in a 50 mg/kg dose, the pain response latency time was noticed to increase by a factor of 7.1 ($p < 0.05$) compared to the control group and by a factor of 1.7 compared to the ketorolac group. Based on the overall data obtained, the pain response time shortened by 1.4 times ($p < 0.05$) relative to the negative control.

The pronounced analgesic activity of thiowurtzine at 100 mg/kg manifested itself as a statistically significant increase in pain response latency, a decrease in paw shakes by 4.0 times ($p < 0.01$), a decrease in total licking time by 2.8 times ($p < 0.01$), and a decrease in pain response time by 1.9 times relative to the corresponding indicators of the control group.

In case thiowurtzine was dosed at 200 mg/kg, the difference in all test indicators was statistically significant between this group and the control group. For instance, the pain response latency increased by 7.0 times ($p < 0.05$) and paw licks and shakes decreased by 1.8 times ($p < 0.05$) and 3.5 times ($p < 0.01$), respectively, with total licking time being reduced by 4.2 times ($P < 0.01$) and pain response time being reduced by 2.5 times ($p < 0.01$) compared to the negative control. The mechanism of pain reaction development is associated with ion channels of nociceptors in tissues when exposed to chemical and mechanical injury. A capsaicin injection, a direct agonist of TRPV1 channels, can successfully provoke such an injury [9].

The findings obtained herein indicate that the test compound blocked pain progress in capsaicin-injected mice, at that the observed analgesic effect was dose-dependent, reached its maximum when thiowurtzine was dosed at 200 mg/kg, and was commensurable with the ketorolac effect.

The TRP ion channel family whose discovery was an important step in exploring the nature of pain sensation at the molecular level is currently considered as the pharmacologically most urgent biotargets of the human nervous system for analgesia [13–16]. Searching for modulators and agonists of TRP ion channels and designing high-efficacy analgesics with minimum side effects on their basis is among the priority focal areas of research labs around the world [13, 16]. The discovered properties of thiowurtzine actualize further development of unique thiowurtzine-based drugs intended for therapy of acute and moderate pain of different genesis. The pronounced anti-nociceptive activity of thiowurtzine even one hour post injection suggests a potential use of thiowurtzine-based drugs to alleviate pain syndrome in extreme cases.

CONCLUSION

Thiowurtzine when administered in a preventive single *per os* dose of 100 and 200 mg/kg was found to effectively block nociceptive reactions caused by activation of TRPA1 and TRPV1 ion channels. That being said, the thiowurtzine analgesic activity turned out to be comparable and/or superior to ketorolac and diclofenac, depending on the model situation. Besides, thiowurtzine (200 mg/kg *per os*) was found to be equivalent to diclofenac sodium (10 mg/kg *per os*) and superior in anti-inflammatory expressiveness to ketorolac (6 mg/kg *per os*) in the formalin test. The test results can assert that the pronounced anti-nociceptive action of the innovative compound in the capsaicin and formalin tests is due to the interaction with the TRPV1 and TRPA biotargets.

Moreover, all the data obtained previously may indicate that the activity modulation of TRP ion channels is one of the mechanisms of thiowurtzine's analgesic action. By the combination of the revealed key characteristics typical of an analgesic, thiowurtzine is a unique chemical entity with a high therapeutic and innovation potential.

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Authors contribution

Krylova S.G. – development of the concept and design of experiments, experimentation, critical revision for important intellectual content. Lopatina K.A. – arrangement and experimentation, statistical analysis, writing. Zueva E.P. – substantiation of the manuscript and critical revision for important intellectual content. Safonova E.A. – experimentation. Povet'eva T.N. – conceptualization, experimental design, experimentation. Suslov N.I. – critical revision for important intellectual content. Nesterova Yu.V. – experimentation, statistical analysis. Afanas'eva O.G. – experimentation. Kul'pin P.V. – experimentation. Sysolyatin S.V. – synthesis of thiowurtzine. Kulagina D.A. – synthesis and provision of thiowurtzine. Zhdanov V.V. – analysis of pharmacological activity prediction results, final approval of the manuscript for publication.

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