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Research Article

Comparative assessment of the analgetic activity of new partially hydrogenated pyridines, α-cyanothioacetamidederivatives, in experiments of orofacial trigeminal pain and hot plate test

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Abstract

Introduction: Experimental studies were carried out to determine the antinociceptive activity of new partially hydrogenated pyridines, α-cyanothioacetamide derivatives, in experiments of orofacial trigeminal pain and hot plate test, implemented on white outbred laboratory rats.

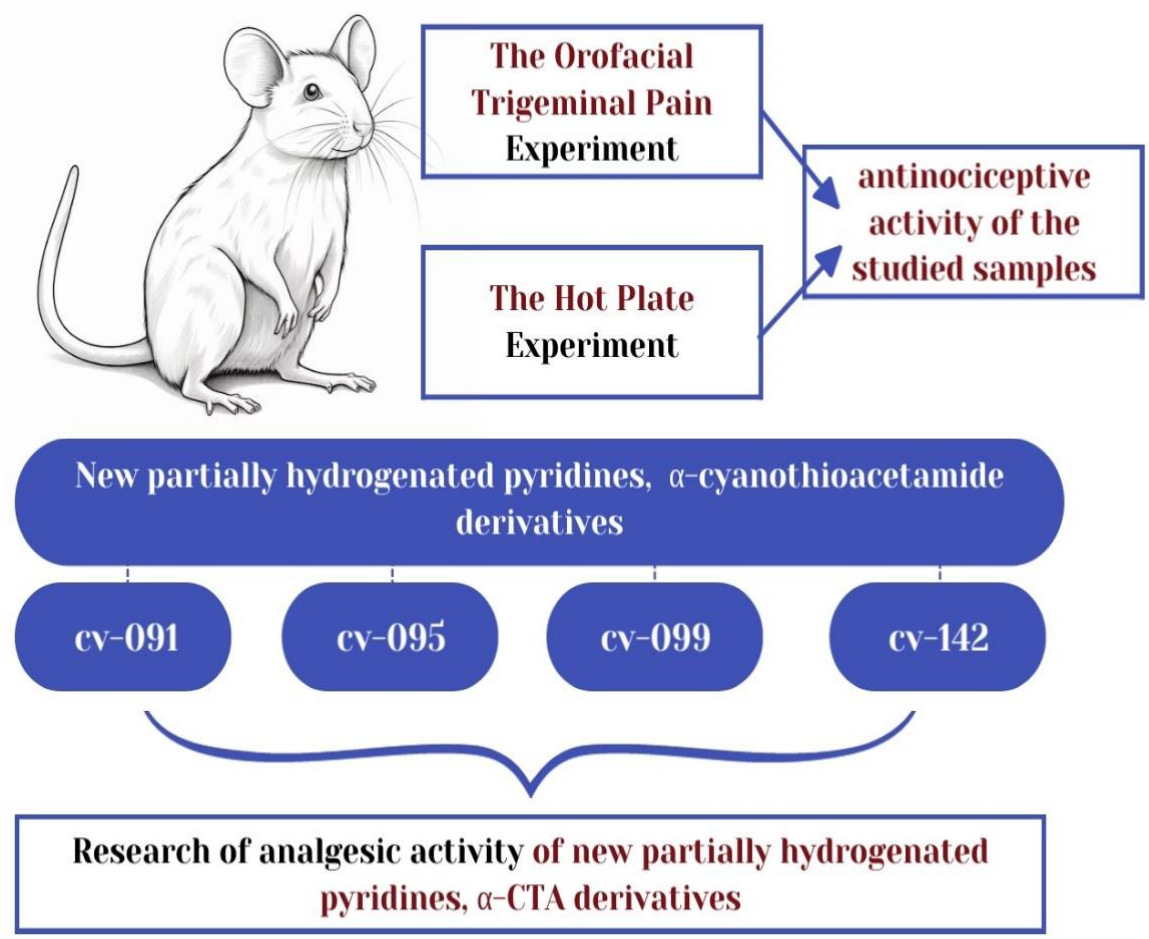
Materials and Methods: Controlled randomized studies of orofacial trigeminal pain and hot plate were carried out on white outbred laboratory rats in the conditions of the scientific research laboratory of Saint Luka Lugansk State Medical University of the Ministry of Health of the Russian Federation. New partially hydrogenated pyridines, α-cyanothioacetamide derivatives (α-CTA) with laboratory codes **cv-091**, **cv-095**, **cv-099**, and **cv-142**, which are promising considering preliminary studies in silico, were used as test samples. The analgesics metamizole sodium and mefenamic acid were used as comparison drugs. The experiment was implemented on 128 outbred sexually mature male rats. The animals received experimental samples and reference drugs intragastrically. Statistical processing of experimental data was carried out using the nonparametric Wilcoxon T-test. The normality of the distribution was determined using the Shapiro-Wilk criterion.

Results: According to the data obtained during the experiment, it was determined that new partially hydrogenated pyridines, α-cyanothioacetamide derivatives, with laboratory codes **cv-091**, **cv-095**, **cv-099**, **cv-142**, have analgesic activity, which is confirmed by a decrease in the dynamics of the number of scratching movements of the vibrissae area with the front paws in rats, relative to the control group, as well as prolonging the nociceptive response time in the hot plate test.

Discussion: In the course of the experimental study, data were obtained indicating the presence of pronounced analgesic activity in new partially hydrogenated pyridines, α-cyanothioacetamide derivatives (α-CTA). The biological activity of compounds with the lab codes **cv-091, cv-095, cv-099,** and **cv-142** is confirmed by data obtained in preliminary studies in silico-biological screening, where the main biological targets that determine the antinociceptive activity of the studied compounds are identified. The leading sample in orofacial trigeminal pain and hot plate tests is compound **cv-099**, injected intragastrically at a dosage of 5 mg per kg of animal body weight.

Conclusion: New partially hydrogenated pyridines, α-cyanothioacetamide derivatives, showed pronounced antinociceptive activity at a dosage of 5 mg per kg of animal body weight with a single intra-gastric injection in experiments of orofacial trigeminal pain and a hot plate test, which is confirmed by the results obtained during the tests. Compounds with laboratory codes **cv-091, cv-095, cv-099, cv-142** are recommended for further preclinical studies due to their practical prospects.

Graphical Abstract



Keywords

antinociceptive activity, pain, orofacial trigeminal pain, hot plate test, 1,4-dihydropyridines, α-cyanothioacetamide

Introduction

Pain is an unpleasant sensory and emotional experience associated with existing or possible tissue damage, or similar to such an experience, which is the most common symptom of various diseases. Based on this definition recommended by the International Association for the Study of Pain (Yakhno et al. 2020), it can be concluded that pain syndrome is a kind of indicator of various disorders occurring in the human body, as well as possible mental distress. This is one of the major medical and socio-economic problems worldwide.

The cerebral cortex plays a major role in the subjective perception and awareness of pain (Grachev et al. 2019).

There are two ways in which signals are transmitted to the brain: the neospinal pathway (for forming a rapid response to an irritant) and the paleospinal pathway (for slow pain). These pathways are primarily associated with the work of the I (fast pain), II and III (slow pain) posterior horns of the spinal cord (Kobelyatsky and Shaida 2015).

Nociception is the process of identifying damage to tissue or tissues caused by various thermal, mechanical and/or chemical stimulations, and information about the corresponding effect is transmitted through fibers A, δ and C (Ovechkin 2012). The threshold of sensitivity of nociceptors is quite high, so the excitation of primary sensory neurons is caused by sufficiently strong stimulation. Nociception is a physiological mechanism of pain transmission, not related to its emotional component.

Almost half of the world's population experiences acute and chronic pain on a daily basis, including recurrent pain (Andreeva et al. 2014). This symptom accompanies almost 70% of all known diseases and pathological conditions.

Currently, the algorithm for pain syndrome therapy includes the use of the following groups of drugs: local anesthetics (for short-term local relief of pain), narcotic and non-narcotic analgesics, as well as drugs that indirectly affect pain perception (sedatives of herbal and synthetic origin, tranquilizers, etc.) together with auxiliary (adjuvant) drugs (Osipova et al. 2010).

The most effective drugs listed above in terms of pain relief are narcotic analgesics containing active ingredients in their structure – natural and synthetic alkaloids (Krylov and Bobyrev 1999). However, the frequency and variety of adverse events, addiction and dependence, even with short-term use, the peculiarities of the selling of medicines of this group at pharmacies, limits the possibility of using narcotic analgesics in the daily treatment of pain syndrome (Abramov 2013; Ismailova et al. 2014; Mamedov and Asadov 2019).

The drugs of choice are non-narcotic analgesics, which, in addition to their analgesic activity, also exhibit antipyretic and anti-inflammatory properties. This group of drugs includes antipyretic analgesics (metamizole sodium, ketorolac, paracetamol), as well as nonsteroidal anti-inflammatory drugs (acetylsalicylic acid, diclofenac sodium, nimesulide, indomethacin, meloxicam, etc.). Unlike narcotic analgesics, they do not have sedative and hypnotic effects, do not cause euphoria, addiction and drug dependence when rationally used. Along with their high therapeutic activity, non-narcotic analgesics also exhibit many undesirable (side) effects, such as hemorrhages, thrombocytopenia, inhibition of leukopoiesis, sodium and water ion retention, methemoglobinemia, the development of Ray's syndrome (especially in children), Vidal syndrome, etc.

There is a high risk of allergic reactions, bronchospasm, as well as proven nephrotoxicity, hepatotoxicity, hematotoxicity, pronounced ulcerogenic effect against the background of the use of nonsteroidal anti-inflammatory drugs, from the point of view of "risk-benefit" in some cases calls into question the use of a drug from this pharmacotherapeutic group. In addition, the availability of drugs in pharmacies for patients of this group, over-the-counter prescription of most of them, self-medication and unjustified polypharmacy only exacerbate the existing situation. One of the vectors of modern pharmacological research is aimed at finding new active and effective, safe pharmaceutical substances with high specificity relative to the proposed biotargets.

Such potential substances are partially hydrogenated pyridines, α-cyanothioacetamide derivatives, which have high analgesic and anti-inflammatory activity. These properties determine the prospects of studying the biological activity of new compounds.

Previous studies on a significant number of α-cyanothioacetamide derivatives confirm their polyfunctionality and biological activity (Bibik et al. 2020, 2021; Bocheva 2023; Bochev 2024; Subbota 2025).

The aim of this research was to conduct a pharmacological preclinical study of the presence of antinociceptive activity of new organic compounds, alpha-CTA derivatives with lab codes **cv-091, cv-095, cv-099,** and **cv-142.**

Materials and Methods

Theoretical background

Alpha-cyanothioacetamide derivatives with laboratory codes **cv-091, cv-095, cv-099,** and **cv-142** was synthesized on the basis of the Scientific Research Laboratory ChemEx (Lugansk, LPR, Russia). These derivatives participated in preliminary in silico studies to determine their prospects in terms of biological activity, as well as other pharmacokinetic parameters.

As a result of the conducted biological screening using the Swiss Target Prediction program, potential biological targets were identified and, in accordance with the data obtained, basic ideas about the potential pharmacological activity of new samples were formed. Using the ProTox 3.0 program (Charité – University Medicine Berlin, Germany), the toxicity class of compounds with codes **cv-091, cv-095, cv-099,** and **cv-142**, as well as their estimated toxicological parameters, was determined. The main pharmacokinetic parameters were determined using the SwissADME program. Taking into account the integrated approach to in silico research, comparing the results of molecular docking, as well as taking into account the peculiarities of the organic synthesis of new samples, α -CTA derivatives, compounds with codes **cv-091, cv-095, cv-099,** and **cv-142** were selected for further preclinical tests on laboratory animals. Evaluating the overall results of these studies, it is worth noting that the studied derivatives, according to biological screening data, inhibit phosphodiesterase 10A (PDE10A), and also affect the group of tyrosine protein

kinases (SRC, JAK3, JAK2, FYN, SYK, TEK). They affect lipoprotein-associated phospholipase A2 (PLA2G7), and arachidonate-5-lipoxygenase (ALOX5), as well as the enzyme cyclooxygenase-2 (PTGS2), are potential targets of the studied samples.

According to preliminary toxicological screening, it was determined that derivatives with codes **cv-091, cv-095,** and **cv-099** belong to the 4th class of predicted toxicity as low-risk substances, and compound **cv-142** belongs to the 5th class of toxicity, being a relatively safe substance, according to the international classification.

The data obtained projected vectors of in vivo studies related to the determination of the potential antinociceptive activity of α-cyanothioacetamide derivatives with laboratory ciphers **cv-091, cv-095, cv-099,** and **cv-142.**

Animals

The experiments were carried out in the spring 2025 at the specialized scientific research laboratory of Saint Luka Lugansk State Medical University (Russia) and were approved by the Bioethics Commission of Saint Luka Lugansk State Medical University of the Ministry of Health of the Russian Federation (Minutes No. 5 dated 12 October 2022). The total number of animals sampled for studies No. 1 and No. 2 was 128 individuals – white mongrel male rats, which were obtained from the vivarium of Saint Luka Lugansk State Medical University of the Ministry of Health of the Russian Federation, then examined and selected according to the following criteria: age and weight, condition of the coat, appearance and physical activity. The study included white sexually mature rats at the age of two months without visible pathologies in physical development and injuries, damage to the skin and coat, traumatization and suppuration in the area of the vibrissae. The average weight of the animal was 280±10 grams.

The animals were kept in conditions recommended by the ARRIVE manual (Animal Research: Reporting of *in vivo* Experiments) and the rules for working with animals based on the provisions of the Helsinki Declaration and recommendations contained in EU Directive 86/609/ECC and the Council of Europe Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes, and other regulations for this type of work (Bibik et al. 2022: 77-95).

Both studies were controlled and randomized. Randomization was carried out using the "envelopes" method. In Study No. 1 and No. 2, the animals were divided into 8 groups of 8 animals each (intact, control, reference 1, reference 2, experimental groups № 1, № 2, № 3, № 4).

Research design

To carry out a comprehensive assessment of the analgesic activity of the new compounds, two pharmacological techniques were used, the implementation of which was carried out in accordance with the guidelines and methodological recommendations (Mironov 2012).

Study No. 1 is an orofacial trigeminal pain test based on a nociceptive response to a stimulus and visually manifested by itching in the vibrissae area in rats, as well as the appearance of characteristic scratching movements.

Study No. 2 is a test using a hot plate, which is also associated with pain irritation and serves to determine the antinociceptive activity of the studied drugs.

Metamizole sodium and mefenamic acid were used as comparison drugs in Studies No. 1 and No. 2.

Intragastric administration of drugs was carried out using a gastric tube. The substances were previously dispersed in a universal solvent, purified water (Aqua purificata).

Mefenamic acid and experimental samples were administered at a dosage of 5 mg/kg of animal body weight, and animals received metamizole sodium at a dosage of 7 mg/kg. The dosage of the administered substances was calculated relative to the single dose received by an adult with oral administration of these drugs for pain relief in terms of body weight of laboratory animals, as well as in accordance with methodological recommendations (Rybakova et al. 2018).

The design of the orofacial trigeminal pain study was compiled in accordance with the ARRIVE guidelines, study design and is shown in Figure 1.

Study No. 2 is based on behavioral reactions controlled by supraspinal ~~thinal~~ structures in response to pain irritation. The animal was placed on a plate heated to 52 (51-55) °C, surrounded by a cylinder. The time from the moment the animal was placed on a hot plate to the appearance of a behavioral response to nociceptive stimulation in the form of jumping, jerking and licking of the hind legs was recorded. Pharmacocorrection was performed 1.5 hours before the test.

The design of the hot plate study is shown in Figure 2.

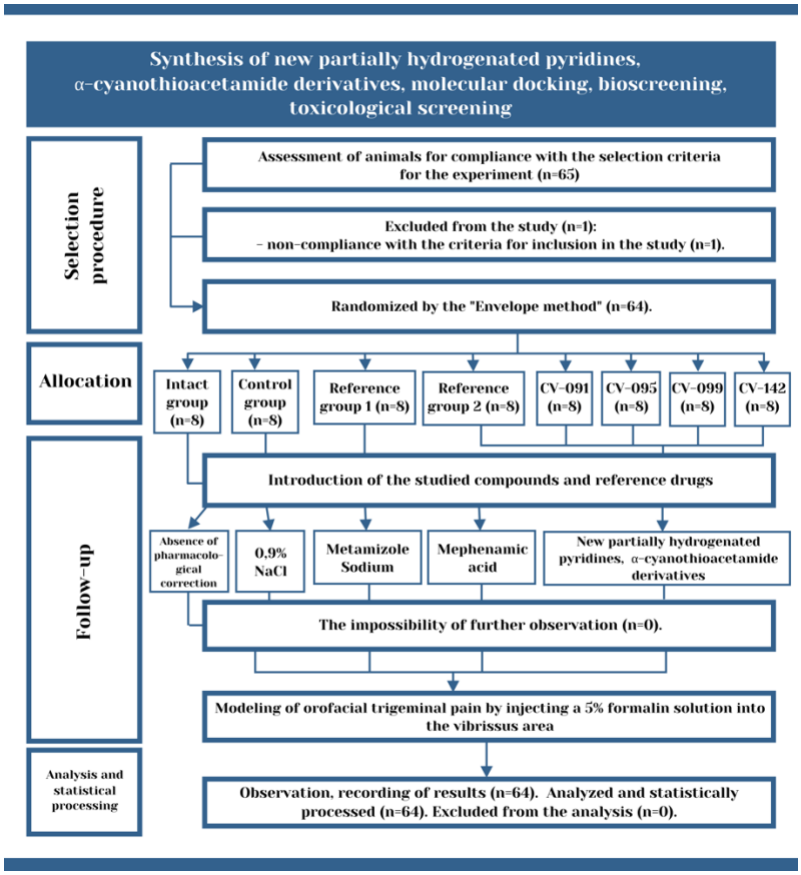


Figure 1. A flowchart for the implementation of an experimental study of orofacial trigeminal pain in white rats.

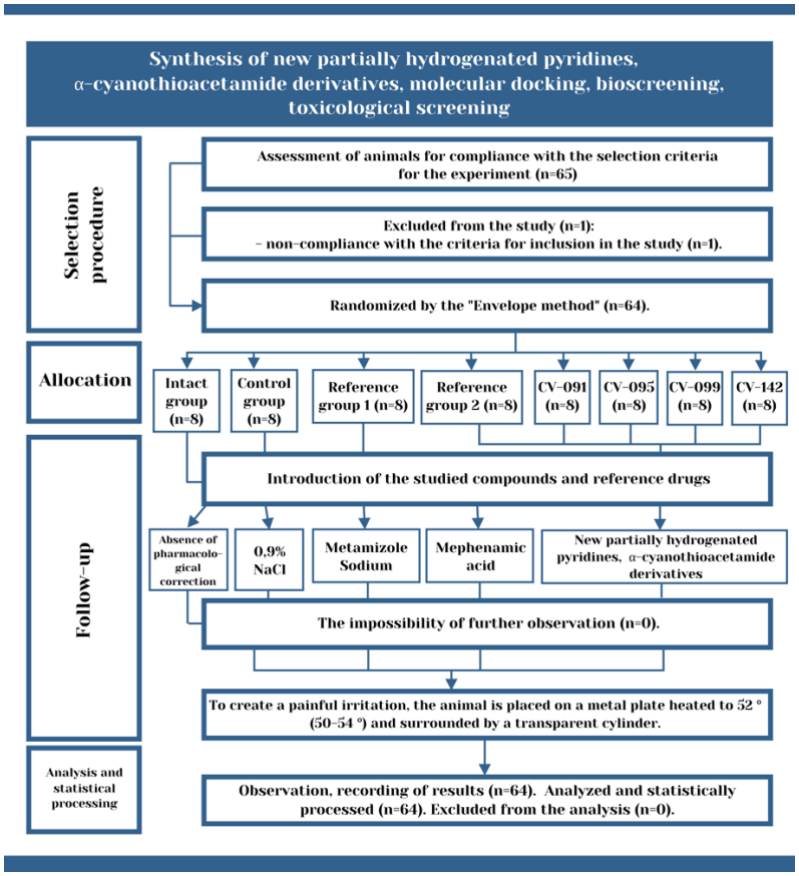


Figure 2. A flowchart for conducting an experimental study in a hot plate test.

Table 1 shows the distribution of drugs administered to animals for pharmacocorrection in Study No. 1 and Study No. 2.

Table 1. The ratio of the number of animals, their distribution into groups and the administered medicinal substances.

№	Animal group	Number of animals in the group	Drug for pharmacocorrection
Study No. 1	Intact	8	without pharmacocorrection
	Control	8	0.9% Sodium chloride solution
	Reference № 1	8	Mefenamic acid
	Reference № 2	8	Metamizole sodium
	Experimental № 1 (cv-091)	8	sample cv-091
	Experimental № 2 (cv-095)	8	sample cv-095
	Experimental № 3 (cv-099)	8	sample cv-099
	Experimental № 4 (cv-142)	8	sample cv-142
Study No. 2	Intact	8	without pharmacocorrection
	Control	8	0.9% Sodium chloride solution
	Reference № 1	8	Mefenamic acid
	Reference № 2	8	Metamizole sodium
	Experimental № 1 (cv-091)	8	sample cv-091
	Experimental № 2 (cv-095)	8	sample cv-095
	Experimental № 3 (cv-099)	8	sample cv-099
	Experimental № 4 (cv-142)	8	sample cv-142

The studied compounds

The structural formulas of the described samples, α-CTA derivatives with laboratory codes **cv-091, cv-095, cv-099,** and **cv-142** are shown in Figure 3. These compounds were carefully selected as a result of a number of in silico studies described in the introduction.

Statistical analysis

To statistically evaluate the data obtained, the Microsoft Excel program was used during the study, as well as the Statistica 10.0 program for Windows in accordance with the recommendations (Agayants 2015; Petri and Sabin 2002). In the statistical processing of quantitative indicators, the normality of the distribution was assessed using the Shapiro-Wilk criterion. As a result of the analysis, it is determined that the distribution is abnormal. The uniformity and reliability of the experimental data were evaluated using the nonparametric analysis method of the Wilcoxon T-test. The experimental study involved nonlinear animals.

Results

The introduction of a 5% aqueous formaldehyde solution led to a nociceptive response, expressed in an increase in the number of scratching movements of the vibrissae area with the front paws in experimental animals. The data obtained is considered in three time projections. Primary data obtained 10 minutes after algogen administration indicate that in the control group, the frequency of scratching movements increases by an average of 90.9 times.

In relation to the control group, the comparison groups mefenamic acid and metamizole sodium reduce the number of carding movements by 2.3 and 1.5 times, respectively. The experimental groups show the following results: the combination with the **cv-091** code reduces the number of scratching movements by 4.5 times compared to those in the control group, **cv-095** – by 4.2 times**, cv-099** – by 4.3 times, and the **cv-142** compound – by 2.1 times.

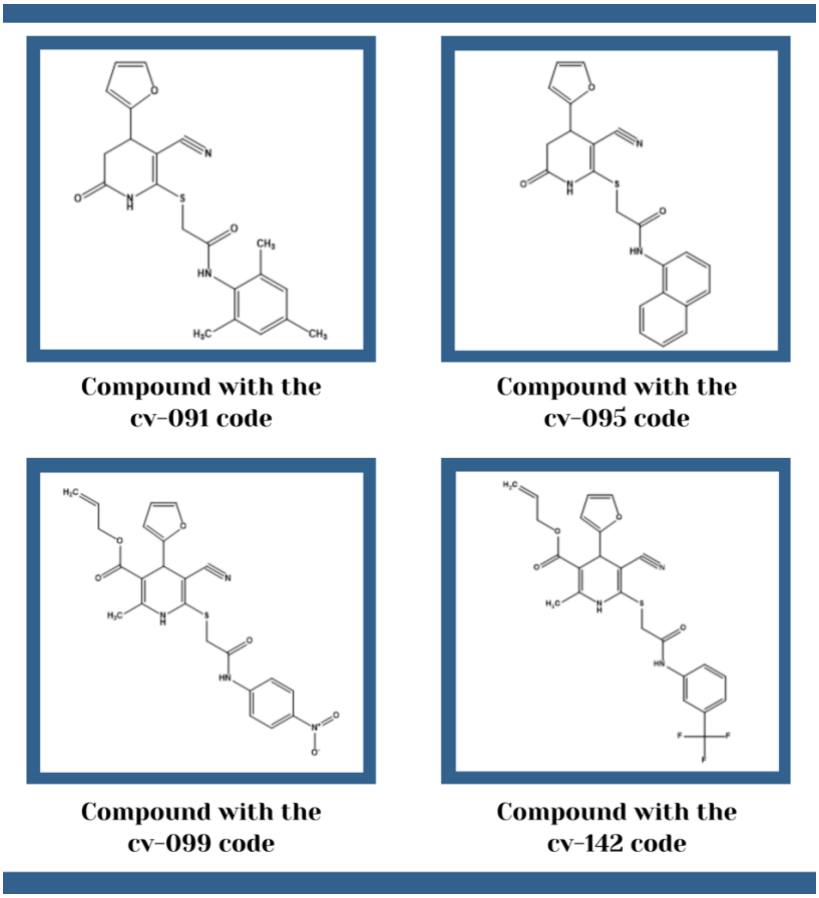


Figure 3. Chemical structure and structural formulas of new partially hydrogenated pyridines, α -CTA derivatives.

Only the **cv-142** sample showed a result that was inferior in activity to the reference drug, mephenamic acid, while surpassing metamizole sodium. The remaining prototypes showed greater activity than the drugs from the comparison groups. According to the numerical data obtained, interpreted and statistically processed, in the time projection after 10 minutes, the alpha-cyanothioacetamide derivative, a compound with the laboratory code **cv-091**, is the leader in antinociceptive activity. In the experimental groups treated with compounds **cv-095** and **cv-099**, high activity is also observed.

As a result of calculating the number of scratching movements 15 minutes after administration of algogen, the following data were obtained: metamizole sodium reduces the number of scratching of the vibrissae zone by 1.7 times compared to that in the control group and mefenamic acid – by 2.5 times. The experimental samples are more effective than the reference drugs. Compound **cv-091** reduces the number of carding movements by 5.6 times, **cv-095** – by 5.3 times, **cv-099** – by 5.9 times, **cv-142 –** by 1.9 times relative to such in the control group. The sample leader in this time projection is the compound with the **cv-099** code.

Analyzing the calculation results obtained 20 minutes after the introduction of a 5% formalin solution into the vibrissae zone, it was determined that metamizole sodium reduces the number of carding movements by 1.9 times and mefenamic acid – by 2.7 times compared to the data obtained in the control group. The new partially hydrogenated pyridines, α-CTA derivatives, show the following results: sample **cv-091** reduces the number of scratching of the vibrissae zone by 3.3 times, sample **cv-095** – by 5.4 times, sample **cv-099** – by 15.4 times, and sample with the code **cv-142** – by 1.8 times relative to the results obtained in the group control.

In this time projection, the connection with the **cv-099** cipher is the leader.

The results of the study are statistically processed and presented in Table 2.

Study No. 2 – the hot plate test was implemented in parallel with the experiment of orofacial trigeminal pain. The pain irritation modeled in the experimental groups led to the emergence of a behavioral reaction caused by pain irritation and a response in the form of twitching and licking of the hind legs by rats in the control group after 18.5 seconds. With respect to this result, prolongation of the reaction time was regarded as the degree of manifestation of the analgesic effect of the experimental samples.

Table 2. Statistical data obtained during the experiment of orofacial trigeminal pain

Value	Intact group	Control group	Metamizole sodium	Mefenamic acid	cv-091	cv-095	cv-099	cv-142
10 minutes after injection of algogen								
Arithmetic mean (seconds)	0	90.9	60.0	40.3	20.38	22.13	21.38	44.13
Median	0	91.5	61.5	42.0	20.5	20.0	21.5	42.5
The variance of values around the arithmetic mean σ^2	-	70.36	33.3	73.19	4.98	14.36	21.73	75.36
Standard deviation	-	8.39	5.8	8.55	2.23	3.79	4.66	8.68
Coefficient of variation (K)	-	9.23%	9.6%	21.25%	10.96%	17.13%	21.81%	19.67%
p	-	-	0.00781 3	0.00781 3	0.00781 3	0.01415	0.00781 3	0.01415
15 minutes after injection of algogen								
Arithmetic mean (seconds)	0	63.3	37.6	25.1	11.25	12.0	10.75	33.13
Median	0	63.0	37.5	24.5	10.5	11.0	10.0	33.0
The variance of values around the arithmetic mean σ^2	-	53.19	6.5	65.11	6.19	9.0	3.69	77.86
Standard deviation	-	7.29	2.5	8.07	2.49	3.0	1.92	8.82
Coefficient of variation (K)	-	11.53%	6.8%	32.12%	22.11%	25.0%	17.86%	26.64%
p	-	-	0.01415	0.00781	0.00781	0.00781	0.01391	0.00781
20 minutes after injection of algogen								
Arithmetic mean (seconds)	0	36.7	19.7	13.5	3.25	5.38	2.38	20.88
Median	0	32.5	19.5	10.5	3.0	5.5	2.0	22.0
The variance of values around the arithmetic mean σ^2	-	90.48	84.0	44.75	5.94	8.98	3.98	51.6
Standard deviation	-	9.51	9.2	6.69	2.44	2.99	1.99	7.18
Coefficient of variation (K)	-	5.97%	6.5%	9.55%	4.98%	5.8%	4.05%	4.41%
p	-	-	0.00781	0.02071	0.01415	0.01415	0.00781	0.01563

Note: * – the result is significant if $p < 0.05$. During statistical processing, it was determined that the differences are significant.

In the animals in the comparison group treated with mefenamic acid, the result was 1.5 times higher than the corresponding values in the control group, while in the comparison group receiving metamizole sodium, – 1.3 times, respectively.

In the groups where animals received experimental samples, the following data were obtained: the introduction of a compound with the **cv-091** code prolonged the occurrence of a behavioral response by 1.3 times, **cv-095** – by 1.5 times, **cv–099** – by 1.8 times, and **cv-142** – by 1.6 times, respectively.

It is worth noting that the **cv-091** sample, according to the data obtained, did not exceed the activity of the result recorded in the group receiving the comparison drug mefenamic acid. In comparison with the data from the group of the reference drug metamizole sodium, it is worth noting similar data without a significant advantage.

The leader sample, according to the received data, is the compound with the **cv-099** cipher.

The results of the study are presented in Table 3.

Table 3. Statistical data obtained during the hot plate experiment

Indicator	Intact group	Control group	Metamizole sodium	Mefenamic acid	cv-091	cv-095	cv-099	cv-142
Arithmetic mean (seconds)	17.9	18.5	24.8	26.8	23.9	28.6	32.8	29.6
Median	17.5	18.5	24.5	26.5	23.0	27.5	34.0	30.0
The variance of values around the arithmetic mean σ^2	5.8	18.3	1.6	19.6	13.6	15.1	23.9	12.6
Standard deviation	2.4	4.3	1.3	4.4	3.7	3.9	4.9	3.5
Coefficient of variation (K)	13.5 %	23.1%	5.2%	16.6%	15.4%	13.6%	14.9%	12.0%
p	-	-	0.0137	0.0142	0.0234	0.02201	0.01403	0.0142

Note: * – the result is significant if $p < 0.05$. During statistical processing, it was determined that the differences are significant.

Discussion

Analyzing the data obtained during experimental preclinical studies, the high prospects of using partially hydrogenated alpha-cyanothioacetamide derivatives as a basis for the creation of new analgesics have been confirmed, which is especially important in light of the significant limitations of the use of narcotic analgesics and the prevalence of undesirable (side) pharmacological effects of nonsteroidal anti-inflammatory drugs.

The results of predictive analysis in silico confirm the high potential of the studied derivatives of partially hydrogenated pyridines, α-cyanothioacetamide derivatives, as multifunctional analgesics. The suspected molecular targets identified during screening indicate the ability of compounds to affect the molecular mechanisms of nociceptive signal transduction and transmission: from blockade of N-type calcium channels and antagonism to neuropeptide Y receptors (**cv-095**), which reduce neuropathic pain, to effects on the cannabinoid system and potential-dependent sodium channels SCN9A (**cv-142**), regulating pain sensitivity. An important advantage of a number of compounds (**cv-091**, **cv-099**, **cv-142**) is the concomitant anti–inflammatory mechanism through the inhibition of enzymes and receptors (JAK, PDE, ALOX5, PAFR), which makes it possible to break the pathological inflammation-pain relationship.

The data obtained during the in silico studies correlate with the results of the experimental evaluation. When implementing a model of orofacial trigeminal pain in outbred male rats and using the studied compounds intragastrically at a dosag of 5 mg per kg of animal body weight, all experimental samples demonstrated pronounced antinociceptive activity, derivatives **cv-091** (10 minutes after administration of algogen) and **cv-099** (in time projections of 15 and 20 minutes after the painful effect). These samples exceed the performance of the reference drugs. In the hot plate test, the studied derivatives also showed antinociceptive activity in relation to the results obtained in the control group. The reference drugs metamizole sodium and mefenamic acid outperformed three derivatives with **cv-095**, **cv-099**, **cv-142** codes.

Such a multi-target profile, confirmed by the results of preclinical studies, makes these substances promising candidates for the treatment of complex pain syndromes; however, the revealed broad biological activity requires careful experimental verification of selectivity and safety assessment in vitro and in vivo.

Conclusion

As a result of the experimental study on white outbred laboratory rats to determine the antinociceptive activity of new partially hydrogenated pyridines, α-cyanothioacetamide derivatives, it was determined that four samples with laboratory codes **cv-091, cv-095, cv-099,** and **cv-142** exhibit pronounced activity at a dosage of 5 mg per kg of animal body weight with a single intra-gastric injection, which is confirmed by the data presented. In the study of orofacial trigeminal pain, samples with laboratory codes **cv-091, cv-095,** and **cv-099** showed the best results in relation to the control group, as well as groups receiving reference drugs – mefenamic acid and metamizole sodium. The undisputed leader in this experiment is the compound with the **cv-099** code. The results obtained in this group of animals exceeded the results of the comparison groups in all time intervals.

In the hot plate test, the leaders in terms of activity are compounds with the laboratory codes **cv-095, cv-099,** and **cv-142**, which also surpassed the results obtained in the comparison groups. The sample leader is a compound with **cv-099**.

The antinociceptive activity of the samples is confirmed by in silico studies, previously implemented using the Swiss Target Prediction program.

The results obtained confirm the relevance of studying new partially hydrogenated pyridines, α-cyanothioacetamide derivatives, which are promising compounds in terms of their biological activity, which forms new vectors for further scientific researches.

Additional Information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

The study was conducted with financial support for research within the state assignment of the Russian Ministry of Education and Science on the topic: "New Heterocyclic Derivatives of Methylene Active Nitriles, Thioamide and Selenamides: Synthesis, Properties and Biological Activity" (code "FREE-2023-0002"), as well as with financial support for research within the state assignment of the Ministry of Health of the Russian Federation on the topic: "Search for New Highly Effective and Safe Drugs Among the Previously Unknown Domestically Produced Heterocyclic Derivatives of Cyanothioacetamide" (code "ZUNP-2024-0002").

Ethics statement

The experiments were carried out in the spring 2025 at the specialized scientific research laboratory of Saint Luka Lugansk State Medical University (Russia) and were approved by the Bioethics Commission of Saint Luka Lugansk State Medical University of the Ministry of Health of the Russian Federation (Minutes No. 5 dated 12 October 2022).

Data availability

All of the data that support the findings of this study are available in the main text.

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