



# Empirical determination of the degree of analgesic activity of some new 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines based on a complex criterion

Igor V. Bibik<sup>1</sup>, Elena Yu. Bibik<sup>1</sup>, Konstantin A. Frolov<sup>1,2</sup>, Sergey G. Krivokolysko<sup>1,2</sup>, Victor V. Dotsenko<sup>3,4</sup>, Andrey A. Pankov<sup>2</sup>

<sup>1</sup> St. Luke Lugansk State Medical University, 1G Quarter of 50<sup>th</sup> Godovschiny Oborony Luganska, Lugansk 91045 Russia

<sup>2</sup> Vladimir Dahl Lugansk State University, KhimEx Research Laboratory, 20-a Molodezhny Quarter, Lugansk 91034 Russia

<sup>3</sup> Kuban State University, 149 Stavropolskaya St., Krasnodar 350040 Russia

<sup>4</sup> North-Caucasus Federal University, 1 Pushkina St., Stavropol 355017 Russia

Corresponding author: Elena Yu. Bibik ([helen\\_bibik@mail.ru](mailto:helen_bibik@mail.ru))

Academic editor: Oleg Gudyrev ♦ Received 22 January 2023 ♦ Accepted 04 April 2023 ♦ Published 07 May 2023

**Citation:** Bibik IV, Bibik EYu, Frolov KA, Krivokolysko SG, Dotsenko VV, Pankov AA (2023) Empirical determination of the degree of analgesic activity of some new 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines based on a complex criterion. Research Results in Pharmacology 9(2): 67–74. <https://doi.org/10.18413/rrpharmacology.9.10029>

## Abstract

**Introduction:** It is relevant to solve the problem of anesthesia based on the research of new highly effective and safe medicines. Among modern studies, the preparation of heterocyclic compounds starting from cyanothioacetamide with analgesic and anti-inflammatory activities is of considerable interest.

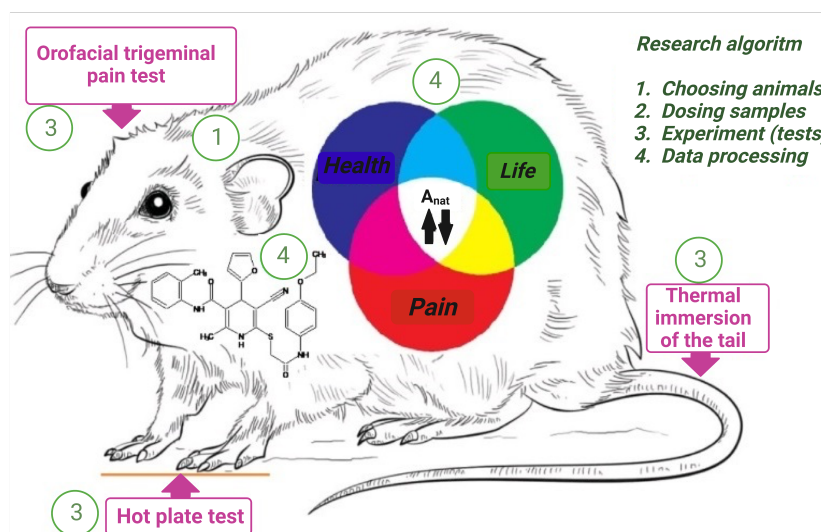
**Materials and Methods:** The analgesic activity of cyanothioacetamide derivatives was determined on the basis of an integrated approach in three tests: orofacial trigeminal pain, thermal immersion of the tail and in the hot plate test. The experimental characteristics in the studies are quantitative indicators – the frequency of scratching movements  $n$  in the first test and the time of the latent period  $a$  and  $t$  in the second and third tests. Statistical processing consisted in a variance analysis of an array of experimental characteristics. A conditional empirical complex criterion of analgesic activity  $A_{nat}$ , inversely proportional to the frequency of scratching movements  $n$  and directly proportional to the time of the latent period  $a$  and  $t$  in the tests is proposed for a comparative assessment of the analgesic activity of the samples.

**Results:** It is shown that all the studied 1,4-dihydropyridines and condensed thieno[2,3-b]pyridines and reveal analgesic activity of varying degrees of severity, based on the values of the criterion  $A_{nat}$ .

**Discussion:** It was found that the most pronounced analgesic activity was shown by three studied samples: compounds AZ-023, AZ-331 and AZ-383. The complex criterion of analgesic activity of  $A_{nat}$  for animals receiving a sample AZ-023 was 50.1, exceeding the indicator in the comparison group by 42 times. The values of this criterion in animals that had received samples AZ-331 and AZ-383 were the highest, namely 64.3 and 68.4, which is 53 and 57 times higher than that of sodium metamizole, respectively.

**Conclusion:** The obtained results and the advantage of the studied samples over the reference drug determine the expediency of further preclinical studies and a detailed study of their acute and chronic toxicity, as well as hepato-, nephro-, hemato- and gastrotoxicity.

## Graphical abstract:



## Keywords

pain syndrome, analgesic activity, thieno[2,3-b]pyridines, complex criterion

## Introduction

The relevance of the research lies in the fact that currently pain syndrome, diverse in its manifestations in intensity and localization, is one of the main complaints of patients. It is pain of various localization as a complaint and a manifestation of acute and chronic pain syndrome that prevails in modern clinical practice.

It is an undeniable fact that pain is a protective biological mechanism, which is based on the mobilization of all functional systems of the body to stop harmful effects. Statistical studies have shown that acute pain is experienced by 9% to 71% of the population. Chronic pain is experienced by 8% to 50% of the population. The frequency of chronic postoperative pain depends on the location of the intervention. As is known, the greatest frequency of pain syndrome was characterized by coronary bypass surgery – from 44 to 56% and pelvic injuries – 48% (Wu and Raja 2011; Yildiz et al. 2016; Brandsborg 2018).

Relief of pain syndrome is a very important task in oncology. Pain is a very frequent and severe symptom of malignant neoplasms (Shtok 2016; Munoz-Farjas 2017; Kogoniya et al. 2018; Pchelincev 2020). The number of patients with malignant neoplasms in the countries of the world, including Russia, is constantly increasing. According to WHO, in 2008, 25 million patients with malignant neoplasms were registered, including 12.7 million new cases of the disease and 7.6 million deaths. According to the forecast of WHO experts, in 2050 there will already be 75 million people with malignant neoplasms in the world, and the number of deaths from oncological diseases will amount from 13 to 17 million cases registered annually (Maksimov 2013; Morozova and Yaroshevskij 2013; Lesnaya 2018).

All this is reflected in the level of working capacity of

people of the older age category, often suffering from diseases that are accompanied by severe pain syndrome. Therefore, scientific research aimed at finding new effective medicines with a high safety profile to eliminate or reduce the severity of pain in the corresponding syndrome is particularly relevant (Filatov and Vejn 1999; Pokrovskij et al. 2011; Karateev 2017).

Analyzing the "benefit/risk" coefficient when using NSAIDs, opioid analgesics, benzodiazepine-type tranquilizers, local anesthetics, anesthesia agents, it is obvious that it is expedient to search for new compounds with pronounced analgesic properties and the absence of ulcerogenic, hepatotoxic, nephrotoxic, cardiotoxic and hematotoxic properties. This is an actual direction of modern research (Karateev et al. 2018; Kukushkin 2016). In the field of scientific interests of modern researchers engaged in the search for new drugs for pain relief, there are biologically active compounds capable of possessing the properties of non-narcotic analgesics, antidepressants, and local anesthetics (Ragulina et al. 2017; Larsen et al. 2018; Schmidt et al. 2018).

Therefore, the solution of the problem of adequate, effective and safe anesthesia in real clinical practice based on the research of new highly effective and safe medicines with analgesic and anti-inflammatory activity is of particular relevance.

The preparation of new heterocyclic compounds starting from cyanothioacetamide belongs to the most promising fields of heterocyclic chemistry and is of considerable interest (Khan et al. 2016). One of the main uses of thioamides is the synthesis of a wide range of *S,N*-heterocyclic compounds. 3-Cyanopyridine-2(1*H*)-thiones belong to the most important groups of *S,N*-heterocyclic compounds. According to a number of studies, cyanothioacetamide derivatives can currently be considered promising for the search for new highly

effective and safe drugs with a variety of pharmacodynamic effects (Krivokolysko et al. 2021; Krivokolysko et al. 2022; Bibik et al. 2021). They are structurally similar to biologically active compounds that were useful for preparation of antihypertensive, antihistamine, antiparkinsonian, diuretic and antitumor drugs. Thus, some new works provide evidence that some of them have high antiviral activity, in particular, against tick-borne encephalitis and Powassan viruses. Some compounds have moderate anti-HIV activity. Some biologically active compounds of a similar chemical structure exhibit pronounced anti-inflammatory and analeptic properties.

### Theoretical background

From the library of 340 heterocyclic samples prepared in ChemEx Research Laboratory of Vladimir Dahl Lugansk State University, ten new cyanothioacetamide derivatives were selected. The selection was performed using virtual bioscreening software Swiss Target Prediction, developed by Swiss Institute of Bioinformatics, (<http://swisstargetprediction.ch/index.php>), on-line software platforms: Online SMILES Translator and Structure File Generator from U.S. National Cancer Institute (<https://cactus.nci.nih.gov/translate/>), OPSIN: Open Parser for Systematic IUPAC nomenclature from University of Cambridge, Centre for Molecular Informatics (<https://opsin.ch.cam.ac.uk/>) to determine the most promising compounds, taking into account the proposed biological targets for pharmacocorrection of pain syndrome (Gfeller et al. 2013).

As a result, 10 samples of new 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines were selected. These samples were recognized as the most promising, taking into account the proposed biological targets for pharmacocorrection of pain syndrome *in vivo*. The selected compounds are marked with library codes **AZ-023**, **AZ-169**, **AZ-213**, **AZ-257**, **AZ-331**, **AZ-420**, **AZ-383**, **AZ-729**, **AU-04271** and **AU-04288**. According to the results of *in silico* preliminary screening, arachidonate-5-lipoxygenase, cyclooxygenase-2, phospholipase A2, phosphodiesterase, and prostanoid, somatostatin, adenosine and cannabinoid receptors are the most likely biological targets.

Thus, according to the results of virtual bioscreening, condensed thieno[2,3-b]pyridine **AZ-023**, will potentially affect prostanoid receptors EP1, EP2 and EP4, cannabinoid receptors CB1 and arachidonate-5-lipoxygenase that may indicate their analgesic activity.

The substituted 1,4-dihydropyridine **AZ-213** is capable to bind to cyclooxygenase-2, prostanoid receptors EP, EP2 and EP4, serotonin, and dopamine transporters.

The compound **AZ-257** of a similar structure shows affinity to arachidonate-5-lipoxygenase, cyclooxygenase-2, somatostatin receptor 3 and prostanoid FP receptor.

The sample encoded **AZ-331** can bind to collagenase-3, phospholipase A2, arachidonate-5-lipoxygenase as well as endothelial receptors of the ET-A and ET-B types.

A new 1,4-dihydropyridine **AZ-383** is potentially capable to bind arachidonate-5-lipoxygenase adenosine receptors A1 and A2b and COX-2.

The compound **AZ-420** affects the activity of serine-threonine protein kinase, phospholipase A2, arachidonate-5-lipoxygenase, and beta-secretase.

Condensed thieno[2,3-b]pyridines **AU-04271** and **AU-04288** have arachidonate-5-lipoxygenase, phospholipase A2, voltage-dependent sodium channels as biological targets, which makes the manifestation of analgesic activity more likely.

The 3-aminothieno[2,3-b]pyridine **AZ-729** can affect prostanoid EP1, EP2 and EP4 receptors, voltage-dependent sodium channels, cannabinoid CB2 receptors, somatostatin receptors 3 and arachidonate-5-lipoxygenase.

The aim of the present study is to identify the effectiveness of newly synthesized condensed 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines as analgesics, based on a complex criterion of analgesic activity.

## Materials and Methods

### Animals

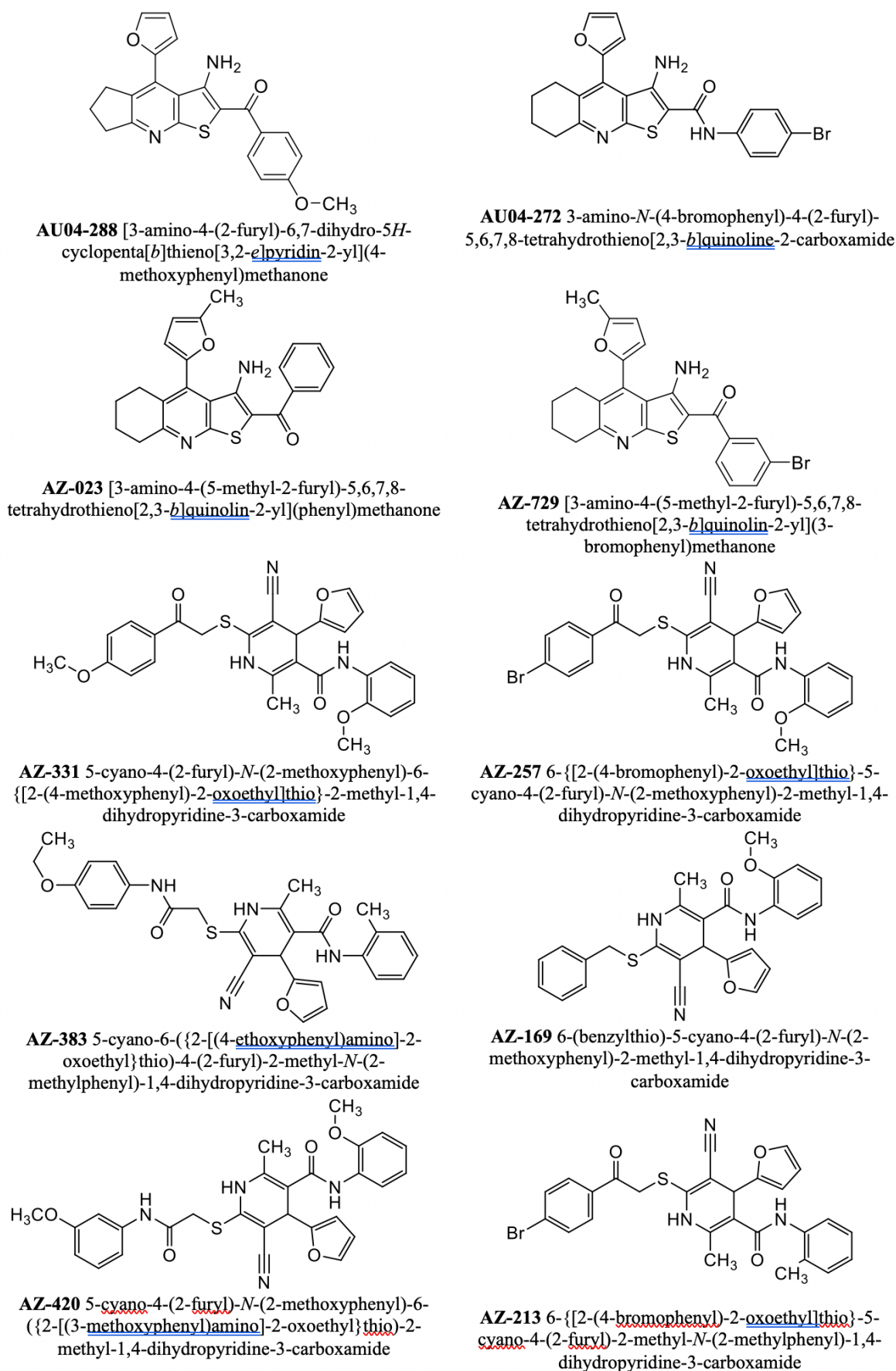
A randomized controlled investigation was carried out in three stages on 390 white mongrel male rats weighing 250-280 grams. The animals were taken from the vivarium of St. Luke Lugansk State Medical University in the autumn-winter period. The experiment did not include animals whose weight differed by more than 50 g. Female rats were not included in the experiment. At the time of inclusion in the study, the experimental animals in all groups were identical in age, sex, body weight, and breed. None had any visible developmental pathologies.

The experiment was implemented in the laboratory of the Department of Fundamental and Clinical Pharmacology of St. Luke Luhansk State Medical University. The studies were conducted in accordance with the rules of high-quality laboratory practice during preclinical studies in the Russian Federation (Order of the Ministry of Health and Social Development of the Russian Federation dated August 23, 2010, №708n). Throughout the entire research period, the animals were monitored with free access to water and food. This corresponds to GOST 33044-2014 "Principles of Good Laboratory Practice" (approved by Order of the Federal Agency for Technical Regulation and Metrology №1700-st, dated November 20, 2014) and in accordance with the protocol of the Ethics Committee of St. Luke Lugansk State Medical University of the Ministry of Health of Russia No. 6 dated November 1, 2021.

Randomization was carried out by the method of envelopes. Groups consisting of 10 animals were used in the experiment. The animals were divided into intact and control groups (rats injected with 2 ml of 0.9% sodium chloride solution intragastrically before the test simulation), a comparison group (receiving sodium metamizole) and 10 experimental groups, according to the number of new of condensed 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines studied.

### The studied compounds

The samples of new cyanothioacetamide derivatives **AZ-023**, **AZ-169**, **AZ-213**, **AZ-257**, **AZ-331**, **AZ-420**, **AZ-383**, **AZ-729**, **AU-04271**, **AU-04288** had been pre-selected according to the results of the virtual bioscreening. The structures of compounds are shown in Fig. 1.



**Figure 1.** The structures of the studied 3-aminothieno[2,3-*b*]pyridines and 1,4-dihydropyridines

### Model of experimental research

Determination of analgesic activity of new cyanothioacetamide derivatives was carried out on the basis of an integrated approach, using three tests, according to (Mironov 2012):

a) orofacial trigeminal pain modeled by subcutaneous injection of 0.1 ml of 5% formalin solution into the vibrissus area in the experimental animals. The analgesic effect was assessed by fixing the number of scratching movements with the front paws of the orofacial area per minute. The number of movements was calculated 10-, 15- and 20-minutes after the introduction of 0.1 ml of 5% formalin solution into the vibrissus area;

b) thermal immersion of the tail. The experiment is based on the spinal flexor reflex in response to the immersion of the rat's tail into water heated to an average of 50-53°C, with the measurement of the time interval of the latent reaction period;

c) hot plate. The animals were placed on a metal plate heated to an average of 52°C, surrounded by a cylindrical wall. The time from the moment of placing the animal on a hot surface to the appearance of a behavioral response to nociceptive stimulation in the form of jumping, pulling back and licking the hind legs was recorded.

The studied thienopyridines and 1,4-dihydropyridines were administered intragastrically at a dose of 5 mg/kg 1.5 hours before modeling acute pain syndrome in an *in vivo* experiment. Metamizole sodium was used as a reference drug. It was administered at a dose of 7 mg/kg intragastrically, respectively.

Indicators of analgesic effect were considered to be: the number of scratching movements in the test of orofacial trigeminal pain and a significant increase in the latent reaction period after the introduction of the studied substances in the tests of thermal immersion of the tail and hot plate.

### Statistical analysis

The experimental characteristics in the studies are quantitative indicators – the frequency of scratching movements  $n$  in the first test and the time of the latent period  $a$  and  $t$ , sec, in the second and third tests, respectively. Therefore, statistical processing of the obtained experimental results was carried out according to the known formulas and methods of mathematical statistics characterizing quantitative variability. When processing experimental data, the following were determined: the arithmetic mean of  $n$ ,  $a$ ,  $t$ ; the variance of values  $s^2$  around the arithmetic mean and the standard deviation  $s$  in the online resource (Arithmetic mean, variance, variation – Access mode: <https://allcalc.ru/node/89>) Due to the individual characteristics of the animals, the uniformity of the obtained experimental data was estimated by the coefficient of variation  $V$  in the specified online resource.

If the coefficient of variation was less than 10%, then the variability of a number of data is considered insignificant, from 10% to 20% – refers to the average variability, and more than 20% and less than 33% – to significant variability. When the coefficient of variation exceeds 33%, this indicates the heterogeneity of information and the need to exclude the largest and smallest values. The values estimation of the excluded data and smoothing of the data series were estimated on the basis of a 95% confidence interval, the size of which

for the experimental groups was determined depending on the values of statistical indicators in the online calculator: Study electronics – Access mode: <https://learningaboutelectronics.com> – and according to (Petri and Sebin 2002; Narkevich 2019).

It was found that the analgesic activity of the samples under consideration is inversely proportional to the frequency of carding movements  $n$  and is directly proportional to the time of the latent period  $a$  and  $t$  in the tests. Therefore, to assess the analgesic activity of new compounds, a conditional empirical complex criterion of analgesic activity  $A_{nat}$  is proposed:

$$A_{nat} = \frac{a+t}{n_{AV}}$$

## Results

The data of experimental studies of analgesic activity in three pharmacological tests for ten original thienopyridines and 1,4-dihydropyridines are presented in Table 1.

## Discussion

Analyzing the data obtained, it can be concluded that the value of the criterion  $A_{nat} = 0.4$  for the control group may be within the measurement error.

Based on the results of experimental studies on three classical pharmacological tests for the detection of analgesic activity, the following has been established. Preliminary administration of the non-narcotic analgesic metamizole sodium 1.5 hours before the modeling of the pain syndrome contributes to a threefold increase in the proposed conditional empirical complex criterion of analgesic activity  $A_{na}$  in comparison with that of the control group without pharmacocorrection.

All the studied thienopyridines and 1,4-dihydropyridines administered for prophylactic purposes before modeling acute pain syndrome, have analgesic activity of varying intensity, based on the values of the  $A_{nat}$  criterion.

Antinociceptive activity similar to that of sodium metamizole is shown by derivatives of thienopyridines **AU-04271** and **AU-04288**. The values of the conditional empirical complex criterion of analgesic activity  $A_{nat}$  for them are determined at the level of 1.54 and 2.00, respectively.

Intragastric administration of the 1,4-dihydropyridine **AZ-169** led to a slight increase in this criterion to a value of 2.2 (Table 1).

In experimental groups of animals which received 1,4-dihydropyridines **AZ-257**, **AZ-213** and **AZ-729**, the values of the proposed conditional empirical complex criterion for analgesic activity of  $A_{nat}$  are 3.93; 4.0 and 4.6, respectively. They are 3.33-3.83 times higher than those values in sodium metamizole.

Four new 3-aminothieno[2,3-b]pyridines and 1,4-dihydropyridines showed high and clearly pronounced analgesic activity according to the results of three classical pharmacological tests for preclinical study of biologically active substances. These are compounds **AZ-420**, **AZ-023**, **AZ-331**, and **AZ-383**.

Thus, the value of the studied conditional empirical integral criterion of analgesic activity  $A_{nat}$  for 1,4-dihydropyridine **AZ-420** is 36.5, which is 30 times higher than the value recorded in the reference group.

Table 1. Results of the dispersion analysis of the indicators of analgesic activity of the studied samples

Groups	Statistical characteristics: quantitative indicators ( $n$ , $a$ , $t$ ), variance $s^2$ , standard deviation $s$ , coefficient of variation $V$ , %					Criteria <i>Anat</i>
	Pharmacological test			Thermal immersion of the tail	Hot plate	
	Orofacial trigeminal pain					
Control	$n = 76.7$ ; $s^2 = 19.4$ ; $s = 4.4$ ; $V = 5.7\%$	$n = 34.8$ ; $s^2 = 127.7$ ; $s = 11.3$ ; $V = 32.4\%$	$n = 20.3$ ; $s^2 = 28.1$ ; $s = 5.3$ ; $V = 26.1\%$	$a = 9.4$ c; $s^2 = 3.6$ ; $s = 1.9$ ; $V = 20.2\%$	$t = 8.0$ c; $s^2 = 3.5$ ; $s = 1.9$ ; $V = 23.8\%$	0.40
	$n_{av} = 43.9$					
Reference (metamizole sodium)	$n = 38.5$ ; $s^2 = 41.0$ ; $s = 6.4$ ; $V = 16.6\%$	$n = 21.2$ ; $s^2 = 15.2$ ; $s = 3.9$ ; $V = 18.4\%$	$n = 12.5$ ; $s^2 = 5.3$ ; $s = 2.3$ ; $V = 18.4\%$	$a = 13.4$ c; $s^2 = 2.3$ ; $s = 1.5$ ; $V = 11.1\%$	$t = 15.6$ c; $s^2 = 11.0$ ; $s = 3.3$ ; $V = 21.2\%$	1.20
	$n_{av} = 24.1$					
AZ-383	$n = 6.5$ ; $s^2 = 1.7$ ; $s = 1.3$ ; $V = 20.0\%$	$n = 2.2$ ; $s^2 = 0.19$ ; $s = 0.44$ ; $V = 20.0\%$	$n = 1.4$ ; $s^2 = 0.2$ ; $s = 0.45$ ; $V = 32.1\%$	$a = 47.5$ c; $s^2 = 127.5$ ; $s = 11.3$ ; $V = 23.8\%$	$t = 171.0$ c; $s^2 = 3102$ ; $s = 55.7$ ; $V = 32.6\%$	64.3
	$n_{av} = 3.4$					
AZ-023	$n = 4.0$ ; $s^2 = 1.7$ ; $s = 1.3$ ; $V = 32.5\%$	$n = 7.3$ ; $s^2 = 9.6$ ; $s = 3.1$ ; $V = 31.7\%$	$n = 7.5$ ; $s^2 = 5.8$ ; $s = 2.4$ ; $V = 32.0\%$	$a_{op} = 29.0$ c; $s^2 = 13.5$ ; $s = 3.7$ ; $V = 12.7\%$	$t = 286$ c; $s^2 = 599$ ; $s = 24.5$ ; $V = 8.6\%$	50.1
	$n_{av} = 6.3$					
AZ-420	$n = 7.0$ ; $s^2 = 3.2$ ; $s = 1.8$ ; $V = 25.7\%$	$n = 3.8$ ; $s^2 = 0.16$ ; $s = 0.4$ ; $V = 10.5\%$	$n = 2.3$ ; $s^2 = 0.25$ ; $s = 0.5$ ; $V = 21.7\%$	$a = 44.6$ c; $s^2 = 182.3$ ; $s = 13.5$ ; $V = 30.3\%$	$t = 116$ c; $s^2 = 639.5$ ; $s = 25.2$ ; $V = 21.9\%$	36.5
	$n_{av} = 4.4$					
AZ-257	$n = 8.0$ ; $s^2 = 6.8$ ; $s = 2.6$ ; $V = 32.5\%$	$n = 11.0$ ; $s^2 = 2.9$ ; $s = 1.7$ ; $V = 15.4\%$	$n = 10.4$ ; $s^2 = 2.9$ ; $s = 1.7$ ; $V = 16.3\%$	$a = 11.4$ c; $s^2 = 6.6$ ; $s = 2.6$ ; $V = 22.8\%$	$t = 27.5$ c; $s^2 = 81.0$ ; $s = 9.0$ ; $V = 32.7\%$	4.00
	$n_{av} = 9.8$					
AZ-213	$n = 8.8$ ; $s^2 = 2.6$ ; $s = 1.6$ ; $V = 18.2\%$	$n = 10.5$ ; $s^2 = 3.6$ ; $s = 1.9$ ; $V = 18.1\%$	$n = 8.0$ ; $s^2 = 3.2$ ; $s = 1.8$ ; $V = 22.5\%$	$a = 14.3$ c; $s^2 = 2.7$ ; $s = 1.6$ ; $V = 11.2\%$	$t = 21.5$ c; $s^2 = 39.0$ ; $s = 6.2$ ; $V = 28.8\%$	3.93
	$n_{av} = 9.1$					
AZ-331	$n = 4.0$ ; $s^2 = 0.25$ ; $s = 0.5$ ; $V = 12.5\%$	$n = 1.75$ ; $s^2 = 0.25$ ; $s = 0.5$ ; $V = 28.5\%$	$n = 3.2$ ; $s^2 = 0.8$ ; $s = 0.9$ ; $V = 28.1\%$	$a = 30.8$ c; $s^2 = 49.2$ ; $s = 7.0$ ; $V = 22.7\%$	$t = 174.3$ c; $s^2 = 6933$ ; $s = 83.3$ ; $V = 47.8\%$	68.4
	$n_{av} = 3.0$					
AZ-729	$n = 11.2$ ; $s^2 = 2.6$ ; $s = 1.6$ ; $V = 14.3\%$	$n = 11.4$ ; $s^2 = 2.9$ ; $s = 1.7$ ; $V = 14.9\%$	$n = 7.6$ ; $s^2 = 0.8$ ; $s = 0.9$ ; $V = 11.8\%$	$a = 11.5$ c; $s^2 = 15.5$ ; $s = 3.8$ ; $V = 33.0\%$	$t = 34.7$ c; $s^2 = 126.3$ ; $s = 11.2$ ; $V = 32.7\%$	4.60
	$n_{av} = 10.1$					
AZ-169	$n = 12.0$ ; $s^2 = 10.9$ ; $s = 3.3$ ; $V = 27.5\%$	$n = 6.0$ ; $s^2 = 2.9$ ; $s = 1.7$ ; $V = 28.3\%$	$n = 3.6$ ; $s^2 = 1.2$ ; $s = 1.1$ ; $V = 30.6\%$	$a = 7.6$ c; $s^2 = 4.9$ ; $s = 2.2$ ; $V = 28.9\%$	$t = 8.3$ c; $s^2 = 1.9$ ; $s = 1.4$ ; $V = 16.8\%$	2.20
	$n_{av} = 7.2$					
AU-04271	$n = 10.4$ ; $s^2 = 2.9$ ; $s = 1.7$ ; $V = 16.3\%$	$n = 10.4$ ; $s^2 = 1.02$ ; $s = 1.01$ ; $V = 9.7\%$	$n = 7.5$ ; $s^2 = 0.64$ ; $s = 0.8$ ; $V = 10.7\%$	$a = 9.9$ c; $s^2 = 2.8$ ; $s = 1.7$ ; $V = 17.2\%$	$t = 4.6$ c; $s^2 = 1.6$ ; $s = 1.3$ ; $V = 28.3$	1.54
	$n_{av} = 9.4$					
AU-04288	$n = 7.3$ ; $s^2 = 5.3$ ; $s = 2.3$ ; $V = 31.5\%$	$n = 10.6$ ; $s^2 = 6.3$ ; $s = 2.5$ ; $V = 23.6\%$	$n = 8.8$ ; $s^2 = 6.8$ ; $s = 2.6$ ; $V = 29.5\%$	$a = 10.8$ c; $s^2 = 4.7$ ; $s = 2.2$ ; $V = 20.4\%$	$t = 6.6$ c; $s^2 = 4.4$ ; $s = 2.1$ ; $V = 31.8\%$	2.00
	$n_{av} = 8.9$					

Three samples showed pronounced analgesic activity in this regard: compounds **AZ-023**, **AZ-331** and **AZ-383**. The proposed empirical complex criterion of analgesic activity proposed by us –  $A_{nat}$  – in experimental groups of animals treated with thienopyridine **AZ-023**, based on the results of three tests was 50.1, exceeding the indicator in the comparison group by 42 times. The value of this criterion in rats of experimental groups who received 1,4-dihydropyridines **AZ-331** and **AZ-383** for prophylactic purposes had the maximum values in this series of experiments. To be more exact, they were 64.3 and 68.4, which is 53–57 times more than that of **metamizole sodium**. It is obvious that such an advantage of the latter over the reference drug determines the expediency of further preclinical research and a detailed study of their acute and chronic toxicity, as well as hepato-, nephro-, hemato- and gastrotoxicity.

## Conclusion

The proposed empirical complex criterion of analgesic activity  $A_{nat}$ , based on the quantitative characteristics of three classical pharmacological tests for the study of analgesic activity *in vivo* allowed us to establish that among the ten new thienopyridines and 1,4-dihydropyridines, three compounds **AZ023**

([3-amino-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-*b*]quinolin-2-yl](phenyl)methanone), **AZ331** (5-cyano-4-(2-furyl)-*N*-(2-methoxyphenyl)-6-[[2-(4-methoxyphenyl)-2-oxoethyl]thio]-2-methyl-1,4-dihydropyridine-3-carboxamide) and **AZ383** ([3-amino-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-*b*]quinolin-2-yl](phenyl)methanone) have the most pronounced analgesic activity. Their values exceed the activity of **sodium metamizole** by 42, 53 and 57 times, respectively.

Further research prospects are to confirm the analgesic activity of the presented samples in other classical tests, to identify the antiexudative effects of these compounds, as well as to study their acute and chronic oral toxicity.

## Funding

The research was financially supported by the Kuban Scientific Foundation (the research project MFI-20.1-26/20, application № MFI-20.1/45).

## Conflict of Interest

The authors declare no conflict of interests.

## References

- Bibik IV, Bibik EY, Dotsenko VV, Frolov KA, Krivokolysko SG, Aksenov NA, Aksenova IV, Shcherbakov SV, Ovcharov SN (2021) Synthesis and analgesic activity of new heterocyclic cyanothioacetamide derivatives. *Russian Journal of General Chemistry [Zhurnal Obshchei Khimii]* 9(2): 154–166. <https://doi.org/10.1134/S107036322102002X>
- Brandsborg B (2012) Pain following hysterectomy: epidemiological and clinical aspects. *Danish Medical Journal* 59(1): B4374. [PubMed]
- Filatov EG, Vejn AM (1999) Pharmacology of pain. *Russian Medical Journal [Rossiiskii Meditsinskii Zhurnal]* 9: 3–11. [in Russian]
- Gfeller D, Michielin O, Zoite V (2013) Shaping the interaction landscape of bioactive molecules. *Bioinformatics* 29(23): 3073–3079. <https://doi.org/10.1093/bioinformatics/btt540> [PubMed]
- Karateev AE (2017) What's new? Review of international publications in 2016 devoted to the problems of efficacy and safety of nonsteroidal anti-inflammatory drugs. *Modern Rheumatology [Sovremennaya Revmatologiya]* 11(1): 38–45. [in Russian]
- Karateev AE, Nasonov EL, Ivashkin VT (2018) Rational use of nonsteroidal anti-inflammatory drugs. Clinical recommendations. *Scientific and Practical Rheumatology [Nauchno-Prakticheskaya Revmatologiya]* 56(1): 1–29. [in Russian]
- Khan MM, Khan S, Saigal S, Iqbal S (2016) Recent developments in multicomponent synthesis of structurally diversified tetrahydropyridines. *RSC Advances. Royal Society of Chemistry (RSC)* 6(48): 42045–42061. <https://doi.org/10.1039/c6ra06767k>
- Kogoniya LM, Voloshin AG, Novikov GA, Sidorov AV (2018) Practical recommendations for the treatment of chronic pain syndrome in cancer patients. *Malignant tumors [Zlokachestvennye opukholi]* 8(3s2): 617–635. <https://doi.org/10.18027/2224-5057-2018-8-3s2-617-635> [in Russian]
- Krivokolysko DS, Dotsenko VV, Bibik EY, Samokish AA, Venidiktova YS, Frolov KA, Krivokolysko SG, Vasilin VK, Pankov AA., Aksenov NA, Aksenova IV (2021) New 4-(2-Furyl)-1,4-dihydropyridinonitriles and 1,4,5,6-Tetrahydropyridinonitriles: synthesis, structure, and analgesic activity. *Russian Journal of General Chemistry [Zhurnal Obshchei Khimii]* 91(9): 1646–1660. <https://doi.org/10.1134/s1070363221090073>
- Krivokolysko DS, Dotsenko VV, Bibik EYu, Samokish AA, Venidiktova YS, Frolov KA, Krivokolysko SG, Pankov AA, Aksenov NA, Aksenova IV (2022) New recent developments in multicomponent synthesis of structurally diversified tetrahydropyridines: Synthesis, recent developments in multicomponent synthesis of structurally diversified tetrahydropyridines. *Russian Journal of Bioorganic Chemistry [Bioorganicheskaya Khimiya]* 48(3): 628–635. <https://doi.org/10.1134/s1068162022030104>
- Kukushkin ML (2016) Algorithm for choosing safe therapy for acute back pain. *Russian Journal of Pain [Rossiiskii Zhurnal Boli]* 3-4(51): 55–63. [in Russian]
- Larsen IM, Drewes AM, Olesen AE (2018) The effect of a combination of diclofenac and methadone applied as gel in a human experimental pain model – a randomized, placebo-controlled trial. *Basic and Clinical Pharmacology and Toxicology* 123(2): 188–194. <https://doi.org/10.1111/bcpt.12993> [PubMed]
- Lesnaya OA (2018) Nonsteroidal anti-inflammatory drugs: more than 30 years at the peak of relevance. *Difficult Patient [Trudnyy Patsient]* 16(11): 45–49. <https://doi.org/10.24411/2074-1995-2018-10030> [in Russian]
- Maksimov ML (2013) Modern approaches to pain syndrome therapy. *Russian Medical Journal [Rossiiskii Meditsinskii Zhurnal]* 34: 1734–1737. [in Russian]
- Mironov AN (2012) Guidelines for conducting preclinical studies of medicines. *Grif i K, Moscow*, 941 pp. [in Russian]
- Morozova OG, Yaroshevskij AA (2013) Pain in the practice of a neurologist: the experience of using the central analgesic acupan. *International Neurological Journal [Mezhdunarodnyi Nevrologicheskii Zhurnal]* 1(55): 149–155. [in Russian]
- Muñoz-Farjas E (1997) Estudios neurofisiológicos en cefaleas [Neurophysiological studies of headaches]. *Revista de Neurologia* 25(146): 1611–1616. [PubMed] [in Spanish]
- Narkevich AN (2019) Methods for determining the minimum required sample size in medical research. *Social Aspects of Public Health [Sotsial'nye Aspekty Zdorov'ya Naseleniya]* 65(6): 10. <https://doi.org/10.21045/2071-5021-2019-65-6-10> [in Russian]
- Pchelincev MV (2020) Pain syndrome in oncology. Possibilities of using dexketoprofen. *Medical Council [Meditsinskii Sovet]* 9: 146–154. <https://doi.org/10.21518/2079-701X-2020-9-146-154> [in Russian]
- Petri A, Sebin K (2002) Visual statistics in medicine. *Geotar-Med, Moscow*, 144 pp. [in Russian]

- Pokrovskij MV, Pokrovskaya TG Korokin MV (2011) Nonsteroidal anti-inflammatory drugs: studies. pharmacology manual for doctors, pharmacists, interns, postgraduates, residents, students of medical and pharmaceutical universities. BelGU, Belgorod, 140 pp. [in Russian]
- Ragulina VA, Kostina DA, Dovgan AP, Burda YE, Nadezhdin SV (2017) Nuclear factor kappa B as a potential target for pharmacological correction endothelium-associated pathology. Research Result: Pharmacology and Clinical Pharmacology 3(1): 114–124. <https://doi.org/10.18413/2500-235x-2017-3-1-114-124>
- Schmidt M, Sorensen HT, Pedersen L (2018) Diclofenac use and cardiovascular risks: series of nationwide cohort studies. British Medical Journal 362: k3462. <https://doi.org/10.1136/bmj.k3426> [PubMed] [PMC]
- Shtok VN (2001) Headache. Medical Information Agency [Meditsinskoe informatsionnoe agentstvo], Moscow, 472 pp. [in Russian]
- Wu CL, Raja SN (2011) Treatment of acute postoperative pain. Lancet 377(9784): 2215–2225. [https://doi.org/10.1016/S0140-6736\(11\)60245-6](https://doi.org/10.1016/S0140-6736(11)60245-6) [PubMed]
- Yildiz E, Feliz M, Yuksel V, Becker E (2016) The Relationship between pain, impairment and disability in the patients with chronic low back pain. Archives of Physical Medicine and Rehabilitation (77): 962.

## Author Contributions

- **Igor V. Bibik**, Candidate of Medical Sciences, Associate Professor of the Department of Surgical Dentistry; e-mail: [dr.bibik.i.v@yandex.ru](mailto:dr.bibik.i.v@yandex.ru); **ORCID ID** <https://orcid.org/0000-0003-4053-1431>. Conducting experiments.
- **Elena Yu. Bibik**, Doctor Habil. of Medical Sciences, Professor, Head of the Department of Fundamental and Clinical Pharmacology; e-mail: [helen\\_bibik@mail.ru](mailto:helen_bibik@mail.ru); **ORCID ID** <https://orcid.org/0000-0001-7470-608X>. Coordination of research, planning and writing of the manuscript text.
- **Konstantin A. Frolov**, Candidate of Chemical Sciences, Associate Professor of the Department of Pharmaceutical Chemistry and Pharmacognosy, Chief Engineer of the ChemEx Research Laboratory; e-mail: [ka.frolov@inbox.ru](mailto:ka.frolov@inbox.ru); **ORCID ID** <https://orcid.org/0000-0002-8045-7582>. Synthesis of new compounds.
- **Sergey G. Krivokolysko**, Doctor Habil. of Chemical Sciences, Professor, Senior Researcher, Head of the Department of Pharmaceutical Chemistry and Pharmacognosy, Scientific Director of the ChemEx Research Laboratory; e-mail: [ksg-group-lugansk@mail.ru](mailto:ksg-group-lugansk@mail.ru); **ORCID ID** <https://orcid.org/0000-0001-9879-9217>. Synthesis of new compounds, scientific idea and verification of the manuscript.
- **Victor V. Dotsenko**, Doctor Habil. of Chemical Sciences, Head of the Department of Organic Chemistry and Technology, Leading Researcher of the ChemEx Research laboratory; e-mail: [victor\\_dotsenko@bigmir.net](mailto:victor_dotsenko@bigmir.net); **ORCID ID** <https://orcid.org/0000-0001-7163-0497>. Synthesis of new compounds.
- **Andrey A. Pankov**, Doctor Habil. of Technical Sciences, Professor of the Department of Transport Technologies; e-mail: [app.post@rambler.ru](mailto:app.post@rambler.ru); **ORCID ID** <https://orcid.org/0000-0003-2813-8231>. Theoretical research, scientific idea, statistical data processing and analysis, manuscript design.