Research Results in Pharmacology

Research Results in Pharmacology 10(3): 61–71
UDC: 547:616.153.455-008.64:616.379-008.64
DOI 10.18413/rrpharmacology.10.483

Research Article

New compounds with a dihydropyridine framework as promising hypolipidemic and hepatoprotective agents

Elena S. Ketova[1], Anna V. Myazina[2], Elena Yu. Bibik[3], Sergey G. Krivokolysko[4,3]

1 BestDoctor Ltd., 27 Vyatskaya St., Bldg 15, Moscow 127015 Russia
2 V.I. Vernadsky Crimean Federal University, 4 Prospekt Vernadskogo, Simferopol 295007 Republic of Crimea, Russia
[3]Saint Luka Lugansk State Medical University of the Ministry of Health of the Russian Federation, 1g Kvartal 50th Godovschiny Oborony Luganska, Lugansk 291045 Russia
[4]Vladimir Dahl Lugansk State University, ChemEx Research Laboratory, 20a Molodezhny Kvartal, Lugansk 291034 Russia

Corresponding author: Elena S. Ketova (ketova_elena@mail.ru)

Academic editor: Oleg Gudyrev ♦ **Received** 01 June 2024 ♦ **Accepted** 21 August 2024 ♦ **Published** 23 September 2024

Citation: Elena S. Ketova, Anna V. Myazina, Elena Yu. Bibik, Sergey G. Krivokolysko (2023) New compounds with a dihydropyridine framework as promising hypolipidemic and hepatoprotective agents Research Results in Pharmacology 10(3): 61–71. https//doi.org/10.18413/rrpharmacology.10.483

Abstract

Introduction: To solve the problem of complex and safe pharmacological correction of metabolic disorders, including hyperlipidemia, hyperglycemia, and liver lesions is currently very important. With this in mind, the new derivatives of cyanothioacetamide with a dihydropyridine framework, with a potential effect on lipid and carbohydrate metabolism and the functioning of the liver are of great interest.

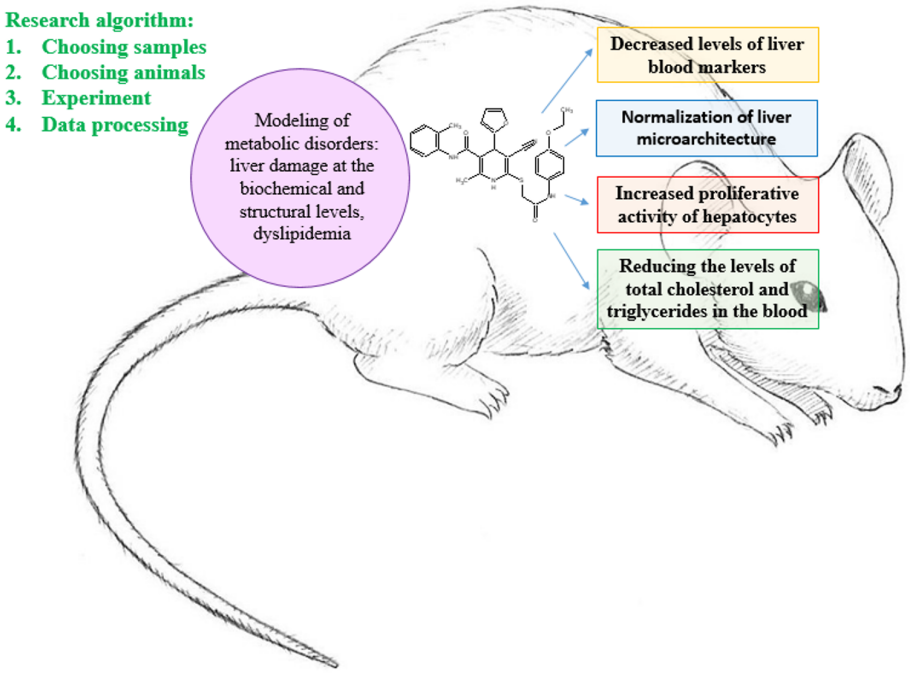
Materials and Methods: To conduct an experiment, three samples were selected from an extensive library of new cyanothioacetamide derivatives, which, according to *in silico* studies, proved promising towards the positive effect on lipid and carbohydrate metabolism, as well as towards the protective effect on the liver. To study the compounds *in vivo*, metabolic disorders were simulated in Wistar rats by long -term alimentary and subsequent dexamethasone loads. The pharmacological efficacy of new compounds AZ-383, AZ-257, AZ-020 (administered in a dose of 1 mg/kg for 14 days) was assessed in comparison with metformin (300 mg/kg for 14 days) and vildagliptin (8 mg/kg for 14 days) by determining the biochemical indicators of blood (ALT, AST, total bilirubin, total cholesterol, triglycerides, and glucose), morphological and morphometric studies of the liver sections, and the study of immunohistochemical indicators of hepatocyte proliferation. The experimental results were statistically processed using the recognized methods of mathematical statistics. When processing the experimental data, the average arithmetic (AA) was determined. The statistical significance of the compared options was determined on the basis of the Student's t-test, with the critical value of the Student's t-test equal to 2.101 and the significance level $\alpha = 0.05$.

Results: The study shows that all the new compounds studied in the experiment – AZ-383, AZ-257, AZ-020 – have hypolipidemic and hepatoprotective properties, which manifested in the reduced levels of liver biochemical markers of blood, which had increased after simulating metabolic disorders, in the reduced concentration of total cholesterol and triglycerides in blood, in the normalization of liver microarchitecture, as well as in the proliferative activity of hepatocytes.

Discussion: The hepatoprotective and hypolypidemic properties of the new derivatives of cyanothioacetamide with a dihydropyridine framework, which were determined while conducting the experiment, can be accounted for by their effects on the biotargets, identified for AZ-383, AZ-257, and AZ-020 *in silico*. According to the results, the most pronounced hepatoprotective activity was found in AZ-383 (intragastrically, 1 mg/kg for 14 days). The Ki-67 proliferation index under the influence of this compound was registered at the level of 1.48±0.03%, which exceeds this indicator in the control animals and proves a significant hepatoprotective activity of AZ-383.

Conclusion: The results show good prospects and high efficacy of the new studied cyanothioacetamide derivatives, such as AZ-383, AZ-257, AZ-020 (in a dose of 1 mg/kg), in terms of comprehensive correction of metabolic disorders. Further study is needed for this class of compounds.

Graphical abstract



Keywords

new cyanothioacetamide derivatives with a dihydropyridine framework, metabolic disorders, hyperlipidemia, hepatoprotective activity, pharmacological correction

Introduction

Over the past decades, the problems of metabolic disorders, including excess body weight, obesity, hyperglycemia, dyslipidemia, as well as concomitant lesions of the hepatobiliary system (Amlaev and Dakhkilgova 2020; Dedov et al. 2021), have gained ground.

Disorders of lipid as well as carbohydrate metabolism are closely linked with the development of cardiovascular diseases and an increased risk of mortality due to them (Kozlovskaya et at. 2006; Wang et al. 2017; Ametov et al. 2018; Chaulin 2020; Chazova et al. 2021; Ray et al. 2022; Polyakova et al. 2023).

At present, the diet of people is becoming increasingly improper and high-fat, among other things due to more foodstuffs containing food palm oil, which are extremely cost-effective, have a long shelf-life and the optimal market quality (Tereshchuk et al. 2014).

Besides, an important fact to stress is an increased polypharmacy in pharmacotherapy of many diseases, especially in the elderly, the share of whom is annually growing according to demographic statistics (Kim and Drapkina 2022).

Speaking of a drug therapy, it is important to specifically mention the pandemic of a new coronavirus infection, which began in 2019 and led to a prescription of a wide range of glucocorticoids, and, therefore, a possible increase in the unwanted reactions – disorders of carbohydrate and lipid metabolism (Lim et al. 2021). Along with their pronounced anti-inflammatory effect, glucocorticoids cause a disorder of gluconeogenesis processes in the liver, the induction of insulin resistance of peripheral tissues, a change in the action of insulin at the receptor and post-receptor levels, and contribute to the development of dyslipidemia. An excessive amount of glucocorticoids stimulates lipogenesis, changes the secretion of adipokines, and stimulates the differentiation of adipocytes, which contributes to the generation of new adipose tissue cells (Liu et al. 2013; Amlaev 2021).

It should be noted that during the clinical course of the COVID-19-related infection, the liver damage was found in 2/3 of the patients, especially in those in serious or critical conditions, the therapy for whom reasonably included glucocorticoids.

Taking into account the above pathogenetic links, it can be said that disorders of lipid and carbohydrate metabolism can happen both against the background of alimentary factors and after taking certain drugs, including glucocorticoids. These exogenous effects can also have a negative impact on the liver, causing the development of hepatobiliary pathology.

Metabolic disorders are the cause of the development of both acute and chronic diffuse liver diseases (Balukova et al. 2018; Meldekhanov et al. 2019). Hepatobiliary pathology can result from taking medications, including glucocorticoids, improper and high-fat diet, and excessive use of alcohol -containing drinks (Balukova et al. 2018). For instance, mortality from drug-induced liver damage in the world, according to various sources, is from 5 to 12% (Balukova et al. 2018). In Europe and the USA, side effects of drugs cause jaundice in 2-5% of hospitalized patients and induce 40% of acute hepatitis in patients over 40 years old and 13-25% of cases of fulminant hepatic failure (Meldekhanov et al. 2019).

Currently, of great relevance are the exotoxic lesion of the hepatobiliary system and the concomitant morphological alterations of the liver (Balukova et al. 2018; Meldekhanov et al. 2019). The morphological alterations of the liver are based on the accumulation of fibrous tissue with the subsequent transformation of the pathological process into cirrhosis and the further development of hepatic failure and portal hypertension. These diseases have an extremely poor outlook and cause temporary and persistent disability, as well as deaths of patients of various age groups (Meldekhanov et al. 2019; Litvinova et al. 2021).

There are identified two variants of toxic effects on the liver. The first variant implies therapeutic moieties either directly affecting the structures of hepatocytes, most frequently damaging their cell membranes, or affecting metabolic processes in the liver tissue. The second variant involves the appearance of antibodies resulting from the combination of protein and metabolites of drugs that cause hypersensitivity reaction and, as a result, an idiosyncratic liver damage (Balukova et al. 2018; Meldekhanov et al. 2019; Chulanova et al. 2024).

In this regard, it is relevant to study the features of lipid and carbohydrate metabolism, biochemical and morphological liver alterations after high-fat and dexamethasone loads, as well as to search for new effective drugs with hypolipidemic, hypoglycemic, and hepatoprotective properties which make it possible to restore the liver structure after the exotoxic impact.

In this direction, new heterocyclic compounds with a dihydropyridine framework from some derivatives of cyanothioacetamide are of scholarly interest (Litvinov 2003; Dyachenko et al. 2018). Some papers state that a number of cyanothioacetamide derivatives are characterized by pronounced antivirus properties (Osolodkin et al. 2013). The derivatives of tetrahydropyridines and hexahydroquinolines have certain anti-inflammatory, antidepressant and analeptic effects, exceeding those in the reference drugs (Bibik et al. 2017). There are reasons to assume that biologically active compounds of a similar chemical structure will be in high demand by rheumatologists, neurologists, combustiologists, and surgeons as agents inhibiting autotoxin (Rice and Turpin 1996) and the β-amyloid peptide formation (Mullan et al. 2007).

The drugs currently used in real clinical practice to correct metabolic disorders, including hypolipidemic and hypoglucemic drugs, drugs for treating obesity, along with their positive effect on carbohydrate or lipid metabolism, are associated with a number of adverse reactions. In addition, their use does not cover the whole pathogenetic range of the development of metabolic disorders, and often does not lead to hepatoprotective activity, which is an important aspect of a comprehensive pharmacological correction (Druk and Ryapolova 2016; Vatutin et al. 2020; Druzhilov et al. 2021; Medvedeva et al. 2023). This fact makes it imperative to look for new agents with hypolipidemic, hypoglycemic and hepatoprotective properties that can benefit body weight.

All the above means that is vital to study the characteristics of lipid and carbohydrate metabolism, biochemical and morphological alterations of the liver under the influence of the well-known antihyperglycemic agents from groups of biguanides and DPP-4 inhibitors, as well as new heterocyclic compounds with a dihydropyridine framework from a number of cyanothioacetamide derivatives after simulating an alimentary load and administering glucocorticoids.

The aim of this research was to study new synthesized compounds with a dihydropyridine framework under laboratory codes AZ-383, AZ-257, AZ-020 as promising hypolipidemic and hepatoprotective agents on the model of metabolic disorders in Wistar rats.

Materials and Methods

Theoretical background

From the library, including over 300 representatives of new heterocyclic samples synthesized on the basis of the ChemEx Research Laboratory of Vladimir Dahl Lugansk State University, three compounds with a dihydropyridine framework were selected from a number of cyanothioacetamide derivatives.

The selection was made using the Swiss Target Prediction software developed by scientists from Swiss Institute of Bioinformatics [http://swisstargetprediction.ch/index.php] and on-line resources: Online SMILES Translator and Structure File Generator by U.S. National Cancer Institute [https://cactus.nci.nih.gov/translate/],

OPSIN: Open Parser for Systematic IUPAC Nomenclature by The University of Cambridge, Centre for Molecular Informatics [https://opsin.ch.cam.ac.uk/]). This way, the compounds under laboratory codes AZ-383, AZ-257, and AZ-020 were selected, which turned out to be the most promising in terms of correction of metabolic disorders and the presence of hepatoprotective activity, with the intended biotargets in view.

According to the results of the *in silico* pre-screening, the biological targets of these compounds with a dihydropyridine framework were the following: AZ-383 is highly likely to affect orexin 1- and 2-type receptors; G protein-coupled bile acid receptors 1; glycogen synthase kinase 3; nicotinamide phosphoribosyltransferase, and A1, A2a, A2b adenosine receptors.

A derivative of 1,4-dihydropyridine under laboratory code AZ-257 is presumed to influence: glucose-dependent insulinotropic receptors, glucokinase, somatostatin 3 receptors, ceramide glucosyltransferase, CB1 cannabinoid receptors, and A1 adenosine receptors.

A compound from a range of derivatives of thieno[2,3-b]quinoline under laboratory code AZ-020 had a positive effect on: neuropeptide Y 5 receptors, glucokinase, ceramide glucosyltransferase, acetyl coenzyme carboxylase, ghrelin receptors, HSP 90-alpha, and A2a adenosine receptors.

These biotargets identified *in silico* for the studied samples are promising in relation to the potential correction of *in vivo* disorders of lipid and carbohydrate metabolism, as well as organ-protective effects on the liver.

Animals

The design of the experiment was considered and approved at a meeting of the Ethical Committee of Voronezh State Medical University named after N.N. Burdenko of the Ministry of Health of the Russian Federation, Minutes No. 5 dated 18 October, 2022.

The experiment was performed at the Research Institute of Experimental Biology and Medicine (RIEBM) of Voronezh State Medical University named after N.N. Burdenko of the Ministry of Health of the Russian Federation on 56 sexually mature Wistar rats. The rats were obtained from the Stolbovaya nursery branch of the Federal State Budgetary Institution of Science "Researcg Center for Biomedical Technologies of the Federal Medical and Biological Agency" of Russia. Upon arrival at RIEBM, the animals underwent adaptation for 14 days. All the rats had the initial weight of 234.9±5 g and were kept in plastic cells, 4 animals in each, in the conditions of a natural lighting regime at a temperature of 22-24ºC and relative air humidity of 40-50%.

All the manipulations with the laboratory animals were carried out in accordance with the principles of bioethics, good laboratory practice (GLP), the requirements of the Federal Law of the Russian Federation of 14 may 1993 N 4979-1 "On Veterinary Medicine" (as amended on 2 July 2021), Directive 2010/63/EU of the European Parliament and the Council of the European Union "On the Protection of Animals Used for Scientific Purposes", GOST No. 33216-2014 "Guidelines for the Maintenance and Care of Laboratory Animals. Rules for Maintenance and Care of Laboratory Rodents and Rabbits", GOST 33215-2014 "Guidelines for Maintenance and Care of Laboratory Animals. Rules for Equipping Premises and Organizing Procedures", and GOST 33044-2014 "Principles of Good Laboratory Practice".

The study was carried out in the autumn to exclude the influence of seasonal rhythms on the animals. Every day, the behavior and general condition of the animals were monitored. All the studies were carried out in accordance with the guidelines for preclinical trials of drugs (Guidelines for Experimental (Preclinical) Trials of New Pharmacological Substances, Moscow, 2005; Guidelines for Conducting Preclinical Trials of Drugs, Moscow, 2012).

At the first stage, sexually mature males of Wistar rats were selected for the studies, without external signs of diseases and anatomical defects and which had gone through the required quarantine and adaptation periods at RIEMB of Voronezh State Medical University named after N.N. Burdenko of the Ministry of Health of the Russian Federation. The studies involved the experimental animals subjected to simulation of metabolic disorders by giving them excess palm oil in the amount of 30 mg/kg of weight, additionally to the daily diet, for 8 weeks and subsequent intraperitoneal administration of 0.125 mg/kg dexamethasone for 13 days.

The animals the weight of which was more than 50 g different, as well as female animals, were not included in the experiment.

The animals with aggressive behavior and injured animals were excluded from the experiment.

The animals were randomized through envelope randomization.

Research design

The research design involved the simulation of metabolic disorders and their subsequent pharmacological correction by administering new compounds with a dihydropyridine framework and the reference drugs. The influence of new cyanothioacetamides under laboratory codes AZ-383, AZ-257, and AZ-020 was evaluated by their hypoglycemic, hypolipidemic, hepatoprotective activity, and their effect on the structural and functional organization of the liver.

Seven groups of 8 animals each were formed: intact – rats received a standard daily diet and water ad libitum; control – animals in addition to the daily diet received excess palm oil for 8 weeks, then 0.125 mg/kg dexamethasone was administered intraperitoneally for 13 days; comparison groups No. 1 and No. 2 – after the formation of metabolic disorders through a high-fat diet and steroid load, animals were orally administered with metformin in the amount of 300 mg/kg per body weight and vildagliptin in a dose of 8 mg/kg per body weight, respectively, for 14 days; experimental groups No. 1, No. 2, and No. 3 included the animals that after the formation of metabolic disorders (high-fat load for 8 weeks and the administration of dexamethasone for 13 days) received AZ-383, AZ-257, AZ -020, respectively, for pharmacological correction, in a dose of 1 mg/kg per body weight. The duration of the pharmacological correction stage with new derivatives of alpha-cyanothioacetamide was 14 days.

After sacrificing the animals from the experiment, blood tests were conducted to determine the concentration of total cholesterol, triglycerides, ALT, AST, total bilirubin, and glucose.

In addition, parts of the liver biomaterial were

sampled according to standard sampling methods. Then, 4-µm thick sections were made for staining with Gill's hematoxilin and eosin. Microslides were evaluated on a software and hardware complex for biological research with an imaging system based on an upright research microscope ZEISS Axo Imager.A2. The sample representativeness was achieved by assessing at least 40 visual fields.

For immunohistochemical analysis, 2-µm thick sections of the liver were made. Ki-67 (SP6) immunohistochemical detection was performed by rabbit monoclonal antibodies (Cat#MA5-14520, USA, dilution 1:250).

The hepatoprotective activity of the new derivatives of cyanothioacetamide was evaluated by determining the average number of hepatocytes in the visual field, the average size of hepatocytes, the area of the cytoplasm and hepatocyte nuclei, and the calculation of a nuclear/cytoplasmic ration. In addition, the morphological pictures of liver sections of various animal groups, as well as the hepatocyte proliferation index, were analyzed.

The studied compounds

The samples of new cyanothioacetamide derivatives with a dihydropyridine framework were pre-selected according to the results of virtual bioscreeing. The structures of the compounds are shown to Figure 1.

Statistical analysis

For medical studies, the confidence level was adopted at $p = 0.95$ and $p = 0.98$. The number of replicate measurements n in the series of experiments with $p = 0.95$ (the significance level a = 0.05) and the allowable error $\varepsilon = \pm 3\,\sigma$ was adopted at 3, and for $p = 0.98$–n = 4.

The necessary sample size according to the online calculator (medstatistic.ru/calculators/calcsize.html) for studies with an accuracy of 0.3 at $p = 0.95$ and the confidence coefficient $t = 2.0$ is at least 44, and at $p = 0.98$ and the confidence coefficient $t = 2.2$ – at least 70.

The results of the experiment were statistically processed using the using the recognized methods of mathematical statistics, characterizing quantitative variability. When processing the experimental data, the average arithmetic (AA) was determined. The statistical significance of the compared options was determined on the basis of the Student's t-test (basing on the online-resource https://medstatistic.ru/calculators/averagestudent.html), with the critical value of the Student's t-test equal to 2.101 and the significance level α = 0.05.

Discard data assessment and data smoothing were carried out on the basis of a confidence interval; the size of which for the experimental groups was determined according to AA and the sample size.

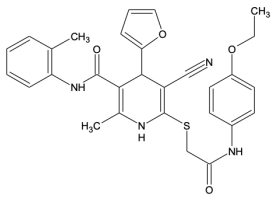
Results

The obtained biochemical indicators of blood of the experimental animals, under the combined sequential effect of alimentary and glucocorticoid loads, as well as the efficacy results of pharmacological correction of metabolic disorders, are represented in Table 1.

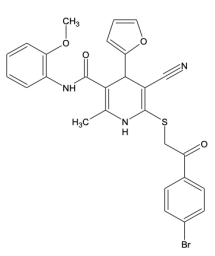
The combined sequential simulation of alimentary obesity and steroid diabetes was reflected in disorders of carbohydrate and lipid metabolism, and hepatotoxic effects. For instance, aminotransferases: ALT and AST – increased by 77% and 31%, respectively. The levels of total bilirubin increased by 118%, glucose – by 45%, total cholesterol – by 54%, and triglycerides – by 171%.

Further pharmacological correction, following the period of modeling metabolic disorders, led to a decrease in the laboratory indicators under study. The dynamics of normalization of the indicators in response to the drugs was different. The ALT level in all the comparison and experimental groups reached the indicators in intact animals. AST under the action of vildagliptin, as well as new derivatives of cyanothioacetamide, reached the targets, whereas in the treatment of metformin it tended to decrease, but remained at a level exceeding the indicators in intact rats by 24%. The best dynamics in normalizing the level of total bilirubin was demonstrated by metformin, vildagliptin and a new heterocyclic compound AZ-383. In the experimental groups treated for metabolic disorders with compounds AZ-257 and AZ-020, the level of total bilirubin steadily tended to decrease, but failed to reach the targeted intact values.

AZ-383
5-cyano-6-({2-[(4-ethoxyphenyl)amino]-2-oxoethyl}thio)-4-(2-furyl)-2-methyl-N-(2-methylphenyl)-1,4-dihydropyridine-3-carboxamide



AZ-257
6-{[2-(4-bromophenyl)-2-oxoethyl]thio}-5-cyano-4-(2-furyl)-N-(2-methoxyphenyl)-2-methyl-1,4-dihydropyridine-3-carboxamide



AZ-020
3-amino-N-(3-methoxyphenyl)-4-(5-methyl-2-furyl)-5,6,7,8-tetrahydrothieno[2,3-b]quinoline-2-carboxamide

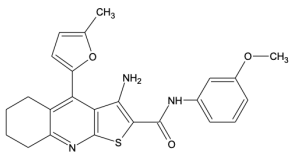


Figure 1. Graphic formulas of the compounds from among cyanothioacetamide derivatives.

Table 1. The results of the biochemical analysis of blood of the experimental Wistar rats upon completion of the modeling of all the stages of the experiment and the correction of metabolic disorders

Indicator	ALT, u/L	AST, u/L	Total bilirubin, µmol/L	Glucose, µmol/L	Total cholesterol, µmol /L	Triglycerides, µmol /L
Intact group (standard daily diet)	$p = 0.5$ x_{av}=.59. Me = 59.6 σ (S) = 8.23 V, % = 13.8	$p = 0.37$ x_{av} = 146.5 Me=147.7 σ(S)=16.83 V, %=11.5	$p = 0.89$ x_{av} = 10.9 Me = 10.9 σ (S) = 6.44 V, % = 59.1	$p = 0.52$ x_{av} = 7.9 Me = 7.9 σ (S) = 0.65 V, % = 8.20	$p = 0.82$ x_{av} = 1.28 Me = 1.25 σ (S) = 0.21 V, % = 16.4	$p = 0.26$ x_{av} = 0.67 Me = 0.62 σ (S) = 0.27 V, % = 40.3
Control group (excess palm oil and dexamethasone)	$p = 0.09$ x_{av} = 105.3* Me = 108.9 σ (S) = 9.91 V, % = 9.40	$p = 0.65$ x_{av} =192.6* Me =190.5 σ (S) =8.97 V, %=4.60	$p = 0.86$ x_{av} = 23.7* Me = 23.6 σ (S) = 3.46 V, % = 13.6	$p = 0.52$ x_{av} = 11.4* Me = 11.8 σ (S) = 1.09 V, % = 9.60	$p = 0.56$ x_{av} = 2.0* Me = 2.1 σ (S) = 0.22 V, % = 11.0	$p = 0.31$ x_{av} = 1.91* Me = 2.01 σ (S) = 0.37 V, % = 19.4
Comparison group №1 (Metformin)	$p = 0.43$ x_{av} = 57.5 Me = 55.9 σ (S) = 6.61 V, % = 11.5	$p = 0.34$ x_{av}=181.7* Me =180.0 σ (S) =10.9 V, % = 6.0	$p = 0.43$ x_{av} = 10.7 Me = 10.7 σ (S) = 1.12 V, % = 10.5	$p = 0.40$ x_{av} = 7.3 Me = 7.3 σ (S) = 0.55 V, % = 7.5	$p = 0.02$ x_{av} = 1.6 Me = 1.6 σ (S) = 0.19 V, % = 11.9	$p = 0.15$ x_{av} = 0.88 Me = 0.83 σ (S) = 0.24 V, % = 0.27
Comparison group №2 (Vildagliptin)	$p = 1.00$ x_{av} = 53.2 Me = 53.8 σ (S) = 6.39 V, % = 12.0	$p = 0.91$ x_{av} = 156.2 Me =156.6 σ (S) =8.53 V, %=5.50	$p = 0.14$ x_{av} = 11.9 Me = 12.5 σ (S) = 1.46 V, % = 12.3	$p = 0.52$ x_{av} = 7.9 Me = 7.9 σ (S) = 0.36 V, % = 4.5	$p = 0.28$ x_{av} = 1.63 Me = 1.65 σ (S) = 0.16 V, % = 9.80	$p = 0.02$ x_{av} = 0.58 Me = 0.52 σ (S) = 0.20 V, % = 34.5
Experimental group №1 (AZ-383)	$p = 0.15$ x_{av} = 56.8 Me = 54.0 σ (S) = 6.56 V, % = 11.5	$p = 0.61$ x_{av} = 155.9 Me =157.8 σ (S) =14.5 V, %=9.30	$p = 0.38$ x_{av} = 11.9 Me = 11.8 σ (S) = 0.69 V, % = 5.80	$p = 0.44$ x_{av} = 7.9 Me = 7.9 σ (S) = 0.40 V, % = 5.1	$p = 0.40$ x_{av} = 1.36 Me = 1.35 σ (S) = 0.11 V, % = 8.1	$p = 0.36$ x_{av} = 0.74 Me = 0.76 σ (S) = 0.24 V, % = 32.4
Experimental group №2 (AZ-257)	$p = 0.91$ x_{av} = 55.8 Me = 54.8 σ (S) = 5.81 V, % = 10.4	$p = 0.63$ x_{av} = 145.3 Me =144.8 σ (S) =12.7 V, %=8.70	$p = 0.56$ x_{av} = 12.5 Me = 12.7 σ (S) = 1.13 V, % = 9.0	$p = 0.22$ x_{av} = 8.6 Me = 8.7 σ (S) = 0.57 V, % = 6.6	$p = 0.57$ x_{av} = 1.19 Me = 1.20 σ (S) = 0.14 V, % = 11.8	$p = 0.50$ x_{av} = 0.66 Me = 0.68 σ (S) = 0.16 V, % = 24.2
Experimental group №3 (AZ-020)	$p = 0.14$ x_{av} = 53.9 Me = 51.6 σ (S) = 5.08 V, % = 9.4	$p = 0.22$ x_{av} = 138.2 Me =134.5 σ (S) =13.3 V, % = 9.6	$p = 0.94$ x_{av} = 12.3 Me = 12.2 σ (S) = 0.84 V, % = 6.8	$p = 0.97$ x_{av} = 8.3 Me = 8.2 σ (S) = 0.53 V, % = 6.4	$p = 0.69$ x_{av} = 1.46 Me = 1.45 σ (S) = 0.21 V, % = 14.4	$p = 0.06$ x_{av} = 0.77 Me = 0.71 σ (S) = 0.33 V, % = 42.8

***Note*:** The normality the distribution at the significance level α = 0.05 and p > α; x_{av} – average value; Me – median; σ (s) – standard deviation; V – coefficient of variation; * – significant differences in comparison with the group of intact animals.

The most pronounced hypoglycemic effect was exerted by metformin, vildagliptin and compound under the laboratory code AZ-383. Under the influence of new cyanothioacetamide derivatives, the levels of total cholesterol and triglycerides decreased to reach the targeted intact values, which demonstrates the hypolypidemic activity of new compounds under laboratory codes AZ-383, AZ-257, and AZ-020.

When studying the micrographs of the liver of the rats which had received excess palm oil for 8 weeks in addition to the daily diet and a subsequent dexamethasone load, a number of defects were discovered to affect both the parenchyma and the organ stroma. The liver structure of the control rats was damaged (Fig. 2). No hepatic cords, hepatic lobules, sinusoids, or bile ducts were identified. There are signs of marked liver dystrophy with the rearrangement of its lobular structure. Boundaries of individual hepatocytes per field of microscope were vague, and the nuclei are deformed with signs of anisocytosis. Intact liver cells had a polygonal shape and a round nucleus. In liver tissue of the rats of the rats of the control group with a combined effect leading to alimentary obesity and steroid diabetes, phenomena of hydropic and adipose degeneration, as well as necrosis, were detected in all the samples.

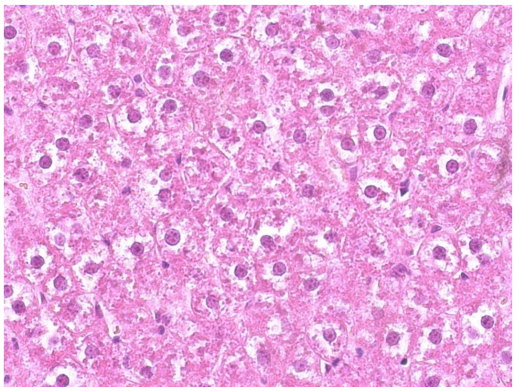


Figure 2. Liver of Wistar control animals with simulated metabolic disorders through alimentary and dexamethason loads (x400, stained with Gill's hematoxylin-eosin).

As can be seen in Fig. 3, the liver of rats of the comparison group that had received metformin to pharmacologically correct metabolic disorders was characterized by broken boundaries of hepatic lobules. Hepatocyte membranes were difficult to identify, in some areas they were hardly visible. In hepatic lobules, focal destruction of hepatocytes with lesion of cell membrane and nuclei is detected. In the cytoplasm of hepatocytes, vacuoles of various sizes can be seen. The foci of hepatocytic necrosis with karyo- and plasmopyknosis phenomena were detected.

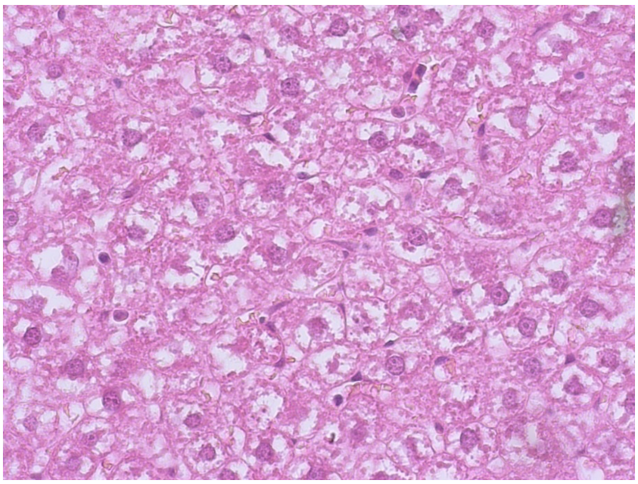


Figure 3. Liver of Wistar rats of the comparison group which received metformin for pharmacological correction of simulated metabolic disorders (x400, stained with Gill's hematoxylin-eosin).

On histological slices of the studied detox organ of the comparison group rats which had received vildagliptin, the cord structure of the liver was preserved. Sinusoids were visible, with formed elements and a typical structure (Fig. 4). Hepatocytes were of the correct shape, with occasional signs of hydropic degeneration.

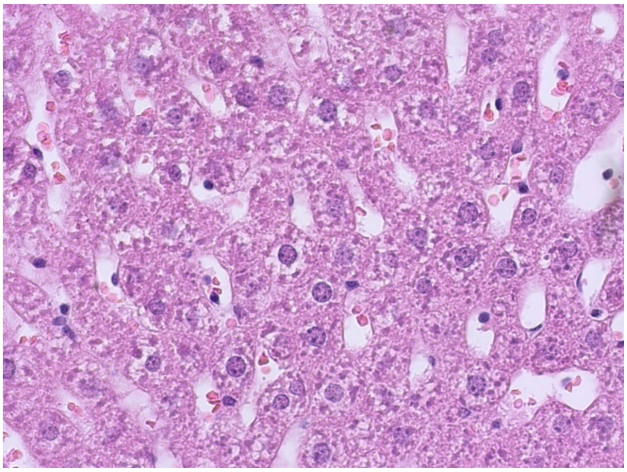


Figure 4. Liver of Wistar rats of the comparison group which received vildagliptin for pharmacological correction of simulated metabolic disorders (x400, stained with Gill's hematoxylin-eosin).

On the liver parenchyma sections of rats of the experimental group which received new derivatives of cyanothiacetamide for pharmacological correction of a number of simulated metabolic disorders, hepatic cords can be clearly seen. Hepatocytes had a polygonal shape,with discernible cytoplasmic membrane. Sinusoids looked like narrow slit-shaped spaces between the hepatic cords, inside of which red blood cells could be identified. Anisocytosis of nuclei was found occasionally' the zones of necrosis and hemorrhages along the periphery of the lobules were significantly fewer. The number of areas of hydropic degeneration and granular degeneration decreased, and cytoplasm had a large number of glycogen inclusions in the form of oval bright pink bodies, unevenly clustered. Round or oval nuclei with one or two nucleoli were located closer to the center of cells and had a well-colored nuclear cell membrane (Fig. 5).

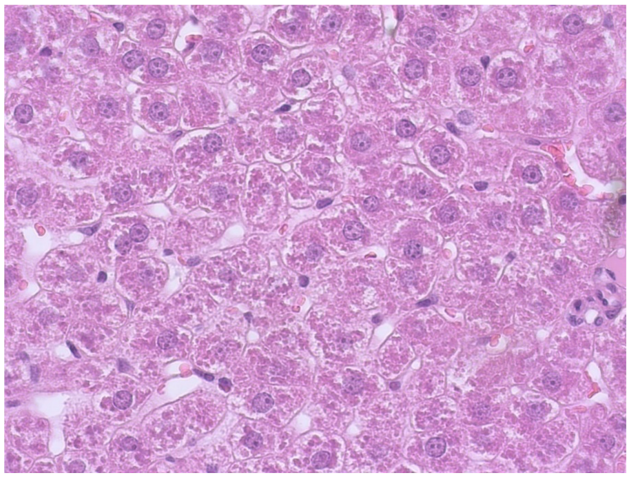


Figure. 5. Liver of Wistar rats of the experimental group which received a new derivative of cyanothioacetamide AZ-383 for pharmacological correction of simulated metabolic disorders (x400, stained with Gill's hematoxylin-eosin).

When comparing the obtained morphometric data, it is clear that the alimentary and dexamethasone loads in the control rats led to a 6.7% increase in the average number of hepatocytes per field of vision relative to the indicators in the intact group. Moreover, the simulated metabolic disorders led to a 11.3% increase in the average size of hepatocytes, a 7.6% increase in their cytoplasm area and a 40% increase in the area of hepatocyte nuclei. As a consequence, the disruption of nuclear-cytoplasmic relationships increased by 30% compared to the indicator of the intact group (Table 2).

Pharmacological correction of metabolic disorders by means of metformin, vildagliptin, and compounds under laboratory codes AZ-383 and AZ-020 led to an increase in the number of hepatocytes by 4.6%, 12%, 17.8%, and 4.7%, respectively, relative to the value of the control group.

The size of hepatocytes under the influence of metformin, vildagliptin, compounds under laboratory codes AZ-383, AZ-257, and AZ-020 statistically reliably reduced by 4%, 4%, 7%, 4.7%, and 4%, respectively, compared with that in the control group animals. The maximum approximation of this morphometric indicator of the liver to the values in the intact group was registered in rats which had received a new dihydropyridine derivative under laboratory code AZ-383.

Also, the results of the experiment showed that at the stage of pharmacological correction there was a decrease in the area of cytoplasm and nuclei of hepatocytes after their increase in simulated metabolic disorders. The most significant decrease in the area of cytoplasm and nuclei of hepatocytes was achieved when using compound under laboratory code AZ-383, with the hepatocyte cytoplasm

Table 2. Morphometric parameters of the liver of rats with a high-fat and steroid load during pharmacocorrection with hypoglycemic agents and new cyanothioacetamide derivatives

Indicator	Group						
	Intact	Control	Comparison group №1	Comparison group №2	Experimental group №1	Experimental group №2	Experimental group №3
Number of hepatocytes per field of vision	40.92 ± 1.04	43.68 ± 0.98*	45.7 ± 0.96*	48.92 ± 1.08*	51.46 ± 1.09*	43.9 ± 1.05*	45.72 ± 0.93*
Cytoplasm area of hepatocytes (μm²)	72.35 ± 0.39	77.82 ± 0.27*	75.9 ± 0.34*	76.19 ± 0.38*	73.79 ± 0.29*	75.68 ± 0.3*	76.04 ± 0.35*
Area of hepatocyte nuclei (μm²)	9.24 ± 0.2	12.97 ± 0.21*	11.2 ± 0.25*	10.98 ± 0.2*	10.54 ± 0.22*	10.86 ± 0.3*	11.13 ± 0.24*
Hepatocyte size (μm²)	81.59 ± 0.59	90.79 ± 0.48*	87.2 ± 0.59*	87.17 ± 0.58*	84.33 ± 0.51*	86.54 ± 0.6*	87.17 ± 0.59*
Nuclear-cytoplasmic relationships	0.13 ± 0.004	0.17 ± 0.004*	0.15 ± 0.003*	0.14 ± 0.003*	0.14 ± 0.004*	0.14 ± 0.003*	0.15 ± 0.005*

***Note*: * – p<0.05 when compared with the intact animals.**

area reducing by 5.2% and the hepatocyte nuclei area by 18.7%, respectively, relative to the values registered in the parenchyma of the liver in the control group rats.

The nuclear-cytoplasmic relationship under the influence of the comparison drugs and new compounds from among cyanothioacetamide derivatives steadily tended to decrease when compared to the indicator of the control group. For instance, pharmacological correction using metformin and compound AZ-020 resulted in a decrease in this relationship by 11.8%. The use of new heterocyclic compounds under laboratory codes AZ-383, AZ-257 and vildagliptin was accompanied by a decrease in the nuclear-cytoplasmic relationship by 17.6%.

When evaluating the Ki-67 proliferation index in the liver, calculated as the percentage of Ki-67-positive nuclei of the total number of hepatocytes, a sudden (4.46-time) inhibition of the hepatocyte proliferation index was recorded on the liver sections of the control group rats, which had received a high-fat load and dexamethasone, when compared to the indicators in the intact animals, as can be seen in Table 3.

Table 3. Immunohistochemical indicators of liver tissue of rats of different experimental groups

Group	Average number of hepatocytes per field of vision	Average number of Ki-67-positive nuclei per field of vision	Proliferation index
Intact group	40.92 ± 1.04	0.75 ± 0.04	1.83 ± 0.04%
Control group	43.68 ± 0.98*	0.18 ± 0.03*	0.41 ± 0.03%*
Comparison group №1	45.7 ± 0.96*	0.54 ± 0.04*	1.18 ± 0.04%*
Comparison group №2	48.92 ± 1.08*	0.46 ± 0.02*	0.94 ± 0.02%*
Experimental group №1	51.46 ± 1.09*	0.76 ± 0.03	1.48 ± 0.03%*
Experimental group №2	43.9 ± 1.05*	0.42 ± 0.03*	0.96 ± 0.03%*
Experimental group №3	45.72 ± 0.93*	0.48 ± 0.04*	1.05 ± 0.04%*

Note: * – p<0.05 when compared with the intact animals.

The most pronounced proliferative activity of the liver is recorded in the rats, which, after a long alimentary and dexamethasone load, were administered for 14 days a new compound from among cyanothioacetamide derivatives under laboratory code AZ-383 (Fig. 6).

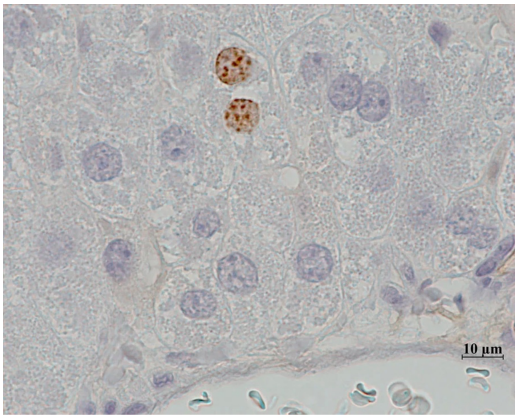


Figure 6. Ki-67-positive hepatocytes at the central vein of liver in microscopic examination of Wistar rats which received AZ-383 for pharmacological correction of simulated metabolic disorders.

Moreover, a high proliferation index (1.05 ± 0.04%) was recorded in the animals, in which pharmacological correction of metabolic disorders included compound AZ-020 for two weeks (Fig. 7).

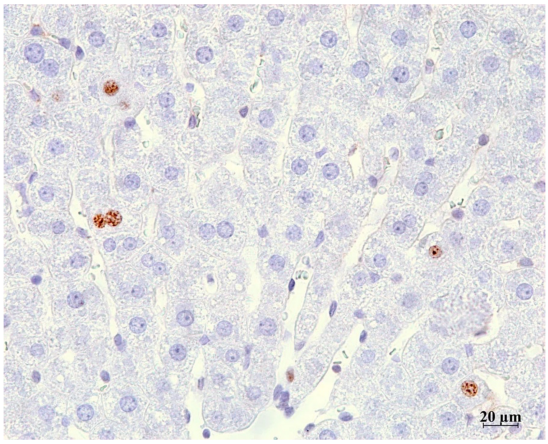


Figure 7. Ki-67-positive hepatocytes in the area of liver inflow in microscopic examination of Wistar rats which received AZ-020 for pharmacological correction of simulated metabolic disorders.

Discussion

Analyzing the biochemical, morphometric and immunohistochemical indicators of the liver function under conditions of an alimentary load and the intake of glucocorticoids, as well as the characteristics of lipid and carbohydrate metabolism, it is possible to notice the presence of hepatoprotective, hypolipidemic and hypoglycemic effects in new heterocyclic compounds under laboratory codes AZ-383, AZ-257, and AZ-020.

Discussing the obtained results, the identified hepatoprotective activity of the compounds under study can be accounted for by the expected biotargets for AZ-383, AZ-257 and AZ-020 discovered *in silico*. For all the compounds, a potential affinity for adenosine receptors was detected, the stimulation of which increases the level of antioxidant protection and contributes to the cytoprotective effect (Chaulin 2019; Muzyko and Perfilova 2022). As for a compound under laboratory code AZ-020, a tentative effect was also detected towards HSP 90-alpha, which have a protective effect on cells, including liver cells.

We link the hypolipidemic properties of the studied samples with a potential effect of compound AZ-383 on bile acid receptors coupled to G-protein 1, an impact of AZ-257 on ceramide glucosyltransferase, and an affinity of AZ-020 for ceramide glucosyltransferase, acetyl coenzyme carboxylase, ghrelin receptors, as well as for serine/threonine protein kinase mTOR.

In addition, there is a direct dependence between the influence of new cyanothioacetamide derivatives with a dihydropyridine framework on blood lipid metabolism and their effect on body weight, which involves the ability to reduce weight.

Bile acid receptors coupled to G-protein 1 according to various scientists are of great interest in the treatment of patients with metabolic syndrome, dyslipidemia, type 2 diabetes, and obesity (Zvenigorodskaya et al. 2016).

Bile acid biosynthesis is one of the important ways of cholesterol excretion. Bile acids are regulators of the expression of genes involved in the metabolism of primary bile acids, cholesterol and triglycerides in hepatocytes and plasma (Zvenigorodskaya et al. 2016). With insulin resistance and type 2 diabetes, the function of bile acids is disrupted, which leads to their decreased absorption, an increased fatty liver infiltration, and the accumulation of triglycerides and low-density lipoproteins. Stimulation of bile acid receptors coupled with G-protein 1 increases the production of insulin by β-cells of the pancreas and helps to reduce insulin resistance of peripheral tissues, to reduce body weight, and to normalize lipid metabolism through a hypocholesteremic effects (Zvenigorodskaya et al. 2016).

The biotargets found in AZ-257 and AZ-020 have a positive effect on lipid metabolism in blood. Ceramide glucosyltransferase is involved in the exchange of lipids by regulating the absorption of edible fats with intestinal endocytes. Acetyl coenzyme carboxylase acts at the intersection of the processes of synthesis and oxidation of lipids and is promising for the development of new methods of treating diabetes, obesity and other manifestations of the metabolic syndrome. A decrease in the activity of ghrelin receptors causes a decrease in appetite, an increase in the insulin level, and a change in the level of glucose and lipid metabolism. Serine/threonine protein kinase mTOR is also involved in carbohydrate and lipid metabolism.

Thus, the significantly pronounced hepatoprotective activity of the new studied compounds under codes AZ-383, AZ-257, and AZ-020 manifested in reduced biochemical hepatic markers of blood after their increase due to simulated metabolic disorders, in positive changes in microscopic and morphometric studies of the liver of rats of the experimental groups, and in the pronounced proliferative properties linked to the restoration of the main detox organ can be associated with the biotargets determined *in silico* and being promising in terms of hepatoprotection.

Hypolipidemic activity of the compounds studied in the experiment, which involves their ability to reduce the levels of total cholesterol and blood triglycerides, can also be explained by their effect on the biotargets involved in lipid metabolism.

Conclusion

The study of new derivatives of cyanothioacetamide with a dihydropyridine frame in the experiment on Wistar rats while simulating metabolic disorders demonstrated the presence of hepatoprotective, hypolipidemic and hypoglycemic activities in the studied compounds. Compounds under laboratory codes AZ-383, AZ-257, and AZ-020 contributed to a decrease in the levels of ALT, AST, total bilirubin in blood of the animals, which had increased after alimentary and dexamethasone loads in the experiment. The new compounds had a positive effect on the morphological and morphometric picture when studying the liver. The leader in hepatoprotective activity among the cyanothioacetamide derivatives was compound AZ-383, which contributed to the pronounced activation of the proliferative properties of the liver after its lesion by the exogenous factors we simulated. Besides, the new compounds under study had a positive effect on lipid metabolism, which was expressed in decreased levels of total cholesterol and blood triglycerides under the influence of AZ-383, AZ-257, and AZ-020 (intragastrical administration of 1 mg/kg for 14 days). The hepatoprotective and hypolipidemic properties of the new compounds with a dihydropyridine framework, which were detected *in vivo*, can be explained by the biotargets detected in them *in silico*, which are promising in terms of liver protection and a favorable effect on lipid and carbohydrate metabolism. The data obtained illustrate the need to further study the new cyanothioacetamide derivatives in terms of their effects on metabolic processes.

Conflict of interest

The authors declare no 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”).

Data availability

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

References

- Ametov AS, Kamynina LL, Litvinenko VM (2018) Hypoadiponectinemia is a marker of glucose and lipotoxicity in patients with type 2 diabetes mellitus and visceral obesity. Endocrinology: News. Opinions. Education 2 (23): 35–45. https://doi.org/10.24411/2304-9529-2018-12003 [PubMed] [PMC]
- Amlaev KR, Dakhkilgova KhT (2020) Obesity: Epidemiology, etiopathogenesis, comorbidity, diagnosis and treatment. Medical Bulletin of the North Caucasus 15 (3): 434–439. https://doi.org/10.14300/mnnc.2020.15104
- Amlaev KR (2021) Dyslipidemia: Epidemiology, clinical picture, diagnosis, prevention and treatment. Doctor [Vrach] 5: 16–20. https://doi.org/10.29296/25877305-2021-05-03 [in Russian]
- Balukova EV, Uspensky YuP, Fominykh YuA (2018) Liver lesions of various origins (toxic, drug-induced, dysmetabolic): from etiological heterogeneity to a single unified therapy for patients. RMJ. Medical Review [Russkij Medicinskij ZHurnal] 26 (1): 35–40. [in Russian]
- Bibik EY, Saphonova AA, Yeryomin AV, Frolov KA, Dotsenko VV, Krivokolysko SG (2017) Search for agents with anti-inflammatory activity among tetrahydropyrido[2,1-b][1,3,5]thiadiazine derivatives. Chemical-Pharmaceutical Journal 51(8): 16–19. https://doi.org/10.30906/0023-1134-2017-51-8-16-19
- Bibik EYu, Saphonova AA, Yeryomin AV et al. (2017) Study of analeptic activity of tetrahydropyrido [2,1-b] [1,3,5] tiadiazine derivatives. Research Result in Pharmacology 3(4): 20–25. https://doi.org/10.18413/2313-8971-2017-3-4-20-25
- Chaulin AM (2020) New groups of lipid-lowering drugs based on inhibition of proprotein convertase subtilisin-kexin type 9 (PCSK9). Part 1. Clinical Medicine [Klinicheskaya Medicina] 98 (11-12): 739–744. https://doi.org/10.30629/0023-2149-2020-98-11-12-739-744
- Chaulin AM (2019) Adenosine and its role in the physiology and pathology of the cardiovascular system. Cardiology: News. Opinions. Education 3(22): 37–45. https://doi.org/10.24411/2309-1908-2019-13004
- Chazova IE, Shestakova MV, Zhernakova YuV (2021) Eurasian recommendations for the prevention and treatment of cardiovascular diseases in patients with diabetes and prediabetes. Eurasian Cardiology Journal [Evrazijskij Kardiologicheskij ZHurnal] 2: 6–61. https://doi.org/10.38109/2225-1685-2021-2-6-61
- Chulanova AA, Smakhtina AM, Mal GS, Bobyntsev II, Mishina ES, Smakhtin MY, Kopeykin PM, Bogomolova EG (2024) Protective effects of thymogen analogues, modified by D-alanine, in hydrazine liver damage. Research Results in Pharmacology 10(2): 11–16. https://doi.org/10.18413/rrpharmacology.10.458
- Dedov II, Shestakova MV, Melnichenko GA (2021) Interdisciplinary clinical recommendations “Treatment of obesity and comorbid diseases”. Obesity and Metabolism [Ozhirenie i Metablizm] 1: 5–99. https://doi.org/0.14341/omet12714 [in Russian]
- Druzhilov MA, Kuznetsova TYu, Chumakova GA (2021) Promising directions for pharmacotherapy of obesity. Russian Journal of Cardiology [Rossijskij Kardiologicheskij ZHurnal] 26(3): 4279. https://doi.org/10.15829/1560-4071-2021-4279 [in Russian]
- Druk IV, Ryapolova EA (2016) Metformin: Updated recommendations and pleiotropic potential. Therapy [Terapiya] 4(8): 44–51. [in Russian]
- Dyachenko VD, Dyachenko IV, Nenaidenko VG (2018) Cyanothioacetamide is a multifunctional reagent with great synthetic capabilities. Advances in Chemistry 87(1): 1–27. https://doi.org/10.1070/RCR4760
- Kim OT, Drapkina OM (2022) The obesity epidemic through the prism of evolutionary processes. Cardiovascular Therapy and Prevention [Kardiovaskulyarnaya Terapiya i Profilaktika] 21(1): 72–79. https://doi.org/10.15829/1728-8800-2022-3109 [in Russian]
- Kozlovskaya SP, Konevalova NYu, Kozlovsky VI, Olenskaya TL (2006) Ezetimibe in the correction of hyperlipidemia. Bulletin of Vitebsk State Medical University [Vestnik Vitebskogo Gosudarstvennogo Medicinskogo Universiteta] 5(2): 11–16. [in Russian]
- Lim S, Bae JH, Kwon HS, Nauck MA (2021) COVID-19 and diabetes mellitus: from pathophysiology to clinical management. Nature Reviews Endocrinology 17 (1): 11–30. https://doi.org/10.1038/s41574-020-00435-4 [PubMed] [PMC]
- Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn ED, Leigh R, Brown JP, Cohen A, Kim H (2013) A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy, Asthma & Clinical Immunology 9: 30. https://doi.org/10.1186/1710-1492-9-30 [PubMed] [PMC]
- Litvinov VP (2003) Multicomponent cascade heterocyclization - a promising route for the targeted synthesis of polyfunctional pyridines. Advances in Chemistry [Uspekhi Himii] 72(1): 75–92. [in Russian]
- Litvinova ES, Konoplya NN, Shulginova AA, Kharchenko AV (2021) Proteins of allogeneic hepatocytes and pharmacological preparations for the correction of immunometabolic disorders in experimental liver pathology. Research Results in Pharmacology 7(2): 83–99. https://doi.org/10.3897/rrpharmacology.7.70314
- Medvedeva EA, Grigorenko EA, Mitkovskaya NP (2023) Innovative lipid-lowering therapy: experience of using inclisiran in the Republic of Belarus. Russian Journal of Cardiology [Rossijskij Kardiologicheskij ZHurnal] 28(4): 5417. https://doi.org/10.15829/1560-4071-2023-5417 [in Russian]
- Meldekhanov TT, Kuttybaev AD, Imanbekova ZhA, Terlikbaeva GA (2019) Toxic drug-induced liver damage. Bulletin of the Kazakh National Medical University [Vestnik Kazahskogo Nacional'nogo Medicinskogo Universiteta] 1: 63–66. [in Russian]
- Mullan MJ, Paris D, Pancham B (2007) Compounds and combinations thereof for inhibiting beta-amyloid production and methods of use thereof. Patent application public. 347 p.
- Muzyko EA, Perfilova VN (2022) The role of adenosine receptors of the A2A subtype in inflammation. Volgograd Scientific and Medical Journal 19(2): 5–11. [in Russian]
- Osolodkin DI, Kozlovskaya LI, Dueva EV, Dotsenko VV, Rogova YV, Frolov KA, Krivokolysko SG, Romanova EG, Morozov AS, Karganova GG, Palyulin VA, Pentkovski VM, Zefirov NS (2013) Inhibitors of tick-borne flavivirus reproduction from structure-based virtual screening. ACS Medicinal Chemistry Letters 4 (9): 869–874. https://doi.org/10.1021/ml400226s [PubMed] [PMC]
- Polyakova OA, Cheremushkin SV, Ostroumova OD (2023) Correction of hypertriglyceridemia as one of the basis for reducing residual cardiovascular risk: focus on fenofibrate. Cardiology: News, Opinions, Training [Kardiologiya: Novosti, Mneniya, Obuchenie] 11 (1): 42–50. https://doi.org/10.33029/2309-1908-2023-11-1-42-50 [in Russian]
- Ray KK, Reeskamp LF, Laufs U, Banach M, Mach F, Tokgözoğlu LS, Connolly DL, Gerrits AJ, Stroes ESG, Masana L, Kastelein JJP (2022) Combination lipid-lowering therapy as first-line strategy in very high-risk patients. European Heart Journal 43(8): 830–833. https://doi.org/10.1093/eurheartj/ehab718 [PubMed]
- Rice WG, Turpin JA (1996) Virus-encoded zinc fingers as targets for antiviral chemotherapy. Reviews in Medical Virology 39(19): 3606–3616. https://doi.org/10.1002/(SICI)1099-1654(199612)6:4<187::AID-RMV176>3.0.CO;2-F [PubMed]
- Tereshchuk LV, Mamontov AS, Starovoyova KV (2014) Palm oil fractionation products in the production of spreads. Equipment and Technology of Food Production [Tekhnika i Tekhnologiya Pishchevyh Proizvodstv] 3: 79–83. [in Russian]
- Vatutin NT, Taradin GG, Rakitskaya IV et al. (2020) Lipid-lowering therapy in special situations. Health, Food & Biotechnology 2(4): 12–27. https://doi.org/10.36107/hfb.2020.i4.s88 [in Russian]
- Wang AL, Iadecola C, Wang G (2017) New generations of dihydropyridines for treatment of hypertension. Journal of Geriatric Cardiology 14 (1): 67. https://doi.org/10.11909/j.issn.1671-5411.2017.01.006 [PubMed] [PMC]
- Zvenigorodskaya LA, Petrakov AV, Nilova TV, Varvanina GG, Lychkova AE (2016) The role of bile acids in the regulation of lipid and carbohydrate metabolism in patients with non-alcoholic fatty liver disease and type 2 diabetes. Experimental and Clinical Gastroenterology [Eksperimental'naya I Klinicheskaya Gastroenterologiya] 11(135): 31–34. [in Russian]

Author Contributions

- **Elena S. Ketova**, Candidate of Medical Sciences, endocrinologist, BestDoctor Ltd.; e-mail: ketova_elena@mail.ru; **ORCID ID** https://orcid.org/0000-0002-4595-8103. Conducting experiments, the analysis and interpretation of the data and the facts obtained.

- **Anna V. Myazina**, Candidate of Medical Sciences, Teaching assistant, Department of Normal Anatomy, V.I. Vernadsky Crimean Federal University; e-mail: anna.krasnodon2009@gmail.com; **ORCID ID** https://orcid.org/0000-0001-5736-2385. The analysis and interpretation of the data and the facts obtained.

- **Elena Yu. Bibik**, Doctor Habil. of Medical Sciences, Professor, Head of the Department of Fundamental and Clinical Pharmacology, Saint Luka Lugansk State Medical University; e-mail: helen_bibik@mail.ru; **ORCID ID** https://orcid.org/0000-0001-7470-608X. Coordination of research, planning and writing of the manuscript text.

- **Sergey G. Krivokolysko**, Doctor Habil. Chemical Sciences, Professor, Senior Researcher, Head of the Department of Pharmaceutical Chemistry and Pharmacognosy, Scientific Director of the ChemEx Research Laboratory, Vladimir Dahl Lugansk State University; e-mail: 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.