Manuscript copyright

SAVATEEVA
EKATERINA ANDREEVNA

SULFUR-CONTAINING HETEROCYCLES DERIVATIVES AS ANTIOXIDANTS AND NONENZYMATIC GLYCOSYLATION OF PROTEINS BLOCKERS FOR CORRECTION OF EXPERIMENTAL DIABETES MELLITUS

03.01.04 - biochemistry

EXTENDED ABSTRACT OF DISSERTATION
for scientific degree of candidate of Biological Sciences

Kazan, 2015

The work is made in Institute of Chemical Engineering of Federal State Autonomous Educational Institution of Higher Professional Education «Ural Federal University named after first President of Russia B.N. Yeltsin»

Scientific advisor: member of the Russian Academy of Sciences,
Doctor of Medical Sciences, professor
Chereshnev Valery Aleksandrovich
Ph. D. in Medicine
Emelyanov Victor Vladimirovich

Official opponents:

Tseilikman Vadim Eduardovich – Doctor of Biological Sciences, professor, head of department of biochemistry in the State Budget Educational Institution of Higher Professional Education «South Ural State Medical University», Chelyabinsk

Shumaev Konstantin Borisovich – Doctor of Biological Sciences, senior research assistant of biochemistry of nitrogen fixation and nitrogen metabolism laboratory, Institute of Biochemistry named after A.N. Bakh, Federal State Institution "Federal Research Center "Fundamental principles of Biotechnology" the Russian Academy of Sciences", Moscow

External reviewer:
Federal State Budget Institution "Endocrinology Research Center," of Ministry of Health of Russia, Moscow

Ph.D. defense will take place «24» September 2015 at 13.00 o'clock at the meeting of the Dissertation Committee D 212.081.08 of the FSAEI of HPE "Kazan (Privolzhsky) Federal University" at the address: 420008, Kazan, Karl Marx St., 74, aud.206. Phone: 7(843)23-37-842.

The dissertation is available at the library named after N. I. Lobachevsky of the FSAEI of HPE "Kazan (Privolzhsky) Federal University" at the address: 420008, Kazan, Kremlyovskaya St., 35.

The electronic version of the extended abstract of the dissertation is available on the official website of the FSAEI of HPE "Kazan (Privolzhsky) Federal University" www.kpfu.ru.

Extended abstract of dissertation dispatched «___» _____ 2015

Scientific Secretary of the Dissertation Committee, Doctor of Biological Sciences, professor  Abramova Z.I.

GENERAL DESCRIPTION OF THE WORK

Relevance of the work. Modern medicine is experiencing a need for new pharmaceuticals to treat diabetes mellitus (DM) – widespread and socially significant disease (Dedov I.I. Innovative technologies in the treatment and prevention of diabetes and its complications // Diabetes mellitus. 2013. Vol. 3. pp. 4 – 10). When searching for new antidiabetic agents, experimental modeling of the disease in animals is widely used. In order to optimize this stage of the search for antidiabetic agents, it is necessary to clearly imagine on what mechanisms the development of experimental and clinical DM a new compound is capable to influence.

Two pathological processes, realized in conditions of chronic hyperglycemia, could be attributed to the number of potential targets for antidiabetic agents - nonenzymatic glycosylation of proteins (NGP) and oxidative stress (Balabolkin M.I. Diabetology // M.: Medicine, 2000. 672 p.; Monnier V.M., Sell D.R. Prevention and repair of protein damage by the Maillard reaction *in vivo* // Rejuvenation Res. 2006. V. 9, № 2. P. 264 – 273; Aldini G., Vistoli G., Stefek M. et al. Molecular strategies to prevent, inhibit, and degrade advanced glycoxidation and advanced lipoxidation end products // Free Radical Research. 2013. V.47, (Suppl. 1). P. 93 – 137).

NGP is a spontaneous chemical reaction between the amino groups of the protein molecule and the carbonyl groups of monosaccharides and the subsequent conversion of the resulting compound, occurring without enzymes. Occurring changes disturb ionic interactions in protein molecules, changing the conformation, solubility, and therefore - the functional properties and sensitivity to proteases. It has been shown that enzymatically glycosylated *in vitro* insulin partially loses the ability to lower blood glucose and to stimulate glucose transport into cells when administered to animals (Hunter S. J., Boyd A. C., O'Harte F. P.M. et al. Demonstration of glycated insulin in human diabetic plasma and decreased biological activity assessed by euglycemic-hyperinsulinemic clamp technique in humans // Diabetes. 2003. V. 52. P. 492 – 498; McKillop A.M., Mooney M.H, Harriott P. et al. Evaluation of glycated insulin in diabetic animals using immunocytochemistry and radioimmunoassay // Biochem. Biophys. Res. Commun. 2011. V. 286. P. 524 – 528).

Complex of metabolic disorders typical for DM, leads to oxidative stress - imbalance between pro- and antioxidants, accumulation of products of free radical oxidation (FRO) of lipids, proteins, nucleic acids (Evans J.L., Maddux B.A., Goldfine I.D. et al. The molecular basis for oxidative stress-induced insulin resistances // Antioxid. Redox Signal. 2005. V. 7, № 7-8. pp. 1040-1052; Menshchikova E.B., Zenkov N.K., Lankin V.Z. et al. Oxidative stress: the pathological conditions and diseases // Novosibirsk: ARTA, 2008. 284 p.; Shumaev K.B., Gubkin S.A., Kumskova I.M. et al. The mechanism of formation of superoxide radical by reacting L-lysine with dicarbonyl compounds // Biochemistry. 2009. Vol. 74, №4. pp. 568-574). There is significant evidence of the effectiveness of sulfur-containing antioxidant and blocker of NGP – lipoic acid (LA) – in the treatment of DM and its complications (Stakhovskaya L.V., Guseva O. α-lipoic acid: pharmacological properties and clinical application. Review of the literature // Moscow, Russian State Humanitarian University, 2003. 63 p .; Ziegler D., Tritschler H.-J., Strokov I.A. Ametov A.S. et al. Treatment of diabetic polyneuropathy with thioctic acid (review) // Farmateka. 2008. V. 17, №171. pp 28 - 35; Shay K.P., Moreau R.F., Smith E.J. et al. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential // Biochim. Biophys. Acta. 2009. V. 1790. P. 1149 – 1160). However, it is an open question whether is possible to improve the results of treatment of DM by use of synthetic sulfur-containing compounds that have properties of antioxidants and blockers of NGP.

In this regard, attention is attracted to synthetic sulfur-containing compounds of class 1,3,4-thiadiazine (1,3,4-TD) and 2,4-substituted thiazoles. Have been reported about antioxidative and radioprotective activity of the representatives of these classes of compounds (Pfeiffer W.-D. 1,3,4-Oxadiazines and 1,3,4-Thiadiazines // Compr. Heterocycl. Chemistry III. 2008. V. 9. P. 401 – 455; Rasina L.N., Perova N.M., Novikova A.P., Chupakhin O.N. Structure, behavior in the body and biological activity of triazole derivatives // Proceedings of the First International Conference "Chemistry and Biological Activity of nitrogen heterocycles and alkaloids"; ed. V.G. Kartsev and G.A. Tolstikov. M., 2001. V.2. P.246). 2-aminothiazole and thiazolidinedione derivatives possess

antidiabetic activity (De S., Adhikari S., Tilak-Jain J. et al. Antioxidant activity of an aminothiazole compound: possible mechanisms // Chem. Biol. Interact. 2008. V. 173. P. 215 – 223; Spasov A.A., Petrov V.I., Cheplyaeva N.I. Lenskaya K.V. et al. Fundamental bases of the drugs research for the treatment of type 2 diabetes // Bulletin of Medical Sciences. 2013. № 2. pp. 43 - 49). It was shown that in aqueous solutions the 1,3,4-TD derivatives can be transformed into thiol derivatives, which according to our hypothesis, can bind glucose and NGP carbonyl intermediates, and possess antioxidant properties. However, antidiabetic activity of the 1,3,4-TD derivatives and 2,4-substituted triazoles have not previously been studied. The abovesaid defined the goal and objectives of this work.

The goal of the present work is evaluation of ability of the sulfur-containing heterocyclic derivatives to exhibit antioxidant activity, to block NGP and to correct the metabolic disorders in experimental DM.

Objectives:

1. To screen compound of classes 1,3,4-TD derivatives and substituted 2,4-thiazoles for ability to block reaction of nonenzymatic glycosylation of bovine serum albumin (BSA) with glucose *in vitro*.
2. To study the antioxidant activity of the 1,3,4-TD derivatives in a model of inhibition of ascorbic acid (AA) oxidation with atmospheric oxygen.
3. To evaluate the ratio of lipo- and hydrophilic properties of the of 1,3,4-TD derivatives with antioxidant and antiglycosylative activity in octanol/water.
4. To study the effect of the 1,3,4-TD derivatives and 2,4-substituted thiazoles on biochemical parameters of rats' blood and internal organs in alloxan DM.
5. To find out the relationship between the structure, lipophilic, antioxidant, antiglycosylation properties of the 1,3,4-TD derivatives and their ability to correct metabolic disorders in experimental DM.
6. To carry out a kinetic analysis of the reaction of NGP and its inhibition with reduced glutathione (G-SH) on the example of genetically engineered human insulin.

The present work was financially supported by Russian Foundation for Basic Research (RFBR grants № 12-04-31852_mol_a and RFBR –"Ural" № 10-04-96097) and "UrFU Development Programme for 2010 – 2020 years".

Scientific novelty. First shown that the 1,3,4-TD and 2,4-substituted thiazole derivatives are able to inhibit the accumulation of NGP products while incubating BSA with glucose. Established that the best antiglycosylation properties have 5-phenyl-1,3,4-TD derivatives with an oxygen-containing hetaryl or alkylamino substituent at 2-position.

The evaluation of lipophilicity of potential NGP inhibitors – 1,3,4-TD derivatives – was performed in octanol/water. Established that the partition coefficient $\lg K_{ow}$ for active NGP inhibitors was 2.90 - 3.19, while for the less active inhibitors was 3.14 - 4.02.

For the first time the ability of 1,3,4-TD derivatives to inhibit the oxidation of ascorbic acid with atmospheric oxygen is demonstrated. The most active compounds are N-32, TD-79, L-92, L-17, L-34.

For the first time in the model of alloxan DM in rats detected antidiabetic activity of 1,3,4-TD derivatives (L-17, L-14, N-32, L-31, L-91) and 2-guanidine-4-pyridinethiazole (2-G-4-PT). Studied 1,3,4-TD derivatives have the ability to correct hyperglycemia, accumulation of products of NGP in blood and internal organs, and also possess antioxidant activity. 2-G-4-PT decreases accumulation of products of NGP and of lipids FRO in the blood and the internal organs, without affecting blood glucose levels.

Proposed and experimentally confirmed the kinetic model of the process of nonenzymatic glycosylation of genetically engineered human insulin *in vitro*. Determined rate constants of the

forward and reverse reactions in the elementary step 2, the ratio of the rate constants of the forward and reverse reactions in the elementary step 1, and thermodynamic equilibrium constants of both steps.

It is found that in the range of physiological concentrations natural thiol G-SH has a dose-dependent ability to inhibit glycosylation of insulin with glucose *in vitro*, kinetic analysis of the process was performed.

Statements to be defended:

1,3,4-TD and 2,4-substituted thiazole derivatives are able to block reaction of NGP. The best antiglycosylation properties have 5-phenyl-1,3,4-TD derivatives with an oxygen-containing hetaryl or alkylamino substituent at 2-position, and having octanol/water partition coefficient lgK_{ow} in a range of 2.90 – 3.19.

1,3,4-TD derivatives are able to inhibit the oxidation of ascorbic acid with atmospheric oxygen. The most active are derivatives of 1,3,4-TD having morpholino substituent at the 2-position and thiophene, phenyl, p-fluorophenyl at 5-position.

The antioxidant and antiglycosylation properties of 1,3,4-TD and 2,4-substituted thiazole derivatives enable to partially correct metabolic disorders in the development of alloxan DM in rats.

As a result of the kinetic study of the reaction of nonenzymatic glycosylation of genetically engineered human insulin (GEHI) and its inhibition with G-SH, proposed the mathematical model of the reaction, verified its compliance with the results of the *in vitro* experiment, calculated rate constants and equilibrium constants for the FA formation steps in the absence and presence of G-SH.

Practical relevance. During the screening have been found compounds able to decrease the accumulation of fructosamine (FA) – primary product of NGP reaction, to inhibit AA oxidation with atmospheric oxygen. Revealed some aspects of "structure-activity" relation, providing to conduct a targeted search and synthesis of compounds with antiglycosylative and antioxidant activities. Use of compounds which combine properties of antioxidant and of NGP blocker, is a promising approach for the pharmacological correction of DM in experiment.

Approbation of the work and publications. 16 scientific papers adapted from the dissertation were published, 3 of them in journals included in the list of HAC. 1 application for an invention was filed, № 2014151103 (priority on December 16, 2014). The results were reported (with the publication of abstracts) in international and Russian conferences:

Russian conference "Actual problems of theoretical and applied biochemistry" (Chelyabinsk, 2009); Annual Conference "Pharmacy and Public Health" (Ekaterinburg, 2010); XX Russian Youth Scientific Conference "Problems of Theoretical and Experimental Chemistry" (Ekaterinburg, 2010); I International scientific and practical conference "High technology, fundamental and applied research in physiology and medicine" (St. Petersburg, 2010); Annual Conference "Pharmacy and Public Health" (Ekaterinburg, 2011); All-Russian scientific and practical conference of biochemists and experts in laboratory medicine "Medical Biochemistry and clinical laboratory diagnostics in terms of modernization of the scientific research" (Omsk, 2011); II International scientific and practical conference "High technology, fundamental and applied research in physiology and medicine" (St. Petersburg, 2011); IX All-Russian Conference "Chemistry and medicine" with the Youth Scientific School on Organic Chemistry (Ufa, 2013); I scientific and practical conference of students and postgraduates of Russia "Chemistry in federal universities" (Ekaterinburg, 2013); Russian scientific and practical conference with international participation "Actual problems of medical biochemistry and clinical laboratory diagnostics" (Kazan, 2013); Ural Scientific Forum "Contemporary Problems of Organic Chemistry" (Ekaterinburg 2014); II All-Russian conference "Fundamental glycobiology" (Saratov, 2014); Russian scientific forum in the Ural "Actual issues of fundamental medicine" (Ekaterinburg 2014).

Personal contribution of the author. The author participated in the planning of the research, performed a literature search, was directly involved in experiments *in vitro*, carried out biochemical

studies of blood and organs of experimental animals, conducted the calculations and experimental verification of the glycosylation reaction kinetic model. The material presented in the abstract, was collected, processed and analyzed by the author personally.

The structure and scope of the work. Dissertation is stated on 160 pages. Dissertation comprises sections: introduction, review of literature, materials and methods, study results, discussion of the results, conclusions, references. The work contains 25 tables and 39 figures. References section comprises 225 sources, including 141 source in foreign languages.

CHAPTER1. REVIEW OF LITERATURE

The review of literature considers the biochemical mechanisms of metabolic disorders development in DM, existing approaches to their pharmacological correction, chemistry and biological activity of natural and synthetic sulfur-containing heterocyclic compounds of classes 1,3,4-TD and substituted thiazoles.

CHAPTER 2. MATERIALS AND METHODS

The study used 1,3,4-TD derivatives and 2,4-substituted thiazole derivatives, synthesized at the Department of Organic Chemistry of Institute of Chemical Engineering of Federal State Autonomous Educational Institution of Higher Professional Education «Ural Federal University named after first President of Russia B.N. Yeltsin» (PhD in Chemistry Sidorova L.P., PhD in Chemistry Perova N.M., PhD in Chemistry Tseitler T.A., PhD in Chemistry Novikova A.P.) under the supervision of the member of the Russian Academy of Sciences O.N. Chupakhin. As reference compounds we used the reduced and oxidized glutathione, aminoguanidine (Sigma, USA) and metformin ("Glucophage", Nycomed, Germany). The ability of the tested compounds to block the reaction of NGP was evaluated by the accumulation of the initial NGP product FA when incubating BSA with glucose in the presence of 1,3,4-TD derivatives and substituted 2,4-thiazoles.

In simulated aqueous solution, comprising BSA at a concentration of 5 g/L and glucose at a concentration of 20 mmol/L, were added abovementioned substances at a concentration of 20 mmol/L as inhibitors of the reaction of NGP. System incubated at 4 °C, *m*-cresol at a concentration of 2.5 mg/mL was added to a solution as a preservative.

After 1, 2, 4, and 8 weeks, samples were taken to determine the concentration of the initial NGP product FA (see Viktorova L.N., Gorodetsky V.K. The colorimetric method for determining the nonenzymatic glycated albumin and hemoglobin // Lab. matter. 1990. Vol. 5. pp. 15 – 18).

Antioxidant activity of 1,3,4-TD derivatives was evaluated by effect on AA oxidation rate with atmospheric oxygen. For that TD-1,3,4 and AA were dissolved in water in ratios 1:1 and 1:2, respectively, and incubated at 37 °C with vigorous stirring for 6 hours. Samples were taken every hour and a concentration of AA in the solutions was established titrimetrically with 2,6-dichlorophenolindophenol (Davies M.B., Partridge D.A., Austin J.A. Vitamin C: Its Chemistry and Biochemistry // M.: Mir, 1999. 176 p.). Based on the findings, graphs of decrease in concentration of AA in the presence of each test substance were plotted. Concentrations decrease rates were calculated by the formula $v = \Delta C/\Delta t$, wherein ΔC is a change of AA concentration, Δt is an incubation time.

To evaluate hydro- and lipophilic properties ratios of 1,3,4-TD derivatives, determined "water/octanol" partition coefficient by slow stirring method (All-Union State Standard 32291-2013).

"Water/octanol" partition coefficient (K_{ow}) is a ratio of equilibrium concentrations of a compound dissolved in a biphasic system consisting of two substantially immiscible solvents. The results are given in the form of a common logarithm ($\lg K_{ow}$) (Rutkowska E., Pajak K., Jóźwiak K. et al. Lipophilicity – methods of determination and its role in medicinal chemistry // Acta Pol. Pharm. 2013. V. 70, №1. P. 3 – 18).

The experiment for evaluation the effect of sulfur-containing heterocyclic compounds on biochemical parameters of blood and internal organs in experimental DM was performed at the Institute of Immunology and Physiology, Ural Branch of Russian Academy of Sciences (Doctor of

Biological Sciences Danilova I.G., Ph.D. in Biology Gette I.F., Junior Researcher Bulavintseva T.S., Director - member of the Russian Academy of Sciences, Doctor of Medical Sciences Chereshnev V.A.)

Alloxan DM was modeled on 50 white mongrel male rats weighing about 200-250 g. The animals were kept in a laboratory vivarium, wherein the temperature was kept constant in the range of 22 - 25 °C and the natural alternation of day and night. All animals had free access to food and water. Cleaning of the cells was carried out daily, disinfection – weekly. All manipulations with animals were carried out in compliance with ethical principles and in accordance with Directive 2010/63 / EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Alloxan DM was modeled by tree-time intraperitoneal administration of alloxan in a total dose of 300 mg/kg animal body weight. Simultaneously with the induction of DM compounds L-17, L-14, H-32, L-31, L-91 and 2-guanidine-4-piridintiazol hydrobromide were administered in a dose of 40 mg/kg animal body weight 3 times a week for 4 weeks. Substances were previously dissolved in injectable water. LA (“Octolipen”, Russia) in similar doses was used as a reference substance.

Upon termination of the experiment concentrations of glucose (by the glucose oxidase method) and of total protein (by biuret method) were determined in the animals' blood (Kamyshnikov VS Handbook of clinical and biochemical laboratory diagnostics: in 2 V. // Minsk: Belarus, 2000 V.1, 495 pp., V.2, 463 pp.). To characterize the activity of NGP concentration of FA in plasma and liver and kidney homogenates was determined in the animals (Viktorova L.N., Gorodetsky V.K. The colorimetric method for determining the enzymatic glycosylated albumin and hemoglobin //Lab. science. 1990.V 5. pp. 15 - 18) and HbA_{lc} in the blood by column chromatography sets "Diabetes test" ("Fosfosorb", Russia). Activity of FOR of lipids was assessed by concentration of MDA in the plasma (Stalnaya I.D., Garishvili T.G. Method for determination of malondialdehyde by using thiobarbituric acid // Modern methods in biochemistry; ed. V.N. Orekhovich. M., 1977, pp. 66 - 68) and catalase activity of whole blood (Bakh A.N., Zubkova S.A. On the question of catalase // Collection of selected works. Leningrad. 1937. pp. 241 - 245).

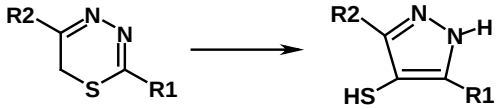
Kinetic analysis of reaction of nonenzymatic glycosylation of GEHI and its inhibition by G-SH was performed in cooperation with an assistant professor of Physical and Colloid Chemistry Department of Chemical Technology Institute of Ural Federal University, Ph.D. in Chemistry N.K. Bulatov.

CHAPTER 3. STUDY RESULTS AND DISCUSSION

Section 3.1. Antiglycosylation and antioxidant activity of 1,3,4-TD derivatives

Section 3.1.1. Study of 1,3,4-TD derivatives’ ability to block reaction of nonenzymatic glycosylation of BSA with glucose *in vitro*

The possibility of using natural and synthetic thiols for correction NGP *in vivo* is limited by their low bioavailability and rapid inactivation by oxidation to disulfides. In this connection, research of activity of synthetic substances, which can be transformed into thiol derivatives in the cells, is evidently promising. This kind of transformation is typical for 1,3,4-TD derivatives, which may explain their wide spectrum of biological activity.



Among the 41 compounds screened, we selected the 12 most active substances, inhibiting accumulation of FA by 20 - 70% compared to control values (see Fig. 1).

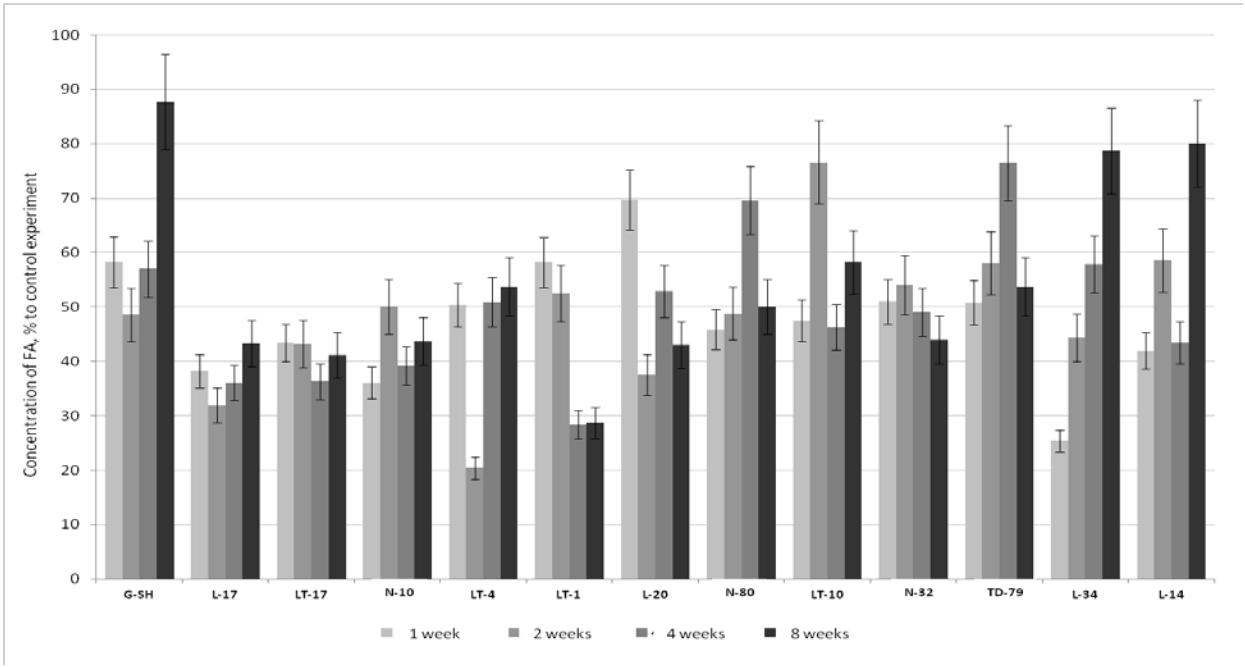
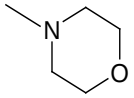
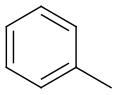
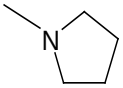
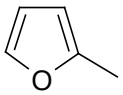
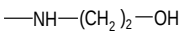
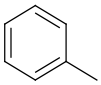
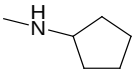
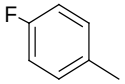
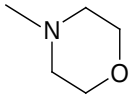
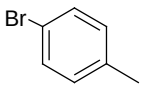
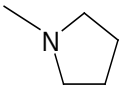
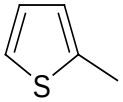
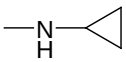
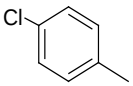
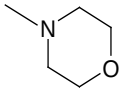
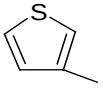
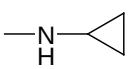
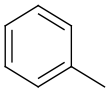
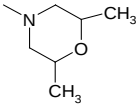
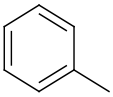
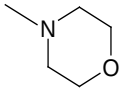
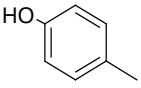
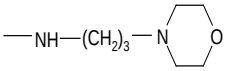
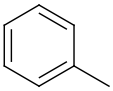


Fig. 1. Accumulation of FA when incubated BSA with glucose in the presence of 1,3,4-TD derivatives, % to the level of the control experiment of the corresponding week of the experiment. Reference compound – G-SH.

The effect of these compounds manifested throughout the experiment in contrast with G-SH, which had lost activity up to 8 weeks. Compounds exhibiting maximum antiglycosilation activity, differ from low active compounds by the nature of the substituents at 2- and 5-positions of thiadiazine cycle (see Table 1).

Table 1

1,3,4-TD derivatives, effectively inhibiting the accumulation of FA

Compound	Substituent		Compound	Substituent	
	R_1	R_2		R_1	R_2
L-17			N-80		
LT-17			LT-10		
N-10			N-32		
LT-4			TD-79		
LT-1			L-34		
L-20			L-14		

Among active inhibitors a combination of phenyl at 5-position and of morpholine or other oxygen-containing alkylamino moiety at 5-position is typical. Among the compounds with the lowest antiglycosilation activity prevail compounds containing dichlorothiophene substituents at 5-position, irrespective of the nature of the substituent at 2-position.

The mechanism of antyglycosylation action of the compounds of 1,3,4-TD class is possibly associated to their ability to be transformed into SH-substituted pyrazoles, as has been observed by heating certain 1,3,4-TD in acidic and alkaline media, or to attach glucose to the moiety of the thiadiazine ring after its opening (see Fig. 3). It can be assumed that the presence of oxygen at 2-position of alkylamine moiety reduces electron-donor properties of nitrogen involved in the p-π conjugation with thiadiazine cycle, thereby contributing to the ring opening with carbon-sulfur bond cleavage.

Section 3.1.2. Study of antioxidant properties of 1,3,4-TD derivatives in a model of AA oxidation with atmospheric oxygen

AA exhibits antioxidant properties synergistically with other natural antioxidants. It is known that ascorbate reduces vitamin E phenoxide radical, being oxidized to dehydroascorbate, and thiol compounds reduce dehydroascorbate to AA. Ascorbate content in the blood of patients with DM is decreased and correlates negatively with the level of HbA_{lc} (Kositsawat J., Freeman V.L. Vitamin C and A_{1c} relationship in the National Health and Nutrition Examination Survey (NHANES) 2003-2006 // J. Am. Coll. Nutr. 2011. V. 30, № 6. P. 477 – 483).

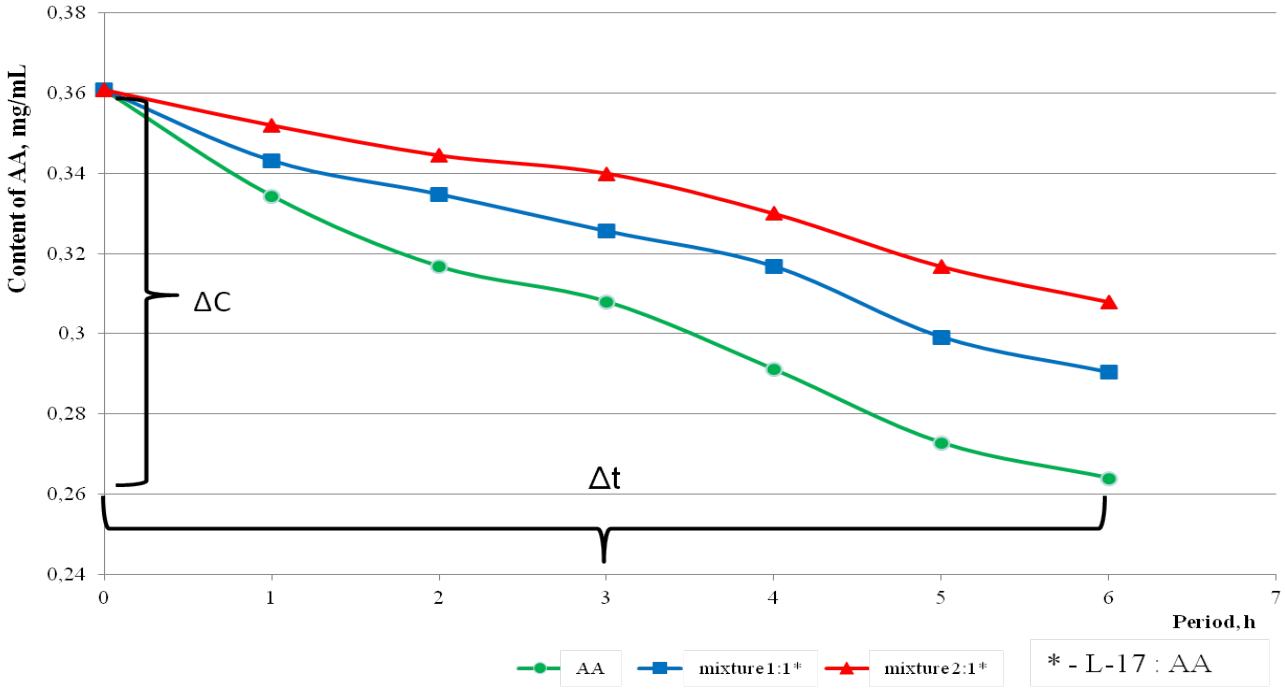


Fig. 2. Evaluation of the L-17 compound effect on the kinetics of the AA oxidation by atmospheric oxygen.

To evaluate antioxidant properties of 1,3,4-TD derivatives, their ability to inhibit AA oxidation with atmospheric oxygen was evaluated.

In the context of compound L-17 (see Fig.2) it can be seen that concentration of AA in the tested solutions decreases over time, but AA oxidation with atmospheric oxygen in the control occurs

faster than in the presence of the compound L-17. AA rate of decay in the absence of the inhibitor was15.0 μg/L·h, in the presence of equimolar amounts of the L-17 it decreased to 11.5 μg/L·h, and in 2-fold excess of the L-17 – to 8.5 μg/L·h. The conducted experiments have shown that the compound L-17 has a dose-dependent ability to prevent the oxidation of the AA.

The experiment was conducted also with seven representatives of the class 1,3,4-TD, LA and glutathione in oxidized and reduced forms. From the data obtained rates of decline in concentrations of AA in tested solutions were calculated (see Table. 2).

Table 2

Oxidation rate of AA with atmospheric oxygen in the presence of the sulfur-containing compounds

Compounds	1:1		1:2	
	v, mg/L·h	% C	v, mg/L·h	% C
GSH	9,78±0,49	50±2,5	2,44±0,12	57±2,8
GSSG	16,56±0,83	72±3,6	14,67±0,73	72±3,6
LA	29,33±1,47	86±4,3	29,33±1,46	86±4,3
TD-79	6,98±0,35	17±0,8	10,48±0,52	30±1,5
L-92	18,33±0,92	78±3,9	10,86±0,54	44±2,2
N-32	16,10±0,81	62±3,1	7,33±0,37	28±1,4
L-31	25,88±1,29	75±3,7	32,59±1,63	100±5
L-34	39,01±1,95	100±5	23,33±1,16	56±2,8
L-17	28,62±1,43	72±3,6	21,46±1,07	53±2,6
LT-1	33,17±1,66	130±6,5	36,67±1,83	123±6,1
L-14	73,33±3,67	650±32,5	71,90±3,59	625±31,2

*% C – percentage to the level of the control experiment

According to the data given in the table, it can be concluded that the highest antioxidant activity has compound TD-79, whereas compounds N-32, L-92, L-17 and G-SH prevent oxidation of AA slightly weaker.

Compounds L-34 and L-31, and oxidized glutathione at two concentrations tested had an ambiguous effect on the kinetics of the AA oxidation and substances LT-1 and L-14 accelerated it altogether.

The possible mechanism of this effect of 1,3,4-TDs we associate with the influence of its transformation products containing a thiol group. These compounds reduce dehydroascorbic acid to AA, while themselves being oxidized to disulfides (see Fig. 3).

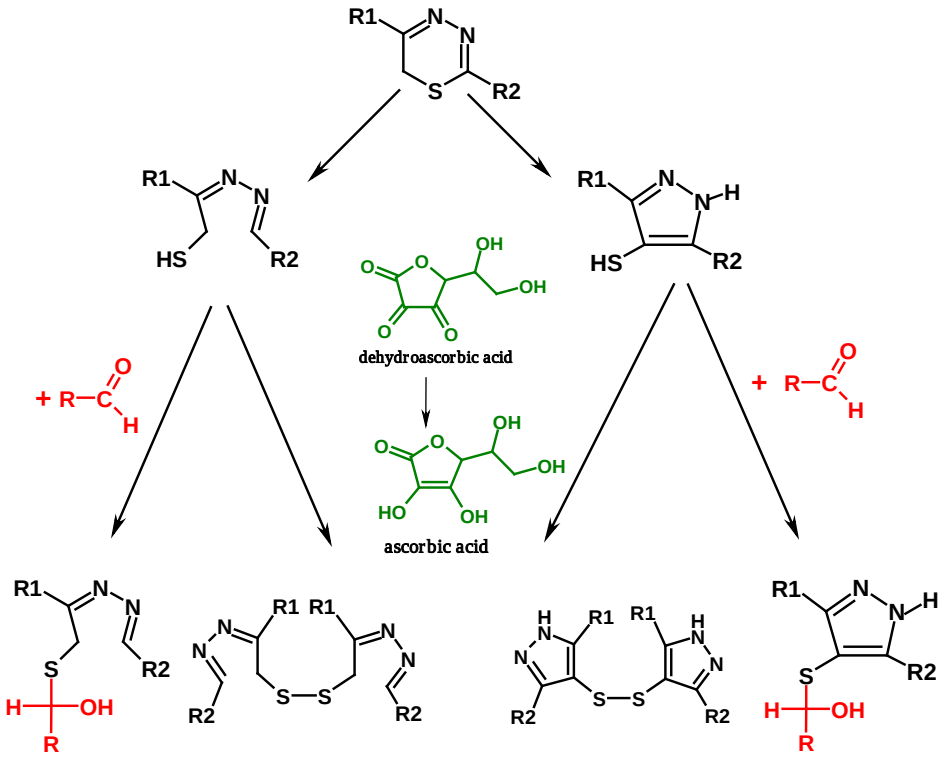


Рис. 3. Binding of carbonyl compounds and reduction of dehydroascorbic acid in presence of 1,3,4-TD derivatives.

Analysis of the "structure-activity" relationship in the series of 1,3,4-TD derivatives changimg the kinetics of AA oxidation with atmospheric oxygen shows the dependence of the activity on the presence and nature of the substituents at the 2- and 5-positions of the thiadiazine cycle. Thus, among the 1,3,4-TD derivatives comprising a phenylic moiety at 5-position, the greatest antioxidant activity possessed by compounds L-17 and L-34 comprising morpholine, and at 2-position comprising dimethylmorpholine, respectively. On the other hand, among the morpholino substituted derivatives of 1,3,4-TD, thiophene derivatives at the 5-position possess the highest antioxidant properties. The highest antioxidant activity among the studied synthetic compounds had 2-morpholino-5-thienyl-1,3,4-TD (TD-79).

Section 3.1.3. Study of the ratios between lipophilic and hydrophilic properties of 1,3,4-TD derivatives with antioxidant and antiglicosilation activity in octanol/water

Interaction between biologically active substances and cellular and subcellular structures occurs in an aqueous medium or nonaqueous layers of membranes formed by hydrophobic lipid fragments. It was shown that increasing of lipophilicity corresponds with decreasing in water solubility, increasing the rate of penetration through the skin, increasing the degree of binding to plasma proteins, the material cumulation, which largely determines the biological activity of the compounds (Lu Y., Kim S., Park K. et al. *In vitro-in vivo* correlation: perspectives on model development // Int. J. Pharm. 2011. V. 418, №1. P. 142 – 148; All-Union State Standard 32291-2013).

Coefficients lgK_{ow} of 10 1,3,4-TD derivatives, distinguished by antiglycosylation activity, were identified by slow stirring method (see Table 3).

Table 3

Values of lgK_{ow} for 1,3,4-TD derivatives in octanol/water

Com-pound	LT-17	LT-4	L-17	TD-79	N-94	L-34	N-32	L-103	L-92	L-14
lgK_{ow}	2,90	3,01	3,03	3,06	3,14	3,17	3,19	3,38	3,41	4,02

When analyzing lgK_{ow} values of studied 1,3,4-TDs, were found expected differences in lipophilicity of compounds differing in the nature of the substituents at the 2- and 5-positions. For example, the compound 2-G-5-P has less bulky substituent at the 2-position of all compounds studied and has lower lipophilicity. Compound L-17 differing from the 2-G-5-P by less polar substituent at the 2-position, has a higher coefficient lgK_{ow}. Compound L-34 has two additional methyl radical in the structure of morpholine moiety and thus has a greater lipophilicity in comparison with the L-17. Finally, the compound L-14 having the most aliphatic and the least polar substituent at the 2-position, naturally has been found to have the highest value lgK_{ow}.

Arranging of 1,3,4-TD derivatives with different antioxidant activity by the ratio of lipophilic and hydrophilic properties failed to demonstrate the relationship between lgK_{ow} and the ability to inhibit the oxidation of AA with atmospheric oxygen. lgK_{ow} values of 1,3,4-TD derivatives with the highest antioxidant activity did not differ from those of the weak antioxidants.

When comparing the lipophilicity and antiglycosylation activity of 1,3,4-TDs, the following facts had been identified. The partition coefficient lgK_{ow} for more active NGP inhibitors was 2.90 - 3.19, while for the less active inhibitors was 3.14 - 4.02. Thus it can be concluded that optimal for manifestation of antiglycosylation activity of derivatives 1,3,4-TD is the value lgK_{ow} = 2.90 - 3.19.

Section 3.2. Study of 2,4-substituted thiazoles' ability to block reaction of nonenzymatic glycosylation of BSA with glucose *in vitro*

Synthetic derivatives of thiazole (thiamine) and guanidine (metformine) are used in the treatment of DM and its complications (Balabolkin M.I., Klebanova E.M., V.M. Kreminskaya Treatment of diabetes and its complications. Guidelines for doctors // M.: Medicine, 2005. 512 p.; Ametov A.S. Type 2 diabetes mellitus. Problems and Solutions // M.: GEOTAR Media, 2011. 704 p.). Aminoguanidine is a reference compound in the experimental evaluation of the ability of compounds to inhibit the NGP. However, guanidine-substituted thiazole derivatives previously were not subjected to studies on antidiabetic activities, and this has caused the formulation of the problem in this Section of the work.

Compounds selected for study were triazole derivatives having guanidine, amino or morpholine at 2-position, and aryl or heteryl at 4-position (see Table 4).

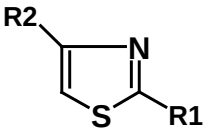
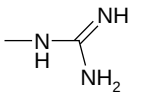
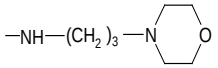
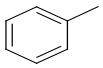
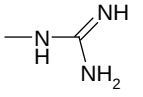
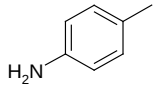
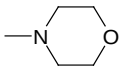
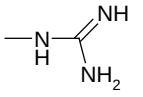
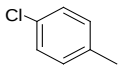
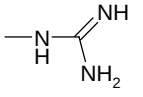
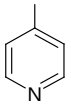
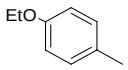
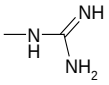
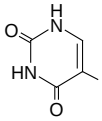
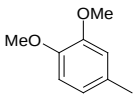


Table 4

Structure of tested 2,4-substituted thiazole derivatives

Compound	Substituent		Compound	Substituent	
	R1	R2		R1	R2
2-guanidine-4-phenylthiazole (2-G-4-PT)			L-90		
2-guanidine-4-*n*-aminophenylthiazole (2-G-4-APT)			N-63		
2-guanidine-4-α-pyridinethiazole (2-G-4-α-PT)			N-107		
2-guanidine-4-γ-pyridinethiazole (2-G-4-γ-PT)			N-108		
2-guanidine-4-(5-uracil) thiazole (2-G-4-UT)			N-109		

Structure of these compounds, particularly presence of guanidine group, makes them probable inhibitors of NGP reaction by competition with the protein in the reaction with glucose and carbonyl intermediates. Moreover, aminoguanidine (AMG) and metformin (MF), whose activity as blockers of the NGP reaction has been proved earlier, were used in the experiment (Ozyazgan S., Unlucerci Y., Bekpinar S., Akkan A.G. Impaired relaxation in aorta from streptozotocin-diabetic rats: effect of aminoguanidine (AMNG) treatment // Int. J. Exp. Diabetes Res. 2000. V. 1, №2. P. 145 – 153; Chang K.C., Hsu K.L., Chou T.F. et al. Aminoguanidine prevents age-related deterioration in left ventricular-arterial coupling in Fisher 344 rats // Br. J. Pharmacol. 2004. V. 142, № 7. P. 1099 – 1104; Lankin V.Z., Konovalova G.G., Tikhaze A.K., Nedosugova L.V. Influence of natural dicarbonyls on the activity of antioxidant enzymes *in vitro* and *in vivo* // Biomed. Chemical. 2012. V. 58, no. 6. pp. 727 – 736). Ability of substituted 2,4-triazoles to inhibit the NGP reaction was investigated similarly to the experiment with 1,3,4-TDs.

From the data obtained (see Fig. 4) it can be concluded that the accumulation of FA in the model system “BSA with glucose” in the presence of guanidine derivatives of thiazole reduced as compared with the control, most of all - in the presence of 2-guanidine-4-phenylthiazole. In the case of *p*-aminophenyl derivative, FA concentration exceeded the control in 3.5 - 4 times during all periods of the experiment. We attribute this to the ability of the compound to form additional quantities of FA at the aminogroup of aminophenyl moiety.

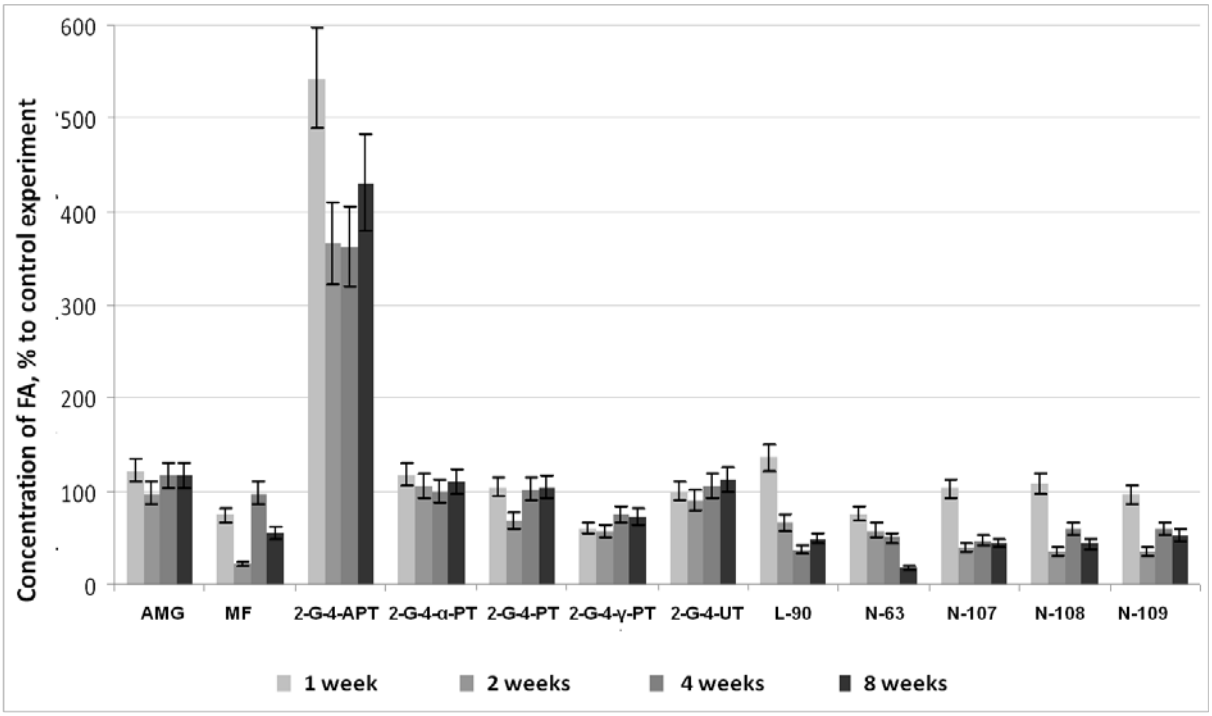


Fig. 4. Accumulation of FA when incubated BSA with glucose in the presence of 2,4-substituted thiazole derivatives, % to the level of the control experiment of the corresponding week of the experiment. Reference compounds – aminoguanidine (AMG) and metformine (MF).

A significant ability to inhibit the accumulation of FA in the later periods of the experiment showed thiazole derivatives containing at 2-position amino or morpholino group and phenyl or substituted phenyl at 4-position. The highest activity among them possesses 2-morpholino-4-phenyl-thiazol (compound N-63). Thus, 4-aryl thiazoles having at 2-position guanidine group or morpholine are prospective for the search for NGP inhibitors group of synthetic sulfur-containing heterocyclic compounds.

Section 3.3. Biochemical factors of blood and organs of rats in the development of alloxan DM on the background of the sulfur-containing heterocyclic compounds administration

In the development of new drugs for the treatment of DM is widely used simulation of this disease in animal experiments. The leading role in the formation of diabetic vascular lesions and nerves plays activation of NGP and FRO of lipids, proteins, and nucleic acids caused by prolonged hyperglycemia. It is known that the natural thiol glutathione corrects condition of endocrine function of β-cells of the pancreas in experimental DM. In the experiment and clinic received numerous reports of successful correction of free radical processes and NGP, enhance the action of insulin in DM by introduction of the LA.

Considering previously identified ability of a number of heterocyclic compounds of 1,3,4-TD and 2-guanidinetiazole class to inhibit the reaction of NGP in the model system, it is of interest to assess the ability of these compounds to effect biochemical factors of peripheral blood in experimental DM.

Table 5

Biochemical factors of blood and organs of rats in the development of alloxan diabetes on the background of the sulfur-containing heterocyclic compounds administration, M± m

Factor	Controle (n=10)	Tested compound						
		LA (n=7)	L-31 (n=5)	L-91 (n=5)	L-17 (n=7)	N-32 (n=5)	L-14 (n=5)	2-G-4-PT (n=6)
Blood								
Glucose, mmol/L	34.6±3.4	14.8±1.7*	10.3±0.9*	13.7±1.9*	23.7±2.2*	25.2±5.2*	29.3±3.0*	33.4±2.5
HbA_{1c}, %	9.1±0.8	5.1±0.9*	6.9±0.3*	5.3±0.3*	6.5±0.7*	5.7±1.7*	7.5±0.7*	9.2±0.7
Fructosamine, μmol/g of protein	4.7±0.5	3.1±0.4*	3.8±0.5	3.6±0.2*	3.2±0.9	3.8±0.8	5.3±0.6	3.2±0.5*
Catalase, ncatal/g Hb	156±11	1094±95*	1025±87*	1222±70*	143±8	79±9*	165±10	138±22
MDA, nmol/L	441±19	370±50	365±45	303 ±43*	106 ± 27*	370±22	620±68	191±28*
Organs								
Fructosamine, kidney, μmol/g of protein	45.2±3.8	16.3±1.3*	37.7±1.5	16.3±0.3*	15.6±2.2	23.9±2.0*	20.7±2.1*	17.3±0.9*
Fructosamine, liver, μmol/g of protein	20.9±8.0	3.7±0.3*	5.1±0.9*	9.3±0.8*	8.6±1.6*	9.4±1.3*	9.5±2.5*	8.3±1.2*

* - statistically significant difference from control experiment, $p < 0.05$

According to data of the Table 5, administration of alloxan led to persistent and severe hyperglycemia, accumulation of glycosylated proteins in the blood and organs of animals. Hyperglycemia and NGP activation could serve as a trigger for oxidative stress, MDA accumulation and decrease in blood catalase activity. Administration of LA significantly mitigated, though not eliminated entirely, biochemical disorders in the developing alloxan DM. Hyperglycemia and its related concentrations of glycosylated blood proteins were 35-55% lower comparing to the control group when administered LA. Contents of FA in homogenates of kidneys and liver was 2.8 and 5.6 times, respectively, less comparing to the control group when administered LA. The antioxidant effect of LA in the present study manifested by increase of catalase activity, however, the content of MDA in the blood has not undergone statistically significant changes.

Studied synthetic sulfur-containing heterocyclic compounds are also able to partially correct metabolic disorders in the development of alloxan DM. However, actions of the 1,3,4-TD derivatives and of 2-G-4-PT were principally different. The 1,3,4-TD derivatives L-17, L-91, N-32, and L-14 caused a decrease of hyperglycemia and HbA_{1c} concentration in the blood, as well as FA in the kidneys and liver of the animals, similar to LA. Compound L-31 differed from the others 1,3,4-TD by the fact that did not reduce the accumulation of FA in the kidneys. The key insight of corrective action of 1,3,4-TD derivatives was the antihyperglycemic effect.

1,3,4-TD derivatives antihyperglycemic effect intensity in the *in vivo* experiment decreased in line L-31 > L-91 > L-17 > H-32 > L-14. By analyzing the relationship between the activity of the 1,3,4-TD derivatives in the experiment *in vivo* and *in vitro,* it should be noted that compounds H-32, L-31 and L-17, which actively inhibited oxidation of AA with atmospheric oxygen, had shown corrective activity *in vivo* greater than compound L-14, which accelerated the oxidation of AA in *in vitro* study and increased MDA accumulation in the blood of the animals.

Administration of 2-G-4-PT did not decrease severity of hyperglycemia and accumulation of HbA_{1c}, but statistically significantly decreased levels of MDA and FA in kidneys and liver. Thus, without having the antihyperglycaemic effect, 2-G-4-PT showed properties of antioxidant and blocker of NGP.

The ability of LA and of the 1,3,4-TD derivatives to mitigate metabolic disorders in the developing alloxan DM we associate primarily with the protection of β-cells of islets of Langerhans against alloxan-induced oxidative stress, leading to the death of pancreatic endocrine cells.

As the biological mechanism of this influence may be considered the ability of LA and of the 1,3,4-TD derivatives to transform into thiol derivatives, increasing the power of the antioxidant defense of β-cells. 2-G-4-PT, not having the ability to replenish the pool of intracellular thiols, apparently devoid of direct effects on β-cells.

The protective effect of the compound at the developing alloxan DM may be explained by considering data of the antioxidant activity of substituted thiazoles and the ability of guanidine derivatives of thiazoles to inhibit NGP by reaction with glucose and carbonyl intermediates of NGP.

Thus, in the current study, for the first time demonstrated the ability of synthetic sulfur-containing heterocyclic compounds from 1,3,4-TD and 2-G-4-PT to correct the metabolic disorders in the development of alloxan DM in rats. Metabolic actions of representatives of two classes of sulfur-containing heterocycles differed: for 1,3,4-TD derivatives was typical antihyperglycemic, antioxidant and antiglycosylative effects, 2-G-4-PT had only the last two mechanisms of action. Efficacy of 1,3,4-TD derivatives in treatment of experimental DM detected parallels to their antioxidant activity *in vitro*.

Section 3.4. Kinetic analysis of reaction of nonenzymatic glycosylation of genetically engineered human insulin

NGP is a complex multi-stage process, which could be conditionally divided into three stages. The initial stage of NGP consists of 2 consecutive steps (see Fig. 5).

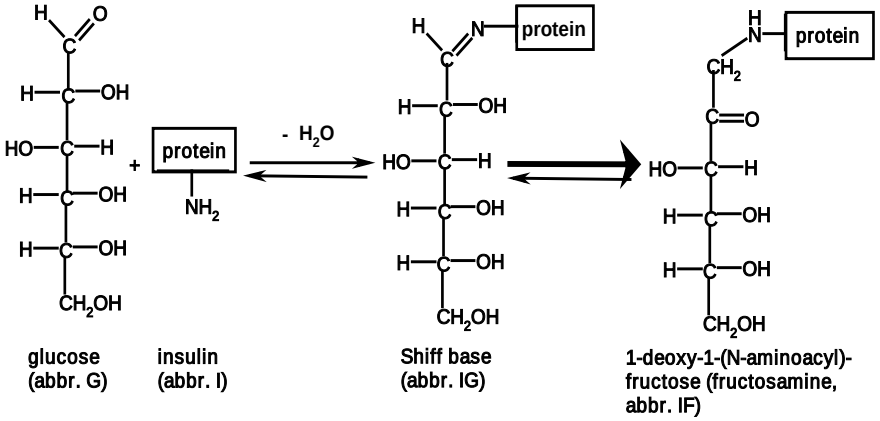


Fig. 5. Initial stage of NGP reaction

The first step is forming of the Shiff's base, which rearranges itself into a more stable Amadori product at the second step. In case of protein reacting with glucose, Amadori product has the structure of 1-deoxy-1-(N-aminoacyl)fructose (so-called "fructosamine", FA). There are different viewpoints about reversibility of the second stage. In this study kinetic analysis had been made in accordance with hypothesis that the second step is reversible.

The second stage is fructosamine transformation into intermediate glycosylation products – carbonyl compounds (glyoxal, methylglyoxal et alias) (see Fig. 6). Formed carbonyl compounds are more active in reaction with protein than initial monosaccharide.

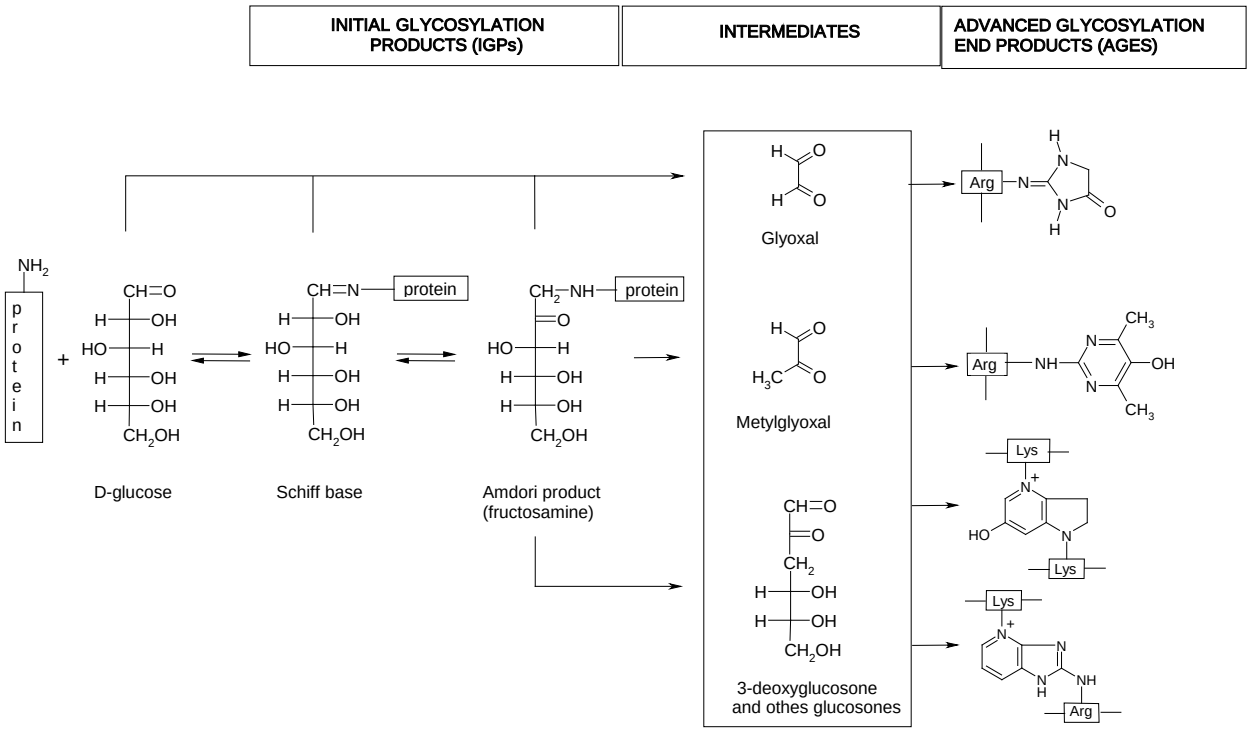


Fig. 6. Scheme of formation certain products of NGP.
Arg and Lys - lysine and arginine residues in the protein.

The final stage of the reaction is formation of advanced glycosylation products through acting between carbonyl compounds and protein molecule (see Fig. 6). Advanced glycosylation products formation irreversibly change native structure of protein. Inner and adjacent cross-linking between protein amino groups mostly contribute to this process.

Knowledge of kinetic principles of nonenzymatic glycosylation of insulin allows to offer a mechanism of interaction between glucose and protein, that will serve as a basis for developing a scientific approach to search and synthesis of NGP inhibitors.

Experimental study of the kinetics of nonenzymatic glycosylation of insulin was carried out in base test solutions comprising GEHI and glucose in various concentrations. The solutions were incubated for 8 weeks, samples were taken periodically to determine the concentration of fructosamine.

Obtained data on c_{IF} concentration changes in tested solutions with the course of time *t* are presented in Fig. 7.

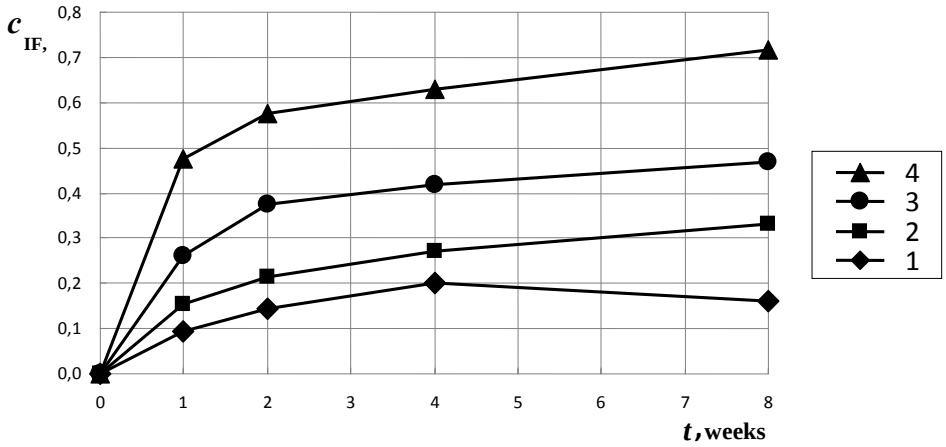


Fig. 7. Kinetic curves $c_{\text{IF}}(t)_n$ (n = 1, 2, 3, 4) for $c_{\text{H}_2\text{O},0}$ = 55400 mol/m^3, $c_{\text{I},0}$ = 0.872 mol/m^3 and various $c_{\text{G},0}$, mol/m^3: 1 – 6.25; 2 – 12.5; 3 – 25.0; 4 – 50. Temperature T = 277 K.

Then linearization of kinetic curves was held for the subsequent mathematical treatment (see Fig. 8), and established a series of equations that characterize the considered reaction step.

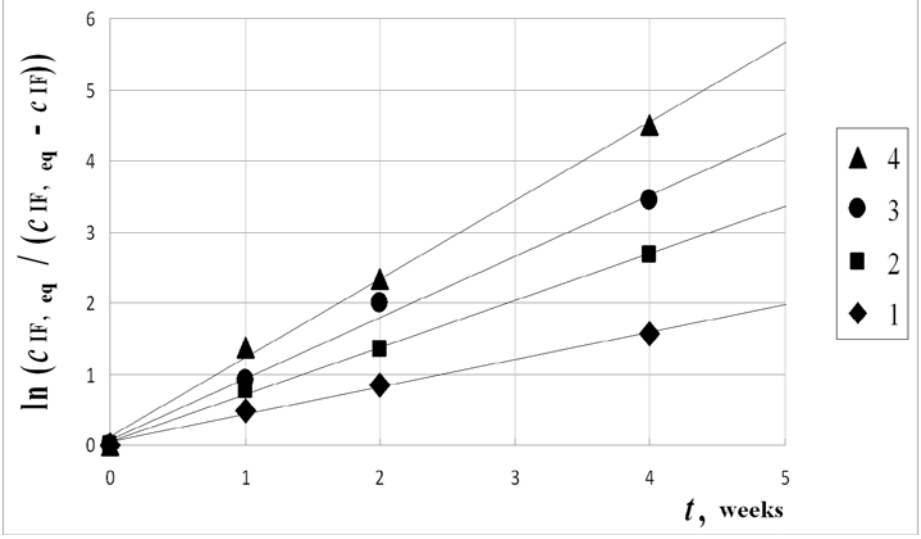


Fig. 8. Linear patterns of kinetic curves $c_{\text{IF}}(t)_n$ (n = 1, 2, 3, 4) (see Fig. 2) in coordinates t and $\ln\left(c_{\text{IF},eq}/\left(c_{\text{IF},eq}-c_{\text{IF}}\right)\right)$ for such choice of $c_{\text{IF},eq}$, mol/m^3: 1 – 0.253; 2 – 0.290; 3 – 0.434; 4 – 0.637

By treatment of the experimental curves $c_{\mathrm{IF}}(t)$ using the kinetic equations of the process of nonenzymatic glycosylation of GEHI *in vitro* were determined: the rate constants of direct and reverse elementary reactions in step 2 (k_2^{dir}=0.044715 1/h; k_2^{rev}=0.002158 1/h), the ratio of the rate constants of the forward and reverse elementary reactions in step 1 ($k_1^{\mathrm{dir}} / k_1^{\mathrm{rev}}$=134.2), and the thermodynamic equilibrium constant of both steps (K_1^c=134.2; K_2^c=21.0). The equilibrium concentrations of all insulin-containing components – participants of the process.

The process at the initial stage of its development is clearly manifested as a combination of two stages, having a bilateral character, the first of which is the interaction of insulin (I) and glucose (G), and leads to the formation of the intermediate - Schiff base (IG), and the second - in the formation of fructosamine (IF):

1. $I + G = IG + H_2O$,
2. $IG = IF$,

wherein the process occurs in quasi-equilibrium regime for the first stage.

Then the model system was complicated by the addition of a third component of the reaction – G-SH – to reveal its ability to inhibit the reaction of NGP. The mathematical treatment of experimental results was carried out similar to the previous experiment.

As a result it was found that G-SH additives do not alter the two-stage mechanism of the process occurring in quasi-equilibrium regime for the first stage, but significantly reduce its pace and a promotion degree due to the binding of insulin by the glutathione in a chemical compound IαY type according to path reaction *p*=3:

3. $\alpha I + Y = I_\alpha Y$,

wherein α is a module of stoichiometric coefficients of insulin.

It was shown that in the presence of G-SH course of the insulin glycosylation process of in its initial stage occurs in quasi-equilibrium regime not only at the first stage but also according to path reaction 3, therefore its course in time *t* still obeys integral kinetic equation of step 2, expressed in logarithmic form: $\ln\left(c_{\mathrm{IF},eq} / \left(c_{\mathrm{IF},eq} - c_{\mathrm{IF}}\right)\right) = at$, wherein parameters $c_{\mathrm{IF},eq}$ and a depend on the initial concentration of glutathione $c_{\mathrm{Y},0}$. This means that G-SH is able to decrease accumulation of FA in the model system in a dose-dependent way.

On the basis of calculations of the equilibrium constant of step 3 $K_3^c(T,[c_k])$, it was found that the absolute value of the stoichiometric coefficient of insulin α = 4. In practice this means that in the model system, one molecule of glutathione reacts with four molecules of insulin. This result is quite unexpected because of the large values of α = 4, so it requires to be further discussed in terms of the mechanism of interaction of insulin with G-SH. Probably, G-SH is able to non-covalently interact with polar functional groups in insulin molecule, thereby changing the conformation of the protein. It is also known that insulin in solution forms aggregates – tetra- and hexamers. Dissociation of these complexes into monomers can increase the availability of amino groups for glycosylation, therefore slowing down of dissociation in the presence of glutathione is capable of inhibiting the process.

Thus, the current study proposed and experimentally confirmed the kinetic model of the non-enzymatic glycosylation of genetically engineered human insulin *in vitro*. It is found that in the range of physiological concentrations natural thiol G-SH has a dose-dependent ability to inhibit glycosylation of insulin with glucose *in vitro*, kinetic analysis of the process was performed.

Conclusions:

1. Shown that the 1,3,4-TD and 2,4-substituted thiazole derivatives are able to inhibit the accumulation of NGP products while incubating BSA with glucose. The best antiglycosylation properties have 5-phenyl-1,3,4-TD derivatives having an oxygen-containing heteryl or alkylamino substituent at 2-position.
2. When evaluating lipophilicity of potential NGP inhibitors – 1,3,4-TD derivatives in water/octanol – was established that the partition coefficient lgK_{ow} for active NGP inhibitors was 2.90 - 3.19, while for the less active inhibitors it was 3.14 - 4.02.
3. 1,3,4-TD derivatives have ability to inhibit the oxidation of ascorbic acid with atmospheric oxygen. The most active compounds are N-32, TD-79, L-92, L-17, L-34.
4. Derivatives of 1,3,4-TD (L-17, L-14, N-32, L-31, L-91) and 2-guanidinethiazole correct metabolic disorders in alloxan DM rats. Studied 1,3,4-TD derivatives decrease hyperglycemia, accumulation of products of NGP in blood and internal organs, and also possess antioxidant activity. 2-G-4-PT exhibits antioxidant and antiglycosylation activity without affecting the severity of hyperglycemia.
5. Sulfur-containing heterocyclic compounds, such as 1,3,4-TD and 2,4-substituted thiazole derivatives are promising for further studies of antidiabetic activity and its biochemical mechanisms in experimental animals.
6. Screening of 1,3,4-TD derivatives for experimental evaluation of antidiabetic action *in vivo* can be performed based on the results of preliminary testing on the antiglycosylation activity *in vitro* (inhibiting of accumulation of fructosamine during incubation of BSA with glucose) antioxidant properties (inhibition of the AA oxidation with atmospheric oxygen) and ratio of the hydrophilic and lipophilic properties (in "octanol/water").
7. Proposed and experimentally confirmed the kinetic model of the non-enzymatic glycosylation of genetically engineered human insulin *in vitro* and its inhibition with G-SH, calculated a number of constants of this reaction.

The main content of the work described in the following publications:

Articles published in peer-reviewed journals outlined by State Commission for Academic Degrees and Titles

1. Savateeva, E.A. Kinetic analysis of reaction of nonenzymatic glycosylation of genetically engineered human insulin *in vitro* / E.A. Savateeva, V.V. Emelyanov, N.K. Bulatov, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Butlerov Communications. 2012. V.30, №6. pp. 94 - 102.
2. Emelyanov, V.V. Prospects for finding new antidiabetic agents among sulfur-containing heterocyclic compounds / V.V. Emelyanov, E.A. Savateeva, A.V. Musalnikova, L.P. Sidorova, N.M. Perova, A.P. Novikova, T.S. Bulavintseva, I.F. Gette, I.G. Danilova, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Allergology and Immunology. 2013. V.14., № 2. pp. 141 - 142.
3. Bulatov, N.K. Kinetic analysis of the inhibition of nonenzymatic glycosylation of genetically engineered human insulin with glutathione *in vitro* / N.K. Bulatov, E.A. Savateeva, V.V. Emelyanov, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Butlerov Communications. 2014. V.38, № 5. pp. 103 - 111.

Other publications

1. Emelyanov, V. Prospects of creation of agents for pharmacological blockade of reaction of nonenzymatic protein glycosylation / V.V. Emelyanov, N.E. Maksimova, N.N.

Mochulskaya, Ya.A. Bulgakova, E.A. Savateeva, V.A. Chereshnev // "Actual Problems of Theoretical and Applied Biochemistry» Russian conference devoted to the 80th anniversary of R.I. Lifshitz, timed to the 65th anniversary of the Chelyabinsk State Medical Academy, 5 - 8 October 2009: collection of conference materials. Chelyabinsk ChelSMA, 2009. pp. 91 - 92.

2. Emelyanov, V.V. Effect of glutathione on nonenzymatic glycosylation of insulin *in vitro* / V.V. Emelyanov, E.A. Savateeva, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Proceedings of the annual conference "Pharmacy and Public Health", February 25, 2010. Collection of conference materials. - Ekaterinburg: USMA, 2010. pp. 27 - 30.
3. Bulgakova, Ya.A. Thiols and guanidine derivatives as potential blockers of reaction of nonenzymatic protein glycosylation / Ya.A. Bulgakova, E.A. Savateeva // Abstracts XIX Russian Youth Scientific Conference "Problems of Theoretical and Experimental Chemistry" dedicated to the 90th anniversary of the Ural State University named after A.M. Gorky. Ekaterinburg, April 20-24, 2010. Ekaterinburg: Ural State University Publishing House, 2010. pp. 480-481.
4. Savateeva, E.A. Changes in intrinsic fluorescence of proteins in nonenzymatic glycosylation *in vitro* / E.A. Savateeva, V.V. Emelyanov, Ya.A. Bulgakova, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Proceedings of the international scientific-practical conference "High technology, basic and applied research in physiology and medicine." Digest of articles. St. Petersburg, 23-26 November 2010 - St. Petersburg: Publishing House of the Polytechnic University Press, 2010. pp. 308 - 309.
5. Emelyanov, V.V. Nonenzymatic glycosylation of blood proteins and oxidative stress in alloxan diabetes against the introduction of immunomodulators / V.V. Emelyanov, E.A. Savateeva, N.E. Maksimova, N.N. Mochulskaya, I.G. Danilova, I.F. Gette, V.A. Chereshnev // Proceedings of the annual conference "Pharmacy and Public Health." Digest of articles. Ekaterinburg, May 19, 2011 – Ekaterinburg: USMA, 2011. pp. 383 - 385.
6. Emelyanov, V.V. Heterocyclic compounds of class 1,3,4-thiadiazine as potential blockers of nonenzymatic glycosylation of proteins / V.V. Emelyanov, E.A. Savateeva, A.V. Musalnikova, E.A. Leontieva, N.E. Maksimova, N.N. Mochulskaya, L.P. Sidorova, N.M. Perova, V.A. Chereshnev // Materials of All-Russian scientific-practical conference of biochemists and experts in laboratory medicine. Digest of articles. Omsk, 20-21 September, 2011 - Omsk: Omsk State Medical Academy, 2011. pp. 89 - 92.
7. Savateeva, E.A. Inhibition with glutathione nonenzymatic glycosylation of genetically engineered human insulin / E.A. Savateeva, V.V. Emelyanov, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Proceedings of the international scientific-practical conference "High technology, basic and applied research in physiology and medicine." Digest of articles. St. Petersburg, October 25-29, 2011 - St. Petersburg: Publishing House of the Polytechnic. University Press, 2011. pp. 281 - 282.
8. Savateeva, E.A. Sulfur-containing heterocyclic compounds as blockers of nonenzymatic glycosylation of proteins / E.A. Savateeva, V.V. Emelyanov, A.V. Musalnikova, L.P. Sidorova, N.M. Perova, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Abstracts of IX All-Russian Conference "Chemistry and medicine" with the Youth Scientific School of Organic Chemistry, Ufa-Abzakovo, June 4-8, 2013 - Ufa: Bashkir State University Publishing House, 2013. p. 101.
9. Savateeva, E.A. Sulfur-containing heterocyclic compounds with potential antidiabetic activity / E.A. Savateeva, V.V. Emelyanov, A.V. Musalnikova, L.P. Sidorova, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Proceedings of the Conference "Chemistry in federal universities." Digest of articles. Ekaterinburg: Ural Federal University, 2013. pp. 142 - 144.

10. Emelyanov, V.V. Effect of sulfur-containing antioxidants on the development of experimental diabetes / V.V. Emelyanov, E.A. Savateeva, A.V. Musalnikova, L.P. Sidorova, A.P. Novikova, T.S. Bulavintseva, I.F. Gette, I.G. Danilova, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Collection of scientific articles of the Russian scientific-practical conference with international participation "Actual problems of medical biochemistry and clinical laboratory diagnostics," dedicated to the memory of academician of Academy of Sciences of Tatarstan Republic Professor D.M. Zubairov. Kazan, September 23-26, 2013 - Kazan: Publishing house "Otechestvo", 2013. pp. 62 – 68.
11. Emelyanov, V.V. 1,3,4-thiadiazines in the experimental treatment of diabetes / V.V. Emelyanov, E.A. Savateeva, L.P. Sidorova, I.F. Gette, T.S. Bulavintseva, I.G. Danilova, N.N. Mochulskaya, O.N. Chupakhin, V.A. Chereshnev // Ural Scientific Forum "Contemporary Problems of Organic Chemistry". Abstracts. Ekaterinburg, 8-12 June 2014 - Ekaterinburg: Ural Federal University, 2014. p. 46.
12. Emelyanov, V.V. Synthetic blockers of nonenzymatic glycosylation of proteins in the experimental treatment of diabetes / V.V. Emelyanov, E.A. Savateeva, L.P. Sidorova, T.S. Bulavintseva, I.F. Gette, I.G. Danilova, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Fundamental glycobiology: conference materials. II All-Russian Conference. Saratov, July 7-11, 2014 – Saratov: "Perspektiva", 2014. p. 30.
13. Savateeva, E.A. Sulfur-containing heterocyclic compounds with potential antidiabetic activity / E.A. Savateeva, V.V. Emelyanov, A.V. Musalnikova, L.P. Sidorova, N.E. Maksimova, N.N. Mochulskaya, V.A. Chereshnev // Chimica Techno Acta. 2014. V.1, №3. pp. 83 - 86.

List of abbreviations

1,3,4-TD – 1,3,4-thiadiazine

2-G-4-PT – 2-guanidine-4-pyridinethiazole

AA – ascorbic acid

AGE – advanced glycosylation end products

BSA – bovine serum albumin

DM – diabetes mellitus

FA – fructosamine

FRO – free radical oxidation

G-SH – glutathione reduced

G-SS-G – glutathione oxidized

GEHI – genetically engineered human insulin

HbA_{lc} – glycosylated hemoglobin

LA – lipoic acid

MDA – malonic dialdehyde

NGP – nonenzymatic glycosylation of proteins

RAGE – receptor for advanced glycosylation end products