Georgian

Medical

News

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

No 7-8 (280-281) 2018

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GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

მლებთან. ამ აქტივობაში ისინი უკავშირდებიან ადიპოკინებს.

ნაშრომის მიზანია განისაზღვროს RANKL-RANKL-ის ოსტეოპროტეგერინის ღერძის ძირითადი რეგულების,და ციტოკინების დამატებითი სარეგულაციო ქსელის კომპონენტების - ადიპონექტინისა და ვისფატინის როლი და ამ ციტოკინების ურთიერთქმედება ვირთაგვის თირკმლის ფუნქციის ქრონიკული უკმარისობის მოდელზე. მოდელი განიხორციელდა მწვავე უკმარისობიდან მე-12 კვირას,რაც გამოწვეული

იყო გლიცერინის 50% ხსნარის ინიექციით კუნთებში. ოსტეოპროტეგერინის, RANKL, ადიპონექტინისა და ვისფატინის დონე განისაზღვრა იმუნოფერმენტული ანალიზით.

დადგენილია,რომ ციტოკინების ღერძის ეფექტი, რომელშიც RANKL ტრიგერს წარმოადგენს, „ძვლოვანი ქსოვილი - თირკმელი" ურთიერთრეგულაციაში ვლინდება ანთებითი პროცესით, ისევე როგორც ვისფატინისა, ხოლო ადიპონექტინი ძვლოვან ქსოვილზე ავლენს საპირისპირო დამცველობით ზემოქმედებას.

SYNTHESIS OF THE NEW 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl) ACETIC ACID DERIVATIVES AND ANALYSIS OF THEIR ANTIOXIDANT ACTIVITY IN NITROSATIVE STRESS MODELS

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Neuroprotection and antioxidant treatment today are a perspective and proved direction because neurodestruction due to activation free radical oxidation can provoke the permanent loss of motor or mental function. Nitric oxide (NO$\cdot$) can play the role of a modulator as active free radical in the central nervous system. In CNS pathology nitric oxide can be a pathogenic factor of changes inside the cells during cerebral ischemia [1,2]. In the acute phase of ischemia NO$\cdot$ plays a neuroprotective role but then especially in reperfusion (recirculation) period NO$\cdot$ effect becomes neurotoxic [3,4]. Especially at that moment in the nerve cells oxidative-nitrosative stress develops where reactive nitrogen species act together with reactive oxygen species. It results in nitration of the metal groups in enzymes and causes their dysfunction. It also causes nucleic acid fragmentation, decreasing NAD$^+$ and ATP level in the cells, inhibition of mitochondrial enzymes activities [5].

Major aggressive factor during this oxidative-nitrosative stress is peroxynitrite (ONOO$\cdot$) due to extra production NO$\cdot$. As a strong oxidant peroxynitrite damages different biologically active molecules and cell organelles. Also ONOO$\cdot$ reacts with superoxide dismutase's active center and produces toxic nitrosonium (NO$^+$) which nitrosizes phenol groups of amino acids (including tyrosine). These impulses in the cells can start their apoptosis [6,7]. So investigation of the antioxidants which will be able to decrease NO$\cdot$ and ONOO$\cdot$ toxicity (especially towards superoxide dismutase) seems to be very of current interest.

[1,2,4]triazino[4,3-*c*]quinazoline represents a scantly explored group of biologically active substances. It was established that derivatives of 3,4,6,7-tetrahydro- and 3,4-dihydro-2*H*-[1,2,4]triazino[4,3-*c*]quinazolines could potentiate barbiturate action and also showed antidepressant, anti-inflammatory and analgesic activity.

So we conducted investigation antioxidant activity of the nine new original derivatives of 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl)acetic acid in vitro and in vivo by using two nitrosative stress models. We studied protective effects of these acid derivatives on superoxide dismutase (SOD) activity under conditions of excessive NO$\cdot$ and ONOO$\cdot$ production.

Materials and methods. *In vitro study*

In the first experimental model, the antioxidant activity of compounds was estimated in vitro with NO$\cdot$ induction [8]. NO$\cdot$ induction was performed under the action of light on sodium nitroprusside sample (the light source was 300W, wavelength > 425nm). The light ray was focused on the sample with the help of a lens. To remove the thermal effect, the ray was directed through the water filter. We used a water solution of sodium nitroprusside $Na_2[Fe(NO)(CN)_5] \times 2H_2O$ (1.0 mM) which was radiated in quartz flask lasting 30min. The efficiency of NO$\cdot$ generation (control) and antioxidant activity of the investigated substances were measured by oxidation rate of ascorbic acid (40 mM, wavelength = 265 nm). The investigated substances were added to the samples before radiation at a concentration 10^{-6}M. Antioxidant activity was expressed in percent of inhibition of ascorbic acid oxidation.

In vivo study. In second experimental model, investigation was carried out in the brain supernatant obtained from the white Wistar rats (male, 200-250g, 4.5 months old). The rats were supplied by the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine, Kyiv. All procedures were conducted accordingly with the Directive 2010/63EU of European Parliament and Council regarding the protection of animals used for experimental and other scientific purposes (Zaporozhye State Medical University Ethics Committee permit No. 62 from 05.IV.2017). The rats were assigned to individual housing in stainless steel, wire-bottomed cages. The quarantine period for all animals was fourteen days. The animals were examined every day regarding the general state, health and death rate. Cages with animals were placed in separate rooms. Lighting was maintained at the 12h light and 12h dark cycles. The temperature was within 19-25C°, humidity – 50-70%. The ventilation was set at 15 air volumes per hour and provided a concentration of CO_2 no more than 0.15%, ammonia – no more than 0.001 mg/l. Water (processed by reverse osmosis) and food were available ad libitum from individual bottles and feeders.

In the day of the experiment after reaching the deep anesthesia with sodium pentobarbital (40.0 mg/kg) rat's cranium was

quickly opened, and brain was extracted. The brain tissue was cooled in isotonic solution of sodium chloride then homogenized in 5 volumes of 50mM Tris-HCl buffer (pH=7.4; t=5°C) containing 0,5mM of EDTA and 1mM of dithiothreitol. The obtained homogenate was centrifuged at 11000g for 30min, t=4°C. For the nitrosative stress modeling dinitrozolic complex of Fe^{2+} and cysteine were utilized. This is a stable $NO^.$ complex which may be considered as a transport form of this radical [9]. $NO^.$-cysteine complex was added to the supernatant (content of protein 5 mg/ml) at a concentration 100mM and was incubated for 10min at 4°C. The investigated substances were introduced before incubation at a concentration $10^{-6}M$.

The concentration of nitrotyrosine in brain homogenate was quantitatively estimated by immunoenzymatical analysis. Samples and standards were incubated in microtiter with antibodies recognizing nitrotyrosine. During this incubation, nitrotyrosine was captured by the antibodies. Unbound material present in the sample was removed by washing. Later biotinylated second antibodies (tracer) to nitrotyrosine were added to the wells. If nitrotyrosine was present in the sample, the tracer antibodies would bind to the captured nitrotyrosine. Excess tracer was removed by washing. Than streptavidin-peroxidase conjugate was applied to the wells and reacted specifically with the biotinylated tracer antibody which was bounded onto the captured nitrotyrosine. The excess streptavidin-peroxidase conjugate was removed by washing. Tetramethylbenzidine was added to the wells. The color developed proportionally to the amount of nitrotyrosine presence in the sample and was measured with a spectrophotometer at 450nm. A standard curve was obtained by plotting the absorbance versus the corresponding concentrations of defined standard (Nitrotyrosine. Elisa Test Kit, HyCult Biotechnology b.v., HK 501).

Protein sedimentation with 20% solution of trichloracetic acid was performed in the supernatant aliquot. 1.0ml of 0.1M 2,4-dinitrophenylhydrazine solution in 2M HCl was added to denature protein and sample was incubated at 37°C for 60min. Then the sample was centrifuged at 3000g for 20min. Sediment was washed with ethanol:ethylacetate mixture (1:1) 3 times. The obtained sediment was dried in thermostatic apparatus at 40°C and then it was dissolved in 3.0ml of 8M urea. For better solubility 0.01ml of 2M HCl was added. The optic density of dinitrophenylhydrazones was registered at wavelength 274nm (aldehydes) and 363nm (carboxyles). The rate of protein oxidative modification was expressed in units of optical density per 1g of protein [8].

This method of SOD activity estimation was based on adrenaline's autooxidation in basic medium leading to superoxide radical formation. At SOD presence this reaction slows down [10]. 4.4 ml of 0.5mM carbonate buffer (pH=10.2) was put into spectrophotometer flask with the optical path of 1cm. 0.1ml of supernatant and 0.5ml of adrenaline solution in citric acid (100ml of distillate contains 192mg of citric acid and 333mg of adrenaline) was added to buffer solution. The kinetics of reaction was registered at 480nm. SOD activity was expressed in units per 1mg of protein per min.

Statistical parameters were calculated using «Statistics» for Windows. For the estimation of significant differences between the groups the Mann-Whitney criteria were used.

Synthesis of original compounds. All materials were purchased from Merck. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer and were within ±0.3 of the theoretical values. Melting points (mp) were determined in open capillary tubes of a Thiele's apparatus. UV spectra (200-400nm) were recorded on a «Specord-200». IR spectra (4000-600cm^{-1}) were recorded on a Bruker ALPHA FT-IR spectrometer using a module for measuring attenuated total reflection (ATR). ^{1}H-NMR spectra were recorded on a Mercury 400 (400MHz) spectrometer

with tetramethylsilane (TMS) as internal standard in dimethylsulphoxide (DMSO-d$_6$) solution. Chemical shifts (δ) are given in ppm units. *J* values are reported in Hz. Electron impact mass spectra (EI-MS) were determined on a Varian 1200L instrument at 70eV. Liquid chromatography-mass spectrometry (LC-MS) results were recorded using a chromatography/mass spectrometric system which consists of high-performance liquid chromatograph "Agilent 1100 Series" equipped with diode-matrix and mass-selective detector "Agilent LC/MSD SL". The structures of new compounds were investigated by combustion elemental analysis and spectroscopic measurements.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid (2) was synthesized by treatment of 4-hydrazinoquinazoline **(1)** (12.85g, 80mmol) in glacial acetic acid or dimethylformamide (DMF) (100mL) with maleic anhydride (9.41g, 96mmol) [11,12]. The resulting mixture was refluxed with stirring for 2-3h. After cooling, a crystalline precipitate was filtered off and washed with EtOH (Fig.).
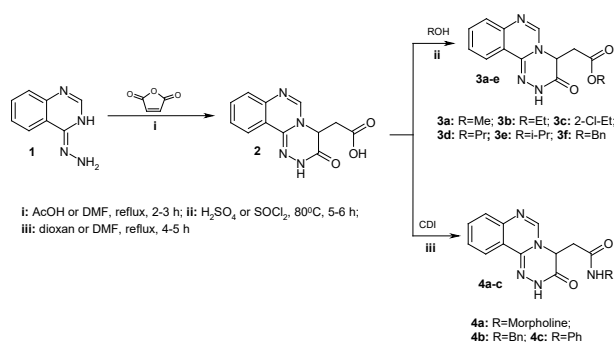
Fig. Synthesis of 2-(3,4-dihydro-3-oxo-2H-[1,2,4] triazino[4,3-c]quinazolin-4-yl) acetic acids (2), esters (3a-f) and amides (4a-c).

AcOH – acetic acid; DMF – dimethylformamide; Me – Methyl; Et – Ethyl; Pr – Propyl; Bn – Benzyl; Ph – Phenyl

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid (2). Yield 77.2%; mp 266-268°C; ^{1}H-NMR δ: 2.93 (d, 2H, *J*=4.8Hz, CH$_2$), 5.06 (t, 1H, *J*=4.8Hz, H-4), 7.4 (m, 2H, H-8,10), 7.56 (t, 1H, *J*=7.6Hz, H-9), 7.81 (s, 1H, H-6), 7.90 (d, 1H, *J*=7.6Hz, H-11), 10.96 (s, 1H, NH); LC-MS *m/z*: 259 (MH$^+$), 213 ([MH–COOH]$^{+}$); EI-MS *m/z*: 258 (M$^{+.}$, 17.0), 213 (31.9), 212 (100.0), 211 (4.1), 200 (4.0), 199 (43.9), 171 (3.8), 156 (18.2), 154 (4.3), 130 (4.4), 129 (51.6), 128 (17.3), 127 (4.5), 116 (3.1), 115 (7.3), 101 (4.1). *Anal.* Calcd for C$_{12}$H$_{10}$N$_4$O$_3$: C, 55.81; H, 3.90; N, 21.70. Found: C, 55.85; H, 3.96; N, 21.64.

The IR spectrum of acid (2) was characterized by stretching vibrations of associated vibrations ν$_{NH}$ and ν$_{OH}$ at 3444-3011cm^{-1}, by the intensive characteristic band at 2784-2469cm^{-1}, by vibrations νc$_{=O}$ at 1738-1706cm^{-1} and γ$_{(OH...O)}$ at 992-917cm^{-1}. Moreover intensive aliphatic CH$_2$ group band of symmetric and asymmetric stretching vibrations at 2978-2855cm^{-1}, stretching vibrations of the carbonyl group of lactam bond at 1712-1662cm^{-1}, ν$_{C=C}$ at 1643-1468cm^{-1} and intensive out-of-plane deformation of CH of the aromatic rings at 846-771cm^{-1} were shown for substance (2).

The ^{1}H-NMR spectrum of acid (2) was presented as: the two protons of the exocyclic methylene group appeared as a doublet at 2.93ppm and the H(4) was found as a triplet at 5.06ppm with coupling constant 4.8Hz. Other signals related to the quinazoline protons in the aromatic region and NH-proton at 10.96ppm. In the LC-MS analysis of compound **(2)** was found the intense peak of quasimolecular ion [MH]$^+$ (*m/z*=259) and [MH-COOH]$^{+.}$ (*m/z*=213). The EI-MS spectrum of compound **(2)** showed its mo-

lecular ion peak at *m/z*=258 which fragmentation corresponds to initial loss of the COOH, HCOOH and CH$_2$COOH groups. Further destruction related to the C(11b)–N(1) and N(5)–C(4) bonds and formation of quinazolinium ion (*m/z*=129). Another fragmentation pattern concerned with N(1)–N(2) and C(4)–C(3) bonds breaking followed by elimination of HNCO.

2-(3,4-Dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl)acetic acid esters (3a–f).

Method A. A mixture of acid (**2**) (1.29g, 5mmol) with alcohol (10mL) and H$_2$SO$_4$ (0.5-1.0mL) was heated at 80°C for 5-6h. The mixture was cooled to room temperature and quenched with NaH-CO$_3$. The formed precipitate was filtered off and washed with H$_2$O.

Method B. SOCl$_2$ (0.54mL, 7.5mmol) was added dropwise to a suspension of acid (**2**) (1.29g, 5mmol) in an alcohol (10mL) and DMF (1 drop). The mixture was heated at 80°C for 5-6h. After cooling till room temperature the solution was quenched with NaH-CO$_3$. The formed precipitate was filtered off and washed with H$_2$O.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl) acetic acid methyl ester (3a). Yield 82.6% (method B); mp 218-224°C (methanol); ^{1}H-NMR δ: 3.03 (d, 2H, *J*=4.8Hz, CH$_2$), 3.52 (s, 1H, CH$_3$), 5.11 (t, 1H, *J*=4.8Hz, H-4), 7.40 (m, 2H, H-8,10), 7.57 (t, 1H, *J*=7.6Hz, H-9), 7.81 (s, 1H, H-6), 7.91 (d, 1H, *J*=7.6Hz, H-11), 11.00 (s, 1H, NH); LC-MS *m/z*: 272 (M$^+$), 213 (M–COOCH$_3$); EI-MS *m/z*: 273 (7.0), 272 (34.1), 254 (12.5), 241 (8.3), 240 (21.2), 239 (5.7), 238 (5.6), 214 (6.0), 213 (51.4), 212 (100), 210 (34.7), 200 (10.8), 199 (53.0), 171 (17.2), 130 (22.0), 129 (50.1), 117 (12.0), 116 (6.0), 115 (8.4), 103 (26.3), 102 (40.9), 90 (19.6), 90 (19.6), 89 (8.8), 88 (6.7), 76 (17.6), 75 (13.2), 63 (6.5), 55 (23.6). *Anal.* Calcd for C$_{13}$H$_{12}$N$_4$O$_3$: C, 57.35; H, 4.44; N, 20.58. Found: C, 57.30; H, 4.39; N, 20.64.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid ethyl ester (3b). Yield 60.0% (method A); mp 184-186°C (methanol); ^{1}H-NMR δ: 1.03 (t, 3H, *J*=7.1Hz, CH$_3$), 3.01 (d, 2H, *J*=4.8Hz, CH$_2$COOH), 3.96 (m, 2H, CH$_2$CH$_3$), 5.12 (t, 1H, *J*=4.8Hz, H-4), 7.40 (m, 2H, H-8,10), 7.57 (t, 1H, *J*=7.7Hz, H-9), 7.82 (s, 1H, H-6), 7.91 (d, 1H, *J*=7.7Hz, H-11), 11.00 (s, 1H, NH); LC-MS *m/z*: 287 (MH$^+$). *Anal.* Calcd for C$_{14}$H$_{14}$N$_4$O$_3$: C, 58.74; H, 4.93; N, 19.57. Found: C, 58.69; H, 4.98; N, 19.61.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid 2-chloroethyl ester (3c). Yield 75.0% (method B); mp 220-224°C (dioxane); LC-MS *m/z*: 321, 323 (MH$^+$). *Anal.* Calcd for C$_{14}$H$_{14}$N$_4$O$_3$: C, 52.4; H, 4.09; Cl, 11.05; N, 17.47. Found: C, 52.53; H, 4.12; Cl, 11.12; N, 17.51.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid propyl ester (3d). Yield 91.9% (method B); mp 189-192°C (propanol-2); ^{1}H-NMR δ: 0.81 (t, 3H, *J*=7.1Hz, CH$_3$); 1.51 (sxt, 2H, *J*=7.1Hz, CH$_2$CH$_3$), 2.91 (d, 2H, *J*=5.0Hz, CH$_2$COOH), 3.93 (t, 2H, *J*=6.6Hz, CH$_2$C$_2$H$_5$), 5.03 (t, 1H, *J*=5.0Hz, H-4), 7.29 (t, 1H, *J*=7.6Hz, H-10), 7.35 (d, 1H, *J*=7.6Hz, H-8), 7.46 (t, 1H, *J*=7.6Hz, H-9), 7.69 (s, 1H, H-6), 7.94 (d, 1H, *J*=7.6Hz, H-11), 10.87 (s, 1H, NH); LC-MS *m/z*: 301 (MH$^+$). *Anal.* Calcd for C$_{15}$H$_{16}$N$_4$O$_3$: C, 59.99; H, 5.37; N, 18.66. Found: C, 60.04; H, 5.32; N, 18.62.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid isopropyl ester (3e). Yield 20.8% (method A), 91.3% (method B); mp 220-224°C (ethanol); ^{1}H-NMR δ: 1.02 (m, 6H, CH(CH$_3$)$_2$), 2.98 (d, 2H, *J*=4.8Hz, CH$_2$), 4.78 (m, 1H, CH(CH$_3$)$_2$) 5.11 (t, 1H, *J*=4.8, H-4), 7.40 (m, 2H, H-8,10), 7.56 (t, 1H, *J*=7.6Hz, H-9), 7.83 (s, 1H, H-6), 7.90 (d, 1H, *J*=7.6Hz, H-11), 11.00 (s, 1H, NH); LC-MS *m/z*: 301 (MH$^+$). *Anal.* Calcd for C$_{15}$H$_{16}$N$_4$O$_3$: C, 59.99; H, 5.37; N, 18.66. Found: C, 60.03; H, 5.40; N, 18.71.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid benzyl ester (3f). Yield 80.5% (method B); mp 200-202°C (dioxane-H$_2$O); ^{1}H-NMR δ: 3.00 (d, 2H, *J*=5.0Hz, CH$_2$+H$_2$O), 5.02 (s, 2H, CH$_2$C$_6$H$_5$), 5.07 (t, 1H, *J*=5.0Hz, *J*=7.6Hz, H-4), 7.21 (m, 5H, H$_{Ph}$), 7.30 (t, 1H, *J*=7.6Hz, H-10), 7.36 (d, 1H, *J*=7.6Hz, H-8), 7.47 (t, 1H, *J*=7.6Hz, H-9), 7.71 (s, 1H, H-6), 7.93 (d, 1H, *J*=7.6Hz, H-11), 10.89 (s, 1H, NH); LC-MS *m/z*: 349 (MH$^+$). *Anal.* Calcd for C$_{19}$H$_{16}$N$_4$O$_3$: C, 65.51; H, 4.63; N, 16.08. found: C, 65.47; H, 4.69; N, 16.02.

Shifting of stretching vibrations in the high-frequency area of the spectrum (1748-1729cm^{-1}), appearance the band of stretching vibrations v_{C-O} at 1360-1300cm^{-1} which had a similar intensity of $v_{C=O}$, and appearance the band of more intensive and wide symmetric and asymmetric aliphatic group stretching vibrations at 2978-2797cm^{-1} revealed for esters (**3a-f**).

From acid (**2**) we obtained esters (**3a-f**) by method A and method B (more manageable and provided better yields). In the spectra of esters (**3a-f**) characteristic triplet of H(4) at 5.12-5.00ppm and two proton doublet of CH$_2$ group at 3.03-2.91ppm were revealed. In addition the signals of alkoxy groups appeared in high-field region.

2-(3,4-Dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl)acetic acid amides (4a-c).

5mmol (1.0g) carbonildiimidazole (CDI) was added to a solution of 5mmol (1.29g) acid (**2**) in 10ml waterless dioxan or DMF. The mixture was refluxed at 60-80ºC temperature for 1-1.5h. Then 5mmol of an appropriate amine was added to the solution and mixture was heated for 6h. After cooling till room temperature H$_2$O was added. The resulting solution was adjusted to pH 6-7 by adding acetic acid. The obtained precipitate was filtered off and dried in air.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid morpholide (4a). Yield 61.9%; mp 220-224°C (propanol-2); ^{1}H-NMR δ: 2.96 (d, 2H, *J*=5.0Hz, CH$_2$+H$_2$O), 3.50-3.38 (m, 8H, morfoline), 5.02 (g, 1H, *J*=3.3Hz, H-4), 7.28 (t, 1H, *J*=7.8Hz, H-10), 7.35 (d, 1H, *J*=7.8Hz, H-8), 7.45 (t, 1H, *J*=7.2Hz, H-9), 7.72 (s, 1H, H-6), 7.93 (d, 1H, *J*=7.0Hz, H-11), 10.80 (s, 1H, NH); LC-MS *m/z*: 328 (MH$^+$); EI-MS *m/z*: 328 (3.7), 327 (22.0), 240 (39.9), 213 (38.9), 212 (100.0), 199 (5.9), 144 (7.4), 130 (13.7), 129 (24.2), 103 (7.1), 102 (9.1), 88 (11.0), 70 (9.6), 56 (7.3), 43 (5.7). *Anal.* Calcd for C$_{16}$H$_{17}$N$_5$O$_3$: C, 58.71; H, 5.23; N, 21.39. Found: C, 58.83; H, 5.36; N, 21.42.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid benzylamide (4b). Yield 51.4%; mp 238-239°C (ethanol–H$_2$O); ^{1}H-NMR δ: 2.78 (m, 2H, CH$_2$+H$_2$O), 4.18 (d, 2H, *J*=2.5Hz, CH$_2$C$_6$H$_5$), 5.07 (t, 1H, *J*=6.6Hz, H-4), 7.11 (m, 3H, H-3, H-4, H-5 Ph), 7.03 (d, 2H, *J*=6.1Hz, H-2, H-6 Ph), 7.40 (t, 1H, *J*=7.4Hz, H-10), 7.43 (d, 1H, *J*=7.5Hz, H-8), 7.59 (t, 1H, *J*=7.4Hz, H-9), 7.69 (s, 1H, H-6), 7.90 (d, 1H, *J*=8.2Hz, H-11), 8.52 (d, 1H, *J*=4.7Hz, NH), 10.97 (s, 1H, NH); LC-MS *m/z*: 348 (MH$^+$); EI-MS *m/z*: 348 (3.8), 347 (25.2), 241 (6.2), 240 (68.3), 239 (23.5), 214 (6.7), 213 (61.3), 212 (100.0), 171 (6.7), 144 (10.3), 130 (12.6), 129 (37.0), 117 (8.1), 105 (6.2), 104 (8.6), 103 (11.7), 102 (14.2), 92 (9.3), 91 (36.3), 89 (5.9), 77 (8.0), 65 (6.9), 55 (6.5), 51 (5.6). *Anal.* Calcd for C$_{19}$H$_{17}$N$_5$O$_2$: C, 65.70; H, 4.93; N, 20.16. Found: C, 65.79; H, 4.99; N, 20.22.

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid phenylamide (4c). Yield 84.6%; mp 272-276°C (propanol-2–H$_2$O); ^{1}H-NMR δ: 2,98 (1H, -CH2, *J*=4.9Hz); 3,09 (1H, -CH2, *J*=4.9Hz), 5,18 (1H, H-4, *J*=4.7Hz), 7,0 (1H, H-4 Ph, *J*=7.4Hz), 7,26 (2H, H-3, 5 Ph), 7,38 (2H, H-8, 10), 7,42 (3H, H-2, 5 Ph), 7,52 (1H, H-9, *J*=7.6Hz), 7,72 (1H, H-6), 7,91 (1H, H-11, *J*=7.4Hz), 10,1 (1H, -C(O)NH), 10,97 (1H, 2-NH); LC-MS *m/z*: 334 (MH$^+$); EI-MS *m/z*: 334 (3.0), 333 (12.9), 240 (37.5), 213 (20.3), 212 (100.0), 171 (7.6), 130 (15.0), 129 (25.4), 117 (7.5), 103 (6.6), 102 (7.7), 93 (10.3), 77 (6.5). *Anal.* Calcd for C$_{18}$H$_{15}$N$_5$O$_2$: C, 64.86; H, 4.54; N, 21.01. Found: C, 64.92; H, 4.51; N, 21.09.

In the IR spectra of amides (**4a-c**) the intensive band of ab-

Table 1: Antioxidant activity of the investigated compounds in sodium nitroprusside photoinduced reaction (in vitro)

Cipher of compound	D of optical density at l=265nm	Antioxidant activity, %
(2)	0.6±0.01	0
(3a)	0.32±0.005*	46.7
(3b)	0.35±0.01*	41.2
(3c)	0.33±0.01	45.0
(3d)	0.35±0.01*	41.7
(3f)	0.3±0.005*	50.0
(4a)	0.35±0.01	41.7
(4b)	0.34±0.005*	48.7
(4c)	0.38±0.01	36.7
Control	0.6±0.01	-

*notes: * – the probability compare to control group (p≤0.05)*

Table 2. Effect of (3f) on SOD activity and content of the products after protein oxidative modification
in the supernatant of rats' brain in the nitrosative stress model (in vivo)

The investigated samples	SOD, units/mg protein/min	Products of POM, units/mg protein		Nitrotyrosine in brain homogenate, nM
		270nm	363nm	
Intact	260.7±7.6	14.0±0.11	22.3±0.17	3.7±0.8
Control (adding of stable NO· and cysteine complex, 100μM)	120.2±5.0	26.2±0.21	36.7±0.10	11.0±0.7
Experimental (adding of stable NO· and cysteine complex + **(3f)**, 100μM)	200.6±4.2*	17.8±0.10*	27.6±0.11*	4.8±0.4

*notes: * – the probability compare to the intact group (p≤0.05)*

sorptions appeared at 3199-3024cm^{-1} and 3387-3149cm^{-1}, and stretching vibrations were shown at 1659-1628cm^{-1} (amide I) and out-of-plane deformation at 1613-1550cm^{-1} (amide II).

The acid (2) was also turned in amides 4a-c after the activation of the carboxylic group by carbonyldiimidazole in a waterless solvent (dioxane or DMF). The spectra of amides (4a-c) displayed characteristic two singlets of 2-NH at 10.97-10.80ppm and amide group proton at 8.52ppm, a triplet of H(4) at 5.07-5.02ppm, two proton doublet of CH$_2$ group at 2.96-2.78ppm. The signals of alkyl and aryl groups appeared in the high-field region.

Analysis of antioxidant activity

Antioxidant activity estimation of the investigated compounds in sodium nitroprusside photoinduction model revealed that the synthesized esters (3a-3f) and amides (4a-4c) of 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acids play a significant role in the inhibition of NO· formation. In this model acid (2) wasn't active while benzyl ester (3f) of acid (2) residue showed the maximal activity in the inhibition of NO· formation (Table 1). We are prone to think that the activity of these compounds is due to substitution inside radical.

The model of nitrosative stress in the supernatant of rats' brain was characterized by the inhibition of antioxidant enzyme (superoxide dismutase) activity due to increasing a formation of products after protein oxidative modification (aldehyde-phenylhydrazones and ketone-phenylhydrazones). These reaction products showed the maximum of absorption at 270 and 363nm (Table 2).

The obtained data does not contradict the data obtained by other investigators which showed that the nitration of thiol groups of protein molecules (receptors, ionic channels) and phenols occurred in nitrosative stress [13]. The activity of antioxidant enzymes is inhibited by formation of nitrocomplexes with metal ions of enzyme's active center. Reduction of SOD activity and thiol level results in significant increase of the free radical oxidation [1-4,6,13-15].

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid benzyl ester (3f) (10^{-6}M) adding into incubating mixture before nitrosative stress modeling resulted in protective activity towards superoxide dismutase which was the most significant compared to other derivatives of acid (2) (Table 2). Also (3f) most effectively reduced the formation of the products after protein oxidative modification and nitrotyrosine. One of the most important mechanisms (3f)'s antioxidant activity is the ability to stabilize NO molecule and to decrease hyperproduction NO radicals. This can protect SOD from deactivation.

Conclusion. In the present paper, the series of new 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl)acetic acids derivatives which were tested for antioxidant activity in the nitrosative stress model were described. Synthesized new compounds displayed high antioxidant activity. 2-(3,4-Dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl)acetic acid benzyl ester **(3f)** is the most active compound. This activity revealed in defending SOD from damage in nitrosative stress.

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SUMMARY

SYNTHESIS OF THE NEW 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl) ACETIC ACID DERIVATIVES AND ANALYSIS OF THEIR ANTIOXIDANT ACTIVITY IN NITROSATIVE STRESS MODELS

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Today we know that NO· and ONOO- are clue pathophysiological factors for progression some ischemic diseases of the central nervous system. So investigation of the antioxidants which will be able to decrease NO· and ONOO- toxicity seems to be very of current interest.

The six esters and three amides of 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid were synthesized for this study, and we showed evidence of antioxidant activity of these new original derivatives.

We studied the effect of 2-(3,4-dihydro-3-oxo-2H-[1,2,4] triazino[4,3-c]quinazolin-4-yl)acetic acid derivatives on superoxide dismutase activity under the condition of excessive NO· and ONOO- production. NO· induction was performed by the action of light on sodium nitroprusside Na2[Fe(NO)(CN)5]×2H2O in vitro. Also, the investigation of the substances was carried out in the brain supernatant obtained from the white Wistar rats in vivo. For nitrosative stress modeling dinitrozolic complex of Fe2+ and cysteine were utilized.

Our data showed that 2-(3,4-dihydro-3-oxo-2H-[1,2,4] triazino[4,3-c]quinazolin-4-yl)acetic acid is not active compound while its esters and amides have antioxidant activity. Compound benzyl ester of this acid revealed the most effective antioxidant activity.

Keywords: quinazoline derivative, antioxidant, nitrosative stress, neuroprotection.

РЕЗЮМЕ

СИНТЕЗ НОВЫХ ПРОИЗВОДНЫХ 2-(3,4-DIHYDRO-3-OXO-2*H*-[1,2,4]TRIAZINO[4,3-*C*]QUINAZOLIN-4-YL) ACETIC ACID И АНАЛИЗ ИХ АНТИОКСИДАНТНОЙ АКТИВНОСТИ

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Известно, что NO· и ONOO· являются ключевыми патофизиологическими факторами прогрессирования некоторых ишемических заболеваний центральной нервной системы, исходя из этого, исследование антиоксидантов, способных снижать токсичность NO· и ONOO·, представляется весьма актуальным.