SYNTHESIS OF THE NEW 2-(3,4-DIHYDRO-3-OXO-2H-[1,2,4]TRIAZINO[4,3-C]QUINAZOLIN-4-YL) ACETIC ACID DERIVATIVES AND ANALYSIS OF THEIR ANTIOXIDANT ACTIVITY IN NITROSATIVE ST....

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адипокинами. Целью данной статьи было исследование роли основных участников оси RANK-RANKL-остеопротегерин и цитокинов дополнительного звена регуляторной сети - адипонектина и висфатина и взаимосвязи этих цитокинов на модели хронической нарушения функции почек у крыс. Модель хронического нарушения функции почек была создана у крыс через 12 недель после острой почечной недостаточности, вызванной внутримышечной инъекцией 50% глицерина. Уровни RANKL, остеопротегерина, адипонектина и висфатина измеряли методом ИФА. Увеличение количества цитокинов и изменения их взаимосвязи выявлены у крыс с моделью хронического нарушения функции почек. Сделан вывод о том, что эффекты оси цитокинов, где RANKL является триггером, в сети регуляции межклеточных связей «костная ткань - почки», имеют воспалительный характер, так же, как и влияние висфатина, а адипонектин оказывает противоположный сохраняющий костную ткань эффект.

SYNTHESIS OF THE NEW 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl) ACETIC ACID DERIVAYIVES 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 ($ONOO^-$) due to extra production $NO^{\cdot}$. As a strong oxidant peroxynitrite damages different biologically active molecules and cell organelles. Also $ONOO^-$ 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 $ONOO^-$ 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 $ONOO^-$ 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 "Agi1ent 1100 Series" equipped with diode-matrix and mass-selective detector "Agi1ent LC/MSD SL". The structures of new compounds were investigated by combustion elemental analysis and spectroscopic measurements.

2-(3,4-Dihydro-3-oxo-2*H*-[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.).

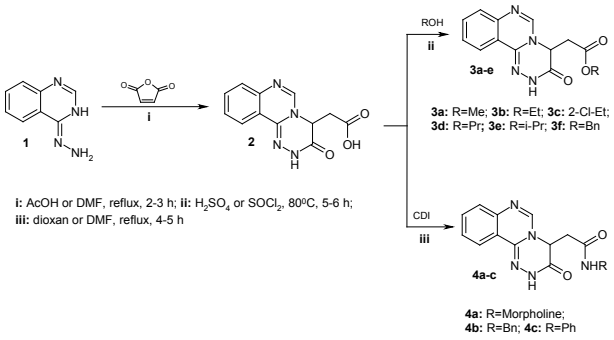

Fig. Synthesis of 2-(3,4-dihydro-3-oxo-2H-[1,2,4] triazino[4,3-c]quinazolin-4-yl) acetic acids (2), esters (3a-f) and amides (4a-c).

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 ($[MH–COOH]^{+\cdot}$); EI-MS *m/z*: 258 ($M^{+\cdot}$, 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 $C_{12}H_{10}N_4O_3$: 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 $\nu c_{=O}$ at 1738-1706cm^{-1} and $\gamma_{(OH...O)}$ 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}, $\nu_{C=C}$ 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 $[MH\text{-}COOH]^{+\cdot}$ (*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_2COOH 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_2SO_4 (0.5-1.0mL) was heated at 80°C for 5-6h. The mixture was cooled to room temperature and quenched with $NaHCO_3$. The formed precipitate was filtered off and washed with H_2O.

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 $NaHCO_3$. The formed precipitate was filtered off and washed with H_2O.

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 $C_{13}H_{12}N_4O_3$: 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_2COOH), 3.96 (m, 2H, CH_2CH_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 $C_{14}H_{14}N_4O_3$: 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 $C_{14}H_{14}N_4O_3$: 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_2CH_3), 2.91 (d, 2H, *J*=5.0Hz, CH_2COOH), 3.93 (t, 2H, *J*=6.6Hz, $CH_2C_2H_5$), 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 $C_{15}H_{16}N_4O_3$: 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 $C_{15}H_{16}N_4O_3$: 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_2O); ^{1}H-NMR δ: 3.00 (d, 2H, *J*=5.0Hz, CH_2+H_2O), 5.02 (s, 2H, $CH_2C_6H_5$), 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 $C_{19}H_{16}N_4O_3$: 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 ν_{C-O} at 1360-1300cm^{-1} which had a similar intensity of $\nu_{C=O}$, 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_2O 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_2O), 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 $C_{16}H_{17}N_5O_3$: 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_2O); ^{1}H-NMR δ: 2.78 (m, 2H, CH_2+H_2O), 4.18 (d, 2H, *J*=2.5Hz, $CH_2C_6H_5$), 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 $C_{19}H_{17}N_5O_2$: 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_2O); ^{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 $C_{18}H_{15}N_5O_2$: 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 $NO^{\cdot}$ and cysteine complex, 100μM)	120.2±5.0	26.2±0.21	36.7±0.10	11.0±0.7
Experimental (adding of stable $NO^{\cdot}$ 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 $NO^{\cdot}$ 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 $NO^{\cdot}$ 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 DERIVAYIVES AND ANALYSIS OF THEIR ANTIOXIDANT ACTIVITY IN NITROSATIVE STRESS MODELS

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Today we know that $NO^{\cdot}$ 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 $NO^{\cdot}$ and $ONOO^{-}$ toxicity seems to be very of current interest.

The six esters and three amides of 2-(3,4-dihydro-3-oxo-2*H*-[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-2*H*-[1,2,4] triazino[4,3-*c*]quinazolin-4-yl)acetic acid derivatives on superoxide dismutase activity under the condition of excessive $NO^{\cdot}$ and $ONOO^{-}$ production. $NO^{\cdot}$ induction was performed by the action of light on sodium nitroprusside $Na_2[Fe(NO)(CN)_5]\times 2H_2O$ 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 Fe^{2+} and cysteine were utilized.

Our data showed that 2-(3,4-dihydro-3-oxo-2*H*-[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 $NO^{\cdot}$ и $ONOO^{-}$ являются ключевыми патофизиологическими факторами прогрессирования некоторых ишемических заболеваний центральной нервной системы, исходя из этого, исследование антиоксидантов, способных снижать токсичность $NO^{\cdot}$ и $ONOO^{-}$, представляется весьма актуальным.

В исследовании синтезированы шесть сложных эфиров и три амида 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*] quinazolin-4-yl)acetic acid, выявлено наличие антиоксидантной активности этих новых исходных производных.

Изучено влияние производных 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl)acetic acid на активность супероксиддисмутазы при избыточной продукции $NO^{\cdot}$ and $ONOO^{-}$. Индукция $NO^{\cdot}$ проводилась путем воздействия света на нитропруссид натрия $Na_2[Fe(NO)(CN)_5]\times 2H_2O$ in vitro. Исследование веществ осуществляли на супернатанте головного мозга, полученном от белых крыс Wistar rats in vivo. Для моделирования нитрозативного стресса использовали динитрозольный комплекс Fe^{2+} и цистеин.

Полученные данные выявили, что 2-(3,4-dihydro-3-oxo-2*H*-[1,2,4]triazino[4,3-*c*]quinazolin-4-yl)acetic acid не является активным соединением, тогда как ее сложные эфиры и амиды обладают антиоксидантной активностью. Соединение бензилового эфира этой кислоты характеризуется выраженной антиоксидантной активностью.

რეზიუმე

ახალი 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl) ძმარმჟავას წარმოებულების სინთეზი და მათი ანტიოქსიდაციური აქტივობის ანალიზი

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ზაპოროჟიეს სახელმწიფო სამედიცინო უნივერსიტეტი, [1]ფარმაკოლოგიის კათედრა; [2]ორგანული და ბიოორგანული ქიმიის კათედრა; [3]ა. ბოგომოლეცის სახ. ნაციონალური სამედიცინო უნივერსიტეტი, კიევი, უკრაინა [4]"ყოვლად წმინდა" უნივერსიტეტი, ფარმაკოლოგიის კათედრა, როზო, დომინიკა

სადღეისოდ ცნობილია, რომ NO და ONOO წარმოადგენენ წამყვან პათოფიზიოლოგიური ფაქტორებს ცენტრალური ნერვული სისტემის იშემიური დაავადებების პროგრესირებაში. აქედან გამომდინარე, მეტად აქტუალურია ანტიოქსიდანტების გამოკვლევა,რომლებიც შეამცირებს NO და ONOO ტოქსიურობას.

აღნიშნული კვლევისთვის სინთეზირდა 6 რთული ეთერი და 3 ამიდი 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl) ძმარმჟავა და ჩვენს მიერ დადასტურდა ამ ახალი წარმოებულების ანტიოქსიდანტური აქტივობა.

ჩატარებული კვლევის შედეგად შესწავლილია 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl) ძმარმჟავას ნივთიერებების ეფექტი სუპეროქსიდდისმუტაზის აქტივობაზე NO და ONOO-ს ჭარბი პროდუქციის (წამოების) პირობებში.

NO-ს ინდუქცია განხორციელდა ნატრიუმის ნიტროპრუსიდზე $Na_2[Fe(NO)(CN)_5]x2H_2O$ შუქის ზემოქმედების ქვეშ in vitro. პარალელურად განხორციელდა ნივთიერების კვლევა თეთრი ვირთხების (Wistar) თავის ტვინზე in vivo. ნიტროზული სტრესის მოდელირებისთვის გამოყენებული იყო Fe^{2+}დინიტროზული კომპლექსი და ცისტეინი.

მიღებულმა მონაცემებმა აჩვენა, რომ 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl) ძმარმჟავა არ წარმოადგენს აქტიურ ნაერთს, მაშინ როდესაც მისი რთული ეთერები და ამიდები ფლობენ ანტიქოსიდაციურ აქტივობას. აღნიშნული მჟავის ბენზილის ეთერთან ნაერთმა გამოავლინა უფრო მკვეთრად გამოხატული ანტიოქსიდაციური აქტივობა.

СРАВНИТЕЛЬНЫЙ АНАЛИЗ АССОРТИМЕНТА ГОМЕОПАТИЧЕСКИХ ЛЕКАРСТВЕННЫХ СРЕДСТВ НА ФАРМАЦЕВТИЧЕСКОМ РЫНКЕ УКРАИНЫ, РОССИЙСКОЙ ФЕДЕРАЦИИ, РЕСПУБЛИК БЕЛАРУСЬ И КАЗАХСТАН

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Современные достижения фармакотерапии приводят не только к положительным результатам в борьбе с самыми распространенными заболеваниями, но и к появлению побочных реакций на лекарственные средства (ЛС), возник-

новение лекарственных болезней, аллергии на ЛС, анти- биотикоустойчивых штаммов микроорганизмов. В такой ситуации поиск безопасных терапевтических методом явля- ются жизненной необходимостью для лиц, страдающих ле- карственной непереносимостью. В связи с чем наблюдается рост интереса к нетрадиционным методам лечения, в част-

ности к гомеопатии, которая является одним из перспектив- ных направлений развития медицины XX в. [2,4,7,8].

На сегодняшний день гомеопатия является самостоятель- ным, оригинальным взглядом на здоровье и болезнь, имеет

свою методологию и технологию коррекции здоровья, подтверждено терапевтическими методами, многолетними клиническими наблюдениями и токсикологической практикой. Актуальным является индивидуальный подход к лечению заболевания с учетом характера болезни [5,6,9].

Согласно информации Всемирной организации здравоохранения, в Австралии, Европе и Северной Америке параллельно с аллопатической медициной все шире используют дополнительную и альтернативную медицину, одной из которых является гомеопатия. Гомеопатические лекарственные средства (ГоЛС) используются в Индии (50% врачей), 40% - в Великобритании, 32% - во Франции, 25% - в Германии, 22% - в Австрии [1,3,8]. По данным зарубежной литературы, ГоЛС составляют более 20% в рецептуре аптек. Таким образом, ГоЛС становятся экономической категорией аптечного ассортимента [1-3].