SYNTHESIS OF 2-(3,4-DIHYDRO-3-OXO-2H-[1,2,4]TRIAZINO[4,3-c]QUINAZOLIN-4-YL)ACETIC ACID DERIVATIVES AS POTENTIAL ANTIOXIDANTS IN NITROSATIVE STRESS MODEL

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Abstract
The esters (3a-f) and amides (4a-c) of 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid were synthesized by prior esterification or aminolysis of the acid 2. The structure of the synthesized compounds was confirmed by spectroscopic investigations. Almost all substances, which have been tested in vitro for the antioxidant activity in nitrosative stress, have shown significant antioxidant activity in comparison with N-acetylcystein (N-ACC). Compound 3f revealed the best antioxidant properties in a nitrosative stress model.

Few weeks after the implementation of the new legislation, it seems that its purpose has at least partially been achieved; a number of “dreams shops” closed or changed their field of activity. However, it is possible that some of the consumers of such products, which have rather moderate risks, will replace them with drugs associated with higher risks, acquired from the black market. Whether the general outcome would be positive or negative will remain to be seen.

Keywords: 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid derivatives, nitrosative stress, antioxidant activity

Introduction
Antioxidant strategy today is a perspective and proved direction in the creation of neuroprotective substances, because of neurodestructive pathologies with subsequent activation of free radical oxidation for a long time. One of the damaging factors of central nervous system (CNS)
pathologies, namely brain ischemia, strokes, epilepsy and others, is the nitrogen monooxide (NO) [12]. Apparently, in acute stroke under neuronal NO-synthase activation, NO has a neuroprotective role, but later, especially in the reperfusion (recirculation) period, NO effect becomes neurotoxic (especially under inducible NO-synthtase activation and L-arginine deficit) [9, 10]. In that moment in the cell a nitrosative stress develops as part of oxidative stress [6]. It results nitration in the thiol group and metal cations of protein molecules which causes their dysfunction. It also causes fragmentation in the nucleic acid, the reduction of NAD$^+$ and a decrease of ATP level in the cell, inhibition of mitochondrial enzymes function [2]. The main neurotoxic factor of nitrosative stress is peroxynitrite (ONOO$^-$), which occurs by NO$^-$ excess and the interaction with a superoxide radical. As a strong oxidant ONOO$^-$ has a high cytotoxicity. It induces damage to enzymes sulphydryl groups of protein fragments, receptors, ionic channels, DNA, and proteolipids oxidative modification. Peroxynitrite reacts with metals from superoxide dismutase’s (SOD) active center and results in the formation of high toxic nitrogen dioxide (NO$_2^-$) which nitrosizes the phenol groups of amino acids (including tyrosine). Then, the mechanism of impulse transmission in CNS and the apoptosis start [2, 5, 17].

Substituted [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-2H-[1,2,4]triazino[4,3-c]quinazolines could potentiate barbiturate action and also showed antidepressant, anti-inflammatory and analgesic activity [11, 13-16]. The two last biological activities were also characteristic for 6-pyridyl-2-alkyl-3,4,6,7-tetrahydro-[1,2,4]triazino[4,3-c]quinazolines [6]. Taking into account the afore-mentioned facts about nitrosive stress, a research of new perspective antioxidants among 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino [4,3-c]quinazolin-4-yl)acetic acids derivatives, which may decrease the toxic action of NO and NOO$^-$ is highly necessary.

Materials and methods

4-Hydrazinoquinazoline (1) was prepared as reported [1]. All starting materials were purchased from Merck and used without purification. Melting points were determined in open capillary tubes in a Thiele’s apparatus. The elemental analyses (C, H, N) were performed using the Perkin-Elmer 2400 CHN analyzer and were within ±0.3 of the theoretical values. UV spectra (200-400 nm) were recorded on a «Specord-200». IR spectra (4000-600 cm$^{-1}$) were recorded on a Bruker ALPHA FT-IR spectrometer using a module for measuring attenuated total reflection.
(ATR). $^1$H and $^{13}$C NMR spectra (500 MHz for $^1$H and 125 MHz for $^{13}$C) were recorded on a Bruker Avance DRX-500 spectrometer with tetramethylsilane (TMS) as internal standard in dimethylsulphoxide (DMSO-d$_6$) solution. Chemical shifts values are reported in ppm units using the $\delta$ scale. Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV. 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” (atmospheric pressure chemical ionization – APCI). The purity of all prepared compounds was checked by $^1$H NMR and LC-MS.

**In vitro studies**

The antioxidant activity was estimated using an experimental model for NO$^\cdot$ induction [4]. NO$^\cdot$ induction was performed under the action of light on sodium nitroprusside sample (the light source 300W, $\lambda > 425$ nm). The light ray was focused on the sample by the lens. The irradiation was directed through the water filter to avoid the thermal effect. Irradiation ($\lambda$=1 mm) in quartz flask lasted for 30 min on water solution of Na$_2$[Fe(CN)$_5$NO]×2H$_2$O (1.0 mM). The efficiency of NO$^\cdot$ generation (control) and the antioxidant activity of the investigated substances was measured by the oxidation rate of ascorbic acid (40 mM, $\lambda$=265 nm). The substances were added to the samples before irradiation at a concentration of 10$^{-6}$ M. The optical density was measured before and after irradiation. Antioxidant activity was expressed in percents of inhibition of the ascorbic acid oxidation.

**In vivo studies**

Antioxidant and protective activity towards SOD was studied in a nitrosive stress model. Wistar white rats of both sexes were supplied by the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine, Kiev. All procedures were carried out in accordance with the Directive 86/609/EEC of November 24th 1986, regarding the protection of animals used for experimental and other scientific purposes. Rats were assigned to individual housing in stainless steel, wire-bottomed cages. The rats were 4.5 months old and the weighting 180-200 g. The quarantine period for all the 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 12-h light and 12-h dark cycles. The temperature was within the limits of 19-25°C, relative humidity – 50-70%. The ventilation was set at 15 air volumes per hour and provided a concentration of CO₂ 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 morning of the experiments after reaching the deep anesthesia with sodium pentobarbital (40.0 mg/kg bw, from «Nizhfarm», Russia), rat cranium was quickly opened, brain was extracted.

The investigation of the synthesized substances was carried out on supernatants obtained from the brain homogenates. In this regard, the brain tissues were cooled in isotonic solution of sodium chloride, then homogenized in 5 volumes of 50 mM Tris–HCl buffer (pH=7.4, t=5°C) containing 0.5 mM of EDTA and 1mM of dithiotreitol. The obtained homogenate was centrifuged at 11000 G for 30 min, at 4°C. For the nitrosative stress modeling, dinitrozolic complex of Fe²⁺ and cystein was used. This one was a stable NO· complex which could be considered as a transport form of this radical with a longer NO inhibition activity [2, 5]. NO· cystein complex was added to the supernatant (content of protein 5 mg/ml) in concentration of 100 mM and was incubated for 10 min at 4°C.

The investigated substances were introduced before incubation at a concentration 10⁻⁶ M. For the nitrosative stress and the investigated substances estimation in vitro protein oxidative modification products were determined. Also the SOD activity in a sample was studied for 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid derivatives protective activity estimation.

The concentration of nitrotyrosine in brain homogenates was determined by immunoenzymatical analysis. Samples and standards were incubated in a microtiter well coated with antibodies recognizing nitrotyrosine. During this incubation, nitrotyrosine was captured by the solid bound antibody. Unbound material presented in the sample was removed by washing. Biotinylated second antibody (tracer) to nitrotyrosine was added to the wells. If nitrotyrosine was present in the sample, the tracer antibodies would bind the captured nitrotyrosine. Excess tracer was removed by washing. Streptavidin-peroxidase conjugate was applied to the wells; this conjugate reacted specifically with the biotinylated tracer antibody bound onto the captured nitrotyrosine. Excess streptavidin-peroxidase conjugate is removed by washing and substrate, tetramethylbenzidine was added to the wells. Color developed proportionally to the amount of nitrotyrosine present in the sample. The enzymatic reaction was stopped by the addition of citric acid and the absorbance at 450 nm was measured using the
spectrophotometer. A standard curve was obtained by plotting the absorbances versus the corresponding concentrations of the defined standard (Nitrotyrosine, Elisa Test Kit, HyCult biotechnology b.v., HK 501).

Protein sedimentation with 20% solution of trichloracetic acid was performed in the supernantant aliquot. Solution (1.0 mL) of 0.1 M 2,4-dinitrophenilhydrazine in 2 M HCl was added to the denaturated protein and was incubated at 37°C for 60 min. Then the samples were centrifuged at 3000 G for 20 min. The 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 3.00 mL of 8 M aqueous solution of urea. For increasing the solubility, 0.01 mL of 2M HCl was added. Optic density of dinitrophenylhydrazones was registered at a wavelength of 274 nm (aldehydes) and 363 nm (carboxyles). The rate of protein oxidative modification was expressed in units of optical density per 1g of protein [3].

This method of SOD activity estimation is based on adrenaline’s autooxidation in basic medium leading to superoxide radical formation. In the presence of SOD this reaction slowed down [8]. 4.4 ml of 0.5 mM carbonate buffer (pH=10.2) was put into the spectrophotometer flask with an optical path of 1 cm. 0.1 mL of supernatant and 0.5 ml of adrenaline solution in citric acid (100.0 mL of bidistilate contains 192.0 mg of citric acid and 333.0 mg of adrenaline) was added to the buffer solution. The kinetics of the reaction was registered at 480 nm. SOD activity was expressed in units per mg 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.

**Results and discussion**

2-(3,4-Dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid (2) was synthesized according to reported procedures by treating 4-hydrazonequinazoline (1) with maleic anhydride in glacial acetic acid (Fig 1) [7]. The synthesis of esters 3a-f was carried out by two methods in the correspondent alcohol in the presence of sulfuric acid (method A) or thionylchloride (method B) (Fig 1). It is necessary to mention that the last method provided quantitative yields and was more convenient. The carboxylic group of the substance 2 was activated by N,N-carbonyldimidazole (CDI), and amides 4a-c were obtained by interaction of proper amines with imidazolide of acid (A) in anhydrous dioxane or dimethylformamide (DMF) (Fig 1).
Experimental

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

Maleic anhydride (9.41 g, 96 mmol) was added to a solution of 4-hydrazinoquinazoline (1, 12.85 g, 80 mmol) in glacial acetic acid or DMF (100 mL). The resulting mixture was refluxed under continuous stirring for 2-3 h. After cooling, a crystalline precipitate was filtered off and washed with EtOH.

**Compound 2.** Yield 77.2%; mp 266-268°C; $^1$H NMR: $\delta$ = 2.93 (d, 2H, $J$ = 4.8 Hz, CH$_2$), 5.06 (t, 1H, $J$ = 4.8 Hz, H-4), 7.4 (m, 2H, H-8,10), 7.56 (t, 1H, $J$ = 7.6 Hz, H-9), 7.81 (s, 1H, H-6), 7.90 (d, 1H, $J$ = 7.6 Hz, H-11), 10.96 (s, 1H, NH); $^{13}$C NMR: $\delta$ = 37.80 (CH$_2$), 54.55 (C-4), 120.20 (C-11a), 123.40 (C-8), 127.10 (C-10), 127.77 (C-11), 132.16 (C-9), 135.85 (C-11b), 143.32 (C-7a), 145.99 (C-6), 161.27 (C-3), 171.20 (COOH); LC-MS: $m/z$ = 259 (MH$^+$), 213 (MH - COOH$^-$); EI-MS, $m/z$ (Irel, %) = 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).

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

**Method A.** A mixture of acid 2 (1.29 g, 5 mmol) in an appropriate alcohol (10 mL) and conc. H$_2$SO$_4$ (0.5-1.0 mL) was heated at 80°C for 5-6
h. The mixture was cooled to room temperature, and quenched with NaHCO₃. The obtained precipitate was filtered off and washed well with H₂O.

*Method B.* SOCl₂ (0.54 mL, 7.5 mmol) was added dropwise to a suspension of acid 2 (1.29 g, 5 mmol) in an appropriate alcohol (10 mL) and DMF (1 drop). The reaction mixture was heated at 80°C for additional 5-6 h. After cooling to room temperature, the solution was quenched with NHCO₃. The obtained precipitate was filtered off and washed well with H₂O.

**Compound 3a.** Yield 82.6% (method B); mp 218-224°C (methanol); ¹H NMR: δ = 3.03 (d, 2H, J = 4.8 Hz, CH₂), 3.52 (s, 1H, CH₂), 5.11 (t, 1H, J = 4.8 Hz, H-4), 7.40 (m, 2H, H-8,10), 7.57 (t, 1H, J = 7.6 Hz, H-9), 7.81 (s, 1H, H-6), 7.91 (d, 1H, J = 7.6 Hz, H-11), 11.00 (s, 1H, NH); ¹³C NMR: δ = 37.65 (CH₂), 52.25 (CH₃), 54.43 (C-4), 120.14 (C-11a), 123.38 (C-8), 127.13 (C-10), 127.84 (C-11), 132.20 (C-9), 135.91 (C-11b), 143.28 (C-7a), 145.90 (C-6), 160.95 (C-3), 170.16 (COOH₃); LC-MS: m/z = 273 (MH⁺), 213 (M – COOH₃); EI-MS, m/z (%): 269 (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).

**Compound 3b.** Yield 60.0% (method A); mp 184-186°C (methanol); ¹H NMR: δ = 1.03 (t, 3H, J = 7.1 Hz, CH₃), 3.01 (d, 2H, J = 4.8 Hz, CH₂COOH), 3.96 (m, 2H, CH₂CH₃), 5.12 (t, 1H, J = 4.8 Hz, H-4), 7.40 (m, 2H, H-8,10), 7.57 (t, 1H, J = 7.7 Hz, H-9), 7.82 (s, 1H, H-6), 7.91 (d, 1H, J = 7.7 Hz, H-11), 11.00 (s, 1H, NH); LC-MS: m/z = 287 (MH⁺).

**Compound 3c.** Yield 75.0% (method B); mp 220-224°C (dioxane); LC-MS: m/z = 321(MH⁺).

**Compound 3d.** Yield 91.9% (method B); mp 189-192°C (propanol-2); ¹H NMR: δ = 0.81 (t, 3H, J = 7.1 Hz, CH₃), 1.51 (sxt, 2H, J = 7.1 Hz, CH₂CH₃), 2.91 (d, 2H, J = 5.0 Hz, CH₂COOH), 3.93 (t, 2H, J = 6.6 Hz, CH₂CH₃), 5.03 (t, 1H, J = 5.0 Hz, H-4), 7.29 (t, 1H, J = 7.6 Hz, H-10), 7.35 (d, 1H, J = 7.6 Hz, H-8), 7.46 (t, 1H, J = 7.6 Hz, H-9), 7.69 (s, 1H, H-6), 7.94 (d, 1H, J = 7.6 Hz, H-11), 10.87 (s, 1H, NH); LC-MS: m/z = 301 (MH⁺).

**Compound 3e.** Yield 20.8% (method A), 91.3% (method B); mp 220-224°C (ethanol); ¹H NMR: δ = 1.02 (m, 6H, CH(CH₃)₂), 2.98 (d, 2H, J = 4.8 Hz, CH₂), 4.78 (m, 1H, CH(CH₃)₂), 5.11 (t, 1H, J = 4.8 Hz, H-4), 7.40 (m, 2H, H-8,10), 7.56 (t, 1H, J = 7.6 Hz, H-9), 7.83 (s, 1H, H-6), 7.90 (d, 1H, J = 7.6 Hz, H-11), 11.00 (s, 1H, NH); LC-MS: m/z = 301 (MH⁺).
**Compound 3f.** Yield 80.5% (method B); mp 200-202°C (dioxane–H₂O); ¹H NMR: δ = 3.00 (d, 2H, J = 5.0 Hz, CH₂H₂O), 5.02 (s, 2H, CH₂C₆H₅), 5.07 (t, 1H, J = 5.0 Hz, J = 7.6, H-4), 7.21 (m, 5H, H₉), 7.30 (t, 1H, J = 7.6 Hz, H-10), 7.36 (d, 1H, J = 7.6 Hz, H-8), 7.47 (t, 1H, J = 7.6 Hz, H-9), 7.71 (s, 1H, H-6), 7.93 (d, 1H, J = 7.6 Hz, H-11), 10.89 (s, 1H, NH); LC-MS: m/z = 349 (MH⁺).

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

5 mmol (1.0 g) of N,N-carbonyldiimidazole (CDI) was added to a solution of 5 mmol (1.29 g) acid 2 in 10 ml anhydrous dioxane or DMF. The resulting mixture was refluxed at 60-80°C temperature for 1-1.5 hours. Then 5 mmol of an appropriate amine was added to the solution and the mixture was heated for 6 hours. After cooling to room temperature excess of H₂O was added. The resulting solution was adjusted to pH 6-7 by adding acetic acid. The obtained precipitate was filtered off and dried.

**Compound 4a.** Yield 61.9%; mp 220-224°C (propanol-2); ¹H NMR: δ = 2.96 (d, 2H, J = 5.0 Hz, CH₂H₂O), 3.50-3.38 (m, 8H, morfoline), 5.02 (g, 1H, J = 3.3 Hz, H-4), 7.28 (t, 1H, J = 7.8 Hz, H-10), 7.35 (d, 1H, J = 7.8 Hz, H-8), 7.45 (t, 1H, J = 7.2 Hz, H-9), 7.72 (s, 1H, H-6), 7.93 (d, 1H, J = 7.0 Hz, H-11), 10.80 (s, 1H, NH); LC-MS: m/z = 328 (MH⁺); EI-MS, m/z (Irel, %) = 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).

**Compound 4b.** Yield 51.4%; mp 238-239°C (ethanol–H₂O); ¹H NMR: δ = 2.78 (m, 2H, CH₂H₂O), 4.18 (d, 2H, J = 2.5 Hz, CH₂C₆H₅), 5.07 (t, 1H, J = 6.6 Hz, H-4), 7.11 (m, 3H, H-3, H-4, H-5 Ph), 7.03 (d, 2H, J = 6.1 Hz, H-2, H-6 Ph), 7.40 (t, 1H, J = 7.4 Hz, H-10), 7.43 (d, 1H, J = 7.5 Hz, H-8), 7.59 (t, 1H, J = 7.4 Hz, H-9), 7.69 (s, 1H, H-6), 7.90 (d, 1H, J = 8.2 Hz, H-11), 8.52 (d, 1H, J = 4.7 Hz, NH), 10.97 (s, 1H, NH); LC-MS: m/z = 348 (MH⁺); EI-MS, m/z (Irel, %) = 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).

**Compound 4c.** Yield 84.6%; mp 272-276°C (propanol-2–H₂O); ¹H NMR: δ = 2.98 (1H, -C₂H₅, J=4.9 Hz), 3.09 (1H, -C₂H₅, J=4.9 Hz), 5.18 (1H, H-4, J=4.7 Hz), 7.0 (1H, H-4, Ph, J=7.4 Hz), 7.26 (2H, H-3, 5, Ph), 7.38 (2H, H-8, 10), 7.42 (3H, H-2, 5, Ph), 7.5 (1H, H-9, J=7.6 Hz), 7.72 (1H, H-6), 7.91 (1H, H-11, J=7.4 Hz), 10.1 (1H, -CO(NH)), 10.97 (1H, 2-NH); LC-MS: m/z = 334 (MH⁺); EI-MS, m/z (Irel, %) = 334 (3.0), 333.
The structures of all synthesized compounds were elucidated by analytical and spectral data. The IR spectrum of acid 2 was characterized by stretching vibrations of associated vibrations $\nu_{\text{NH}}$ and $\nu_{\text{OH}}$ at 3444-3011 cm$^{-1}$, by the intensive characteristic band at 2784-2469 cm$^{-1}$, by vibrations $\nu_{\text{C}=\text{O}}$ at 1738-1706 cm$^{-1}$ and $\gamma_{(\text{OH...O})}$ at 992-917 cm$^{-1}$. Moreover, intensive aliphatic CH$_2$-group band of symmetric and asymmetric stretching vibrations at 2978-2855 cm$^{-1}$, stretching vibrations of carbonyl group of lactam bond at 1712-1662 cm$^{-1}$, $\nu_{\text{C}=\text{C}}$ at 1643-1468 cm$^{-1}$ and intensive out-of-plane deformation of $\text{СН}$ of the aromatic rings at 846-771 cm$^{-1}$ were shown for substance 2. Shifting of stretching vibrations in the high-frequency area of the spectrum (1748-1729 cm$^{-1}$), appearance of the band of stretching vibrations $\nu_{\text{С–О}}$ at 1360-1300 cm$^{-1}$, which had similar intensity of $\nu_{\text{C}=\text{O}}$, and band of more intensive and wide symmetric and asymmetric aliphatic group stretching vibrations at 2978-2797 cm$^{-1}$ revealed for esters 3а-е. In the IR spectra of amides 4а-с opposite to acid 2 the intensive band of absorptions appeared at 3199-3024 cm$^{-1}$ and 3387-3149 cm$^{-1}$, and stretching vibrations were shown at 1659-1628 cm$^{-1}$ (amide I) and out-of-plane deformation - at 1613-1550 cm$^{-1}$ (amide II).

In the $^1$H NMR spectrum of compound 2, two protons of methylene group appeared as doublet at 2.93 ppm and H(4) was found as triplet at 5.06 ppm with a coupling constant of 4.8 Hz. Signals in the aromatic region related to quinazoline protons and the one at 10.96 ppm to NH proton. In the spectra of esters 3а-ф characteristic triplet of H(4) at 5.12-5.00 ppm and two proton doublet of CH$_2$ group at 3.03-2.91 ppm were revealed. In addition, alkoxy group signal appeared in the high-field region. The distinctive features of amides 4а-с $^1$H NMR spectra were the appearance of characteristic signals of the amide group proton at 8.52-10.1 ppm, which was demonstrated as singlet (4с) or doublet (4б). Compound 4а was characterized by the protons of morpholine at 3.50-3.38 ppm. Furthermore, amides 4а-с, as well as compound 2 and 3а-ф were characterized by the triplet of H(4) and doublet or multiplet of CH$_2$ protons.

In the LC-MS analysis, all compounds were characterized by a high-intensive peak of [MH]$^+$, a particularly intense peak of [MH]$^+$ (m/z 259) and [MH – COOH]$^+$ (m/z 213) were found in the spectrum of acid 2. The El-MS-spectrum of compound 2 demonstrated its molecular ion peak at m/z 258, whose fragmentation corresponded to the 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) bond breaking with formation of quinazoline ion (m/z
Another fragmentation pattern happened due to N(1)–N(2) and C(4)–C(3) bond breaking and followed by elimination of HNCO. Mass-spectra (EI) of ester 3a and amides 4a-c were characterized by the ester or amide bond breaking with formation of the ion with m/z 241 and fragmentation by α-bond of carbonyl acid with formation of the ion with m/z 212. Subsequent fragmentation of the above mentioned compounds was similar to acid 2.

In vitro studies

Comparative antioxidant activity estimation of the investigated compounds in sodium nitroprusside photoinduction revealed that the synthesized substances played a significant role in the inhibition of NO-formation (Table I). In this model (3-oxo-3,4-dihydro-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid (2) wasn’t active, while esters (3a-f) of the noted acid revealed activity. Thus, the methyl ester of 3a repressed the peroxynitril radical at 46.7%, exceeding N-acetylcystein (N-ACC) at 26.7%. Subsequent modification of substance 2, namely elongating of the ester fragment (3b-3f), hadn’t influenced the activity. It is important to notice, that amides (4a-4c) also showed a high activity, exceeding the activity of N-acetylcystein at 16.7-28.7%.

Nevertheless when investigating (3-oxo-3,4-dihydro-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid (2) derivatives antioxidant activity in sodium nitroprusside photoinduced reaction, it was found that compounds 3f and 4b had the highest activity in the inhibition of NO-formation (Table I).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Δ of optical density at λ=265 nm</th>
<th>Antioxidant activity (AOA), %</th>
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<tbody>
<tr>
<td>2</td>
<td>0.60±0.01</td>
<td>0</td>
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<tr>
<td>3a</td>
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<td>46.7</td>
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<td>3b</td>
<td>0.35±0.01*</td>
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<tr>
<td>4a</td>
<td>0.35±0.01</td>
<td>41.7</td>
</tr>
<tr>
<td>4b</td>
<td>0.34±0.005*</td>
<td>48.7</td>
</tr>
<tr>
<td>4c</td>
<td>0.38±0.01</td>
<td>36.7</td>
</tr>
<tr>
<td>Control</td>
<td>0.60±0.01</td>
<td>-</td>
</tr>
<tr>
<td>N-acetylcystein</td>
<td>0.480±0.002*</td>
<td>20.0</td>
</tr>
</tbody>
</table>

* – p<0.05 to control.
In vivo studies

The modeled nitrosive stress in brain samples was characterized by the inhibition of antioxidant enzyme (SOD) activity, by increasing the formation of protein oxidative modification products reacting with 2,4-dinitrophenylhydrazine. These reaction products showed the maximum of absorbtion at 270 and 363 nm (Table II). Thus SOD activity had increased to 53.7%. Protein oxidative modification products found at 270 nm were increased to 87.1%, and at 363 nm – to 64.6%. Antioxidant enzymes (their activity was inhibited by the formation of nitrocomplexes with metal ions from the enzymes active center) were another object of nitrosive stress destructive influence. Reduction of SOD activity resulted in a significant increase of the free radical oxidation (Table II).

Introduction of compound 3f into the mixture in a concentration of 10⁻⁶M resulted in unfavorable influence restriction on SOD and proteins (Table II). Thus compound 3f reduced the formation of products in the reaction with 2,4-dinitrophenylhydrazine at 32.0% and 24.8% compared to the control. The value of SOD activity in the mixture with 3f was 66.8% higher than the control value (Table II). The substance 3f also decreased the concentration of nitrotyrosine markers in brain homogenates at 43.6% compared to the control sample (Table II).

<table>
<thead>
<tr>
<th>The investigated samples</th>
<th>SOD units/mg protein/min</th>
<th>POM products, units/mg protein</th>
<th>Nitrotyrosine in brain samples, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference sample</td>
<td>260.7±7.6</td>
<td>14.0±0.11</td>
<td>22.3±0.17</td>
</tr>
<tr>
<td>Control (adding of stable NO and cysteine complex, 100 µM)</td>
<td>120.2±5.0</td>
<td>26.2±0.21</td>
<td>36.7±0.10</td>
</tr>
<tr>
<td>Experimental (adding of stable NO and cysteine complex + 3f, 100 µM)</td>
<td>200.6±4.2*</td>
<td>17.8±0.10*</td>
<td>27.6±0.11*</td>
</tr>
</tbody>
</table>

Table II

Effect of 3f on SOD activity and content of protein oxidative modification (POM) products in brain samples in the nitrosive stress model

* – p<0.05 versus control

Nitrosive stress induced by dinitrozole complex of metal and cystein, inhibited SOD activity in rats’ brain and increased the protein oxidative modification rate. 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 acid (2) had an antioxidant activity in the NO induction experiment. Compound 3f
demonstrated the greatest protective antioxidant activity towards SOD (Table II).

**Conclusions**

In the present paper, a series of new 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetic acid derivatives, which were tested for antioxidant activity in nitrosative stress model were described. Synthesized compounds displayed high antioxidant activity in comparison with N-acetylcystein (N-ACC). The compound benzyl 2-(3,4-dihydro-3-oxo-2H-[1,2,4]triazino[4,3-c]quinazolin-4-yl)acetate (3f) revealed the best antioxidant properties in a nitrosative stress model.

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**References**


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