Chimica Techno Acta
published by Ural Federal University
eISSN 2411-1414; chimicatechnoacta.ru

ARTICLE
2022, vol. 9(2), No. 20229212
DOI: 10.15826/chimtech.2022.9.2.12

# Synthesis and evaluation of cerebroprotective activity of novel 6,7-dimethoxyquinazolin-4(3*H*)-one derivatives containing residues of amino acids and dipeptides

Alexey S. Chiriapkin *, Ivan P. Kodonidi, Dmitry I. Pozdnyakov

Pyatigorsk Medical and Pharmaceutical Institute, Branch of Volgograd State Medical University, Pyatigorsk 357532, Russia
* Corresponding author: prk@pmedpharm.ru

This paper belongs to a Regular Issue.



## Abstract

Neurodegenerative processes of the central nervous system are an important socially significant problem of modern society. They cause many diseases, such as Alzheimer's disease and cerebral ischemia, which significantly reduce the quality of human life and can lead to disability or death. The aim of this study was to synthesize novel 6,7-dimethoxyquinazolin-4(3*H*)-one derivatives with the remains of neuroactive amino acids and dipeptides in order to investigate their cerebroprotective properties. As a result of the study, 13 novel 6,7-dimethoxyquinazolin-4(3*H*)-one derivatives were synthesized. Cerebral ischemia in rats was reproduced by irreversible right-sided occlusion of the middle cerebral artery using the Tamura method, and the area of brain necrosis was evaluated. Cognitive functions were evaluated in the Y-maze test. Among the studied quinazolinone derivatives, compounds **3i**, **3j** and **3k** have the most pronounced cerebrotropic activity, which is not inferior to ethylmethylhydroxypyridine succinate in terms of pharmacological activity, making them promising objects for further research.

## Keywords

quinazolinones
synthesis
medicinal chemistry
cerebroprotective activity
ischemia
amino acid
dipeptide

Received: 15.05.22
Revised: 10.06.22
Accepted: 13.06.22
Available online: 16.06.22

## 1. Introduction

Disorders of the functioning of the central nervous system are an important socially significant problem of modern society. Every year there is an increase in patients with various cognitive impairments, which defines the urgency of developing new methods of their treatment. The main causes of cognitive dysfunctions are vascular pathologies (cerebral ischemia [1]), neurodegenerative processes (such as in the case of Alzheimer's disease) [2], traumatic brain injuries [3] and neuroinfections [4]. It is worth noting that disorders of cerebral circulation are the second most common cause of cognitive dysfunction after Alzheimer's disease [5]. Cerebral ischemia forms insufficient oxygen and glucose delivery for the metabolic processes of neurons, which leads to disruption of cellular homeostasis, oxidative stress, inflammation and death of brain cells [6]. Vascular pathologies of the brain can provoke an ischemic stroke, which often leads the patient to disability or death. The cause of a stroke can also be an acute traumatic brain injury or a brain dysfunction. Modern methods of treatment of ischemic and other pathological conditions of the human central nervous system include intravenous thrombolysis and the use of neuroprotective and anti-inflammatory drugs. Despite this, in most patients with an unfavorable prognosis of the course of the disease, there is a malfunction and rapid death of brain cells [7]. Based on this, an important task for pharmacy and medicine is the development of new highly effective and low-toxic drugs for the treatment of neurodegenerative processes.

One of the most promising classes of organic compounds is quinazolinone derivatives, which have a diverse spectrum of biological activity. So, series of kojyl thioether conjugated to different quinazolinone derivatives were designed, synthesized, and evaluated for their inhibitory activity against mushroom tyrosinase. All derivatives displayed better potency than kojic acid as the positive control [8]. Novel quinazolinone derivatives bear benzenesulfonamide moiety with variable heterocyclic tail. One of them showed antioxidant and hepatoprotective activities in irradiated mice [9]. Novel iodinated quinazolinones bearing sulfonamide were synthesized. Among them there is a compound that acts as a direct antioxidant by scavenging reactive oxygen species and inhibiting radiation-induced oxidative stress [10]. 3-Aryl-8-methylquinazolin-4(3*H*)-ones have promising properties for drug development against keratitis and brain infection causing free-living amoeba, *A.*

*Castellanii* [11]. Antimicrobial activity of substituted-6-methyl-1-thioxo-1,2-dihydro-3*H*-furo[3,2-g]pyrimido[1,6-a]quinazolin-3-ones was evaluated against Gram-positive, Gram-negative bacteria and fungi. Furothiazolo pyrimido quinazolines displayed results excellent for growth inhibition of bacteria and fungi [12].

Most of the different substituted quinazolinones exhibited moderate to high anticonvulsant activity in all seizure models with no symptoms of neurotoxicity and hepatotoxicity [13]. There are data indicating the ability of some quinazolinone inhibit activity of the urease [14]. Novel quinazolinones conjugated with indole acetamide, ibuprofen or thioacetohydrazide were designed to increase cyclooxygenase-2 (COX-2) selectivity. The three synthesized series exhibited superior COX-2 selectivity compared with the previously reported quinazolinones and their nonsteroidal anti-inflammatory drug analogue and had equipotent COX-2 selectivity as celecoxib [15]. Some novel compounds of allyl/benzyl quinazolinone displayed remarkable anti-inflammatory activity as compared to Diclofenac sodium [16]. Also, different 2,7-disubstituted[1,3,4]-tiadiazolov[2,3-b]quinazolin-5(4*H*)-ones were synthesized, and they showed good anti-inflammatory activity [17]. Various 2-(3-aryl-1*H*-pyrazol-1-yl)benzo[d]thiazole incorporated fused thiazolo[2,3-b]quinazolinones exhibited prominent anticancer and antibacterial activities. The potent compounds could serve as templates for further development of anticancer and antibacterial drugs [18]. A series of novel quinazoline derivatives were synthesized and evaluated for their anticonvulsant activity against electrically and chemically induced seizures, compared with that of the standard drugs methaqualone and sodium valproate [19].

A novel series of 4-anilinoquinazoline analogues, DW (1–10), were evaluated for anticancer efficacy in human breast cancer (BT-20) and human colorectal cancer cell lines (HCT116, HT29, and SW620). DW8 may represent a suitable lead for developing novel compounds to treat the human colorectal cancer [20]. The preparation and screening of the focused libraries of 4-anilinoquinolines and 4-anilinoquinazolines for antiviral activity allowed identifying three potent compounds. N-(2,5-dimethoxyphenyl)-6-(trifluoromethyl)quinolin-4-amine effectively inhibited *dengue virus* infection, and N-(3,4-dichlorophenyl)-6-(trifluoromethyl)quinolin-4-amine and N-(3-ethynyl-4-fluorophenyl)-6,7-dimethoxyquinazolin-4-amine successfully inhibited *Venezuelan equine encephalitis virus*. These results provide a prospect of developing a clinical compound against these emerging viral threats [21]. A series of novel histone deacetylase inhibitors using a substituted quinazoline was designed and synthesized as the capping group, attaching 3, 5-dimethyl pentyl as a potential metabolic site protector. One of representatives was proposed as an oral histone deacetylase inhibitor with a potential capacity of treating breast cancer [22]. There is evidence indicating that novel 1,2,3-triazole-quinazolines can be used as antiproliferative agents displaying the extracellular signal-regulated kinase inhibitory activity [23]. Approximately 150 different 6,7-dimethoxyquinazoline-2,4-diamines were synthesized, and via structure-activity relationship studies. Among them 6,7-dimethoxy-N4-(1-phenylethyl)-2-(pyrrolidin-1-yl)quinazo-lin-4-amine exhibits high antimalarial activity as a promising antimalarial drug lead [24]. Two new series of synthesized quinazolinone derivatives were investigated as potential future antileishmanial agents, by assessing their activities against the *Leishmania (L.) donovani* and *L. major species*. The two compounds that were found the most active are the mono quinazolinone and the bisquinazolinone with growth inhibitory efficacies of 35% and 29% for the *L. major* and *L. donovani*, respectively [25].

Based on quinazolin-4(3*H*)-one derivatives, a series of new dual-target inhibitors of poly (ADP-ribose) polymerase-1 (PARP 1) and bromodomain containing protein 4 (BRD4) were successfully synthesized. Among them are compounds that would be promising for the treatment of breast cancer [26]. As a result of the structural modification of the molecular-targeted agent sorafenib, a series of quinazolinyl-arylurea derivatives were synthesized and evaluated for their anti-proliferative activities against six human cancer cell lines. N-(3,4-dichlorophenyl)-N'-(3-((quinazolin-4-ylamino)methyl)phenyl)urea could trigger three different cell death forms including apoptosis, ferroptosis and autophagy. This compound could be a promising lead for molecular-targeted anti-bladder cancer agents discovery [27]. Thus, quinazoline derivatives are promising compounds for the search for biologically active substances, including those with neuroprotective properties.

The most commonly used amino acid for the treatment of diseases of the central nervous system is glycine, acute ischemic stroke [28]. Also, in case of brain disorders, gamma-aminobutyric acid and its cyclic derivatives – racetams are actively used [29]. The scientific literature presents the results of studies that indicate high cerebroprotective properties of a number of low-molecular-weight peptides. So, the novel 14-amino acid peptide, with stress-protein-like sequences exhibiting neuroprotection at unprecedented concentrations was revealed. This peptide prevented neuronal cell death associated with the envelope protein (GP 120) from Human immunodeficiency virus, with excitotoxicity (N-methyl d-aspartate), with the beta amyloid peptide (putative cytotoxin in Alzheimer's disease), and with tetrodotoxin (electrical blockade) [30]. It was found that lipophilic peptide fragments offer bioavailability and stability, providing lead compounds for drug design against neurodegenerative diseases [31]. Cationic arginine-rich peptides (CARPs) are an expanding and relatively novel class of compounds possessing intrinsic neuroprotective properties [32]. The neuroprotective efficacy of different modifications to the poly-arginine-9 peptide (R9) was examined [33]. The new synthetic peptide, CMX-9236, can function as a neuroprotective agent and an activator of a

beneficial signal transduction pathway in in vitro and in vivo models of cerebral ischemia [34].

## 2. Experimental

### 2.1. Chemistry

All chemicals were acquired from Sigma-Aldrich (SigmaAldrich, St. Louis, MO, USA), Carl Roth (Carl Roth, Karlsruhe, Germany) and Merck Chemicals (MerckKGaA, Darmstadt, Germany). Melting points (m.p.) were recorded using the PMP-M1 melting point apparatus (Himlaborpribor, Klin, Russia). All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F254 TLC plates (Merck, Darmstadt, Germany). Spectroscopic data were registered with the following instruments: IR, IR-Fourier FSM 1201 spectrophotometer (Spectrum, Moscow, Russia); UV, SF-2000 device (Spectrum, Moscow, Russia); $^{1}$H NMR and $^{13}$C NMR, Bruker Avance III 400 MHz spectrometer (Bruker, Germany) in DMSO-$d_6$ using tetramethylsilane as the internal standard. Coupling constant (*J*) values were measured in hertz (Hz) and spin multiplets are given as follows: *s* (singlet), *d* (double), *t* (triplete), *q* (quartet), *m* (multiplet).

#### 2.1.1. General procedure for synthesis of amides of 2-amino-4,5-dimethoxybenzoic acid (3, 4)

0.05 mol of the acetyl chloride or benzoyl chloride was slowly added to a solution of 9.85 g (0.05 mol) of 2-amino-4,5-dimethoxybenzoic acid (2) in 35 ml of 10% sodium hydroxide with stirring. As a result, a precipitate was formed, which was filtered and washed with water. Purification of the obtained substances is carried out by recrystallization from ethyl alcohol.

#### 2.1.2. General procedure for synthesis of 2-alkyl-6,7-dimethoxy-3,1-benzoxazine-4-ones (2a–2e)

5.91 g (0.03 mol) of 2-amino-4,5-dimethoxybenzoic acid was dissolved at boiling in 25 ml of the corresponding anhydride and the reaction was carried out for 5 hours. In the case of 2d-2e compounds, the reaction was carried out in a similar way, and 0.03 mol of amides of 2-amino-4,5-dimethoxybenzoic acid (3 and 4) were used as starting substances. The reaction mixture was kept in the refrigerator for a day; the resulting precipitate was filtered and washed with water. The product was purified by recrystallization from ethyl alcohol.

#### 2.1.3. General procedure for synthesis of 6,7-dimethoxyquinazolin-4(3*H*)-one derivatives containing residues of amino acids and dipeptides (3a–3m)

A mixture of 0.01 mole of the corresponding 2-alkyl-6,7-dimethoxy-3,1-benzoxazine-4-one (**2a–2e**) and 0.01 mole of amino acid or dipeptide was dissolved at boiling in 10-15 ml of glacial acetic acid. Next, 0.5 ml of DMF was added and the reaction was carried out with stirring for 1.5 hours. The reaction mixture was kept in the refrigerator for a day; the resulting precipitate was filtered and washed with water. The product was purified by recrystallization from ethyl alcohol.

### 2.2. Pharmacological study

#### 2.2.1. Animals

The study was performed on 96 mature male Wistar rats. The animals were obtained from the laboratory animal nursery "Rappolovo" (Leningrad region) and for the duration of the experiment were kept in a vivarium, 6 individuals per cage at an air temperature of 20±2 °C, relative humidity of 60±5% and a twelve-hour daily cycle (12 hours day/12 hours night). Until the moment of inclusion in the work, the rats were kept in a quarantine room for 2 weeks. Animals received a complete dry food diet (granulated food) and water *ad libitum*. The design of the study and the manipulations performed with the animals were in accordance with the international ethical principles of working with laboratory animals, as set out in the ARRIVE 2.0 guidelines [35].

#### 2.2.2. Cerebral ischemia model

Cerebral ischemia in rats was reproduced by irreversible right-sided occlusion of the middle cerebral artery according to a *Tamura, 1981*. Animals were anesthetized by intraperitoneal injection of chloral hydrate at a dose of 350 mg/kg, the area below and to the right of the eye was depilated, the skin and soft tissues were dissected. Next, a burr hole was made over the intersection of the artery with the olfactory tract, then the artery was coagulated by an electrocoagulator. The soft tissue topography was restored, the wound was sutured. The suture was treated with 10% povidone-iodine [36].

#### 2.2.3. Study design

The cerebroprotective activity of the tested substances was evaluated during therapeutic administration. When setting up the study, the following experimental groups were distinguished: SO – sham-operated animals, to which all sequential procedures were applied with the exception of the coagulation of the middle cerebral artery; NC – a group of rats with cerebral ischemia, but without pharmacological correction (during the experiment, this group of animals received purified water); a group of rats that were treated by the reference drug and groups of rats that received the test substances. A total of 16 groups of 6 individuals each were formed. Ethylmethylhydroxypyridine succinate (EMHPS, «Mexidol», FARMASOFT, Russia) was used as a reference drug at a dose of 100 mg/kg, orally [37]. The test compounds were administered at a screening dose of 40 mg/kg orally. The reference and tested compounds were administered 30 min after ischemia modeling and then for 72 h (one administration per day). On the 4$^{th}$ day of the experiment, the changes of cognitive functions of rats in the Y-maze test were evaluated. After that, the animals were decapitated under anesthesia and the brain was removed, in which the change in the volume of necrotic tissue was assessed.

#### 2.2.4. Y-maze test

The setup consisted of three equal arms connected at an angle of 120°. The animal was placed in the center of the setup, and the number of animal movements between the arms was recorded for 8 minutes. At the same time, spontaneous alternating entries into the arms (1–2–3, 3–1–2, 2–3–1) were recorded. Based on the data obtained, the percentage of spontaneous alternation was determined (formula (1)), which reflects the change in the cognitive abilities of animals [38]:

$$\text{Percentage of spontaneous alternation} = \frac{\text{Number of alternating entries into the arms}}{\text{Total number of movements}} \cdot 100 \quad (1)$$

#### 2.2.5. Brane necrosis zone determination

The size of the necrosis zone was determined by the change in the color intensity of triphenyltetrazolium chloride. The course of determination was as follows: after decapitation of the animals, the brain was removed, the cerebellum was cut off and the hemispheres were separated along the central sulcus. Both hemispheres were weighed, then individually homogenized and placed in weighing bottles. To the homogenate was added 10 ml of a 1% solution of triphenyltetrazolium chloride in phosphate buffer (pH 7.4). The bottles were incubated in a water bath at 37 °C for 20 min. After that, the samples were centrifuged at 5000 RPM/10 min, and the supernatant was removed. 3 ml of phosphate buffer and 3 ml of cold chloroform were added to the precipitate. The mixture was shaken for 2 min and then incubated for 15 min at 4 °C, shaking every 5 min for 30 s, re-centrifuged in the same mode, and its optical density was measured against pure chloroform at 492 nm. The calculation of the necrosis zone was performed according to the formula (2) and expressed as a percentage of the total mass of the hemispheres:

$$x = 100 - \frac{\varepsilon_1 M_1 + \varepsilon_2 M_2}{\varepsilon_1 (M_1 + M_2)} \cdot 100, \quad (2)$$

where $x$ is the size of the necrosis zone as a percentage of the total brain mass; $\varepsilon_1$ is the optical density of the sample with an undamaged hemisphere; $\varepsilon_2$ is the optical density of the sample with the damaged hemisphere; $M_1$ is the mass of the undamaged hemisphere; $M_2$ is the mass of the damaged hemisphere.

#### 2.2.6. Statistical analysis

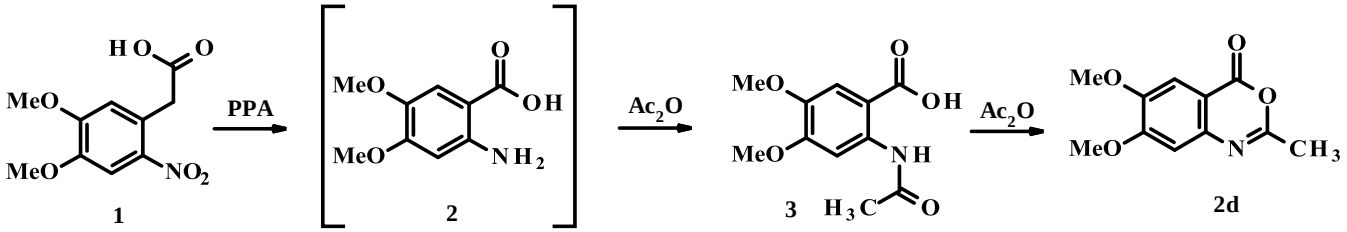
Statistical processing of the obtained results was carried out using the software package STATISTICA 6.0 (StatSoft, USA). The data were expressed as M (mean) ± SEM (standard error of mean). The Gaussian distribution was tested by the Shapiro-Wilk test. The homogeneity of the variance was assessed in the Levene test. One-way analysis of variance (ANOVA) with post-processing by Tukey's test (in the presence of a Gaussian distribution) or Kruskal - Wallis (in the absence of Gaussian distribution), carried out by the comparison of groups. The critical level of significance was taken as $p<0.05$.

## 3. Results and Discussion

### 3.1. Synthesis

A convenient way to synthesize 6,7-dimethoxyquinazoline-4(3*H*)-one is the replacement of the oxygen heteroatom with nitrogen in the intermediate 2-methyl-6,7-dimethoxy-3,1-benzoxazine-4-one (Scheme 1), which was previously developed with the staff of The research-scientific Institute of Physical & Organic Chemistry of Rostov University of Southern Federal University. A new modification of the synthesis was proposed based on the interaction of nitrohomoveratric acid **(1)** and polyphosphoric acid – PPA **(2)** with subsequent treatment of the reaction mixture with a small amount of acetic anhydride, which leads to the formation of 2-methyl-6,7-dimethoxy-3,1-benzoxazine-4-one **(2d)** through the intermediate 2-acetamido-4,5-dimethoxybenzoic acid **(3)** [39].

However, the yield of 2-methyl-6,7-dimethoxy-3,1-benzoxazine-4-one products is low, because a redox reaction of disproportionation occurs at the first stage of obtaining 2-acetamido-4,5-dimethoxybenzoic acid. Another negative factor in obtaining the intermediate 2-methyl-6,7-dimethoxy-3,1-benzoxazine-4-one is the use of acetic anhydride, which is a controlled substance (Narcotic drugs, psychotropic substances and their precursors, controlled in the Russian Federation), and this complicates the possibility of developing laboratory regulations for the synthesis of an active pharmaceutical substance.



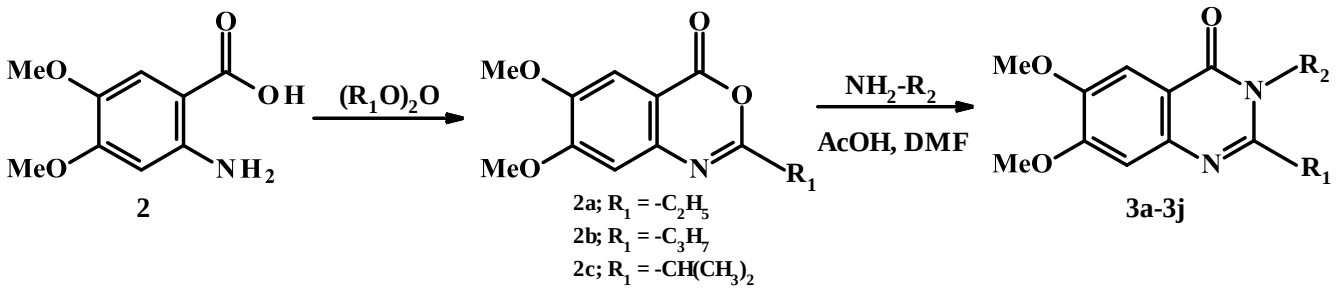

**Scheme 1** Synthesis of 2-methyl-6,7-dimethoxy-3,1-benzoxazine-4-one using acetic anhydride.

Based on this, for the synthesis of 2-substituted ethyl, propionyl and isopropionyl 6,7-dimethoxy-3,1-benzoxazine-4-one, an acylation process with further heterocyclization should be used (Scheme 2). This process proceeds by boiling dimethoxyanthronylic acid in the corresponding anhydride without the release of intermediate amides of dimethoxyanthranylic acid, which leads to the formation of intermediate 2-ethyl, 2-propyl and 2-isopropyl-6,7-dimethoxy-3,1-benzoxazine-4-one. At the first stage of synthesis, 2-amino-4,5-dimethoxybenzoic acid is heated in excess of propionic, butyric and isobutyric anhydride. As a result, 2-ethyl-6,7-dimethoxy-3,1-benzoxazine-4-one **(2a)**, 2-propyl-6,7-dimethoxy-3,1-benzoxazine-4-one **(2b)** and 2-isopropyl-6,7-dimethoxy-3,1-benzoxazine-4-one **(2c)** were obtained with yields of 90%, 84% and 75%, respectively.

According to the yield of 2-substituted 6,7-dimethoxy-3,1-benzoxazine-4-one, the reactivity decreases with the elongation of the carbon chain and even more so with an increase in the branching of anhydrides. Next, the heteroatom was replaced with nitrogen by the interaction of the corresponding benzoxazinone with amino acids or dipeptides.

Compounds **1c-10c** were obtained by replacing the oxygen of the heterocycle with nitrogen by the interaction of the corresponding 6,7-dimethoxy-3,1-benzoxazine-4-one derivatives with glycine, glycylglycine, glycylleucine and glycyltryptophan in the medium of glacial acetic acid with the addition of 0.5 ml of dimethylformamide.

At the same time, it is advisable to synthesize 2-methyl and 2-phenyl-6,7-dimethoxyquinazoline-4(3*H*)-ones **(3k–3m)** through amides of 2-amino-4,5-dimethoxybenzoic acid (**3** and **4**), which in turn was synthesized by acylation of dimethoxyanthranilic acid **(2)** by acetyl chloride or benzoyl chloride (Scheme 3).



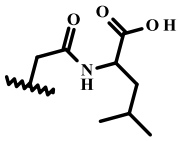

**2**

**2a;** $R_1$ = -$C_2H_5$
**2b;** $R_1$ = -$C_3H_7$
**2c;** $R_1$ = -$CH(CH_3)_2$

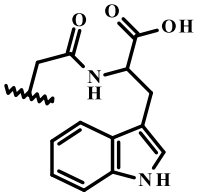
**3a-3j**

**3a;** $R_1$ = -$C_2H_5$, $R_2$ = -$CH_2$-COOH
**3b;** $R_1$ = -$C_2H_5$, $R_2$ = -$CH_2$-C(O)-NH-$CH_2$-COOH

**3c;** $R_1$ = -$C_2H_5$, $R_2$ =

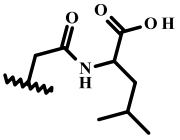


**3d;** $R_1$ = -$C_2H_5$, $R_2$ =

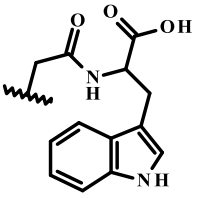


**3e;** $R_1$ = -$C_3H_7$, $R_2$ = -$CH_2$-COOH
**3f;** $R_1$ = -$C_3H_7$, $R_2$ = -$CH_2$-C(O)-NH-$CH_2$-COOH

**3g;** $R_1$ = -$C_3H_7$, $R_2$ =

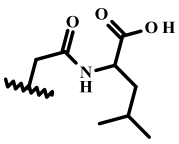


**3h;** $R_1$ = -$C_3H_7$, $R_2$ =

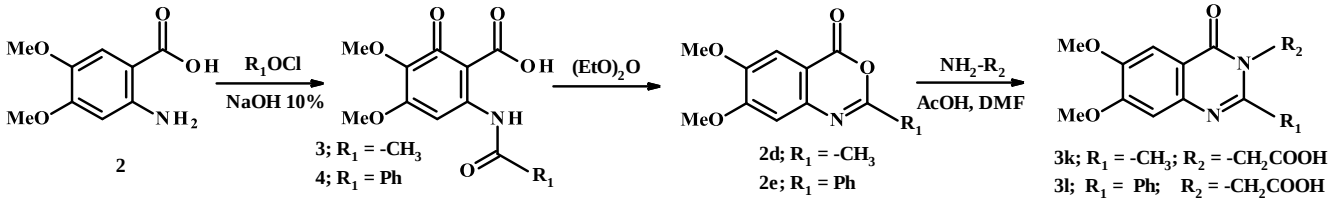


**3i;** $R_1$ = -$CH(CH_3)_2$, $R_2$ = -$CH_2$-C(O)-NH-$CH_2$-COOH

**3j;** $R_1$ = -$CH(CH_3)_2$, $R_2$ =



**Scheme 2** Synthesis of **3a–3j**.




**2**

**3;** $R_1$ = -$CH_3$
**4;** $R_1$ = Ph

**2d;** $R_1$ = -$CH_3$
**2e;** $R_1$ = Ph

**3k;** $R_1$ = -$CH_3$; $R_2$ = -$CH_2$COOH
**3l;** $R_1$ = Ph; $R_2$ = -$CH_2$COOH
**3m;** $R_1$ = Ph; $R_2$ = -$C_2H_4$COOH

**Scheme 3** Synthesis of **3k–3m**.

The 2-methyl-6,7-dimethoxy-3,1-benzoxazine-4-one and 2-phenyl-6,7-dimethoxy-3,1-benzoxazine-4-one were obtained by two-stage synthesis. At the first stage, 2-acetamido-4,5-dimethoxybenzoic acid was obtained by interaction in an alkaline medium by adding the acetyl chloride by drop while stirring. According to the method of the Schotten–Baumann acylation reaction, the pH of the medium should be controlled so that it remains alkaline. Further, the formation of the core of 3,1-benzoxazine-4-one was carried out by boiling 2-acetamido-4,5-dimethoxybenzoic acid in excess of propionic anhydride. The preparation of 2-phenyl-6,7-dimethoxy-3,1-benzoxazine-4-one was carried out in a similar way using benzoyl chloride. The substances **3k–3m** were obtained by replacing oxygen with nitrogen in 3,1-benzoxazine-4-one by the interaction of the corresponding benzoxazinones with glycine or β-alanine in the medium of glacial acetic acid in the presence of catalytic amounts of DMF (Scheme 3).

The mechanism of formation of 6,7-dimethoxyquinazoline-4(3*H*)-one derivatives can be represented through the formation of an intermediate carbocation (Scheme 4). Possible ways of protonation of the initial 2-phenylbenzoxazine-4-one **(I)** were previously considered; the most reliable variant of the reaction involves protonation of the oxygen heteroatom **(II)**, leading to a more stable carbocation **(III)**. The attack by the carbocation amine reagent leads to the formation of another cation **(IV)**, which further cleaves off the proton **(V)**. Deprotonation preceding cyclization allows the formation of an intermediate adduct that cleaves off water molecules with the formation of a heterocycle **(VI)**.

### 3.1.1. 2-acetamido-4,5-dimethoxy-benzoic acid (3)

The grey crystals were obtained. Yield: 77%. M.p.: 136–137 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 231, 261, 324. IR spectrum (KBr), ν, $cm^{-1}$: 3487 (OH), 3375 (NH), 16567 (C=O), 1624 (C=O), 999 (C–O).

### 3.1.2. 2-benzamido-4,5-dimethoxy-benzoic acid (4)

The grey crystals were obtained. Yield: 91%. M.p.: 237–238 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 202, 242, 308. IR spectrum (KBr), ν, $cm^{-1}$: 3450 (OH), 3157 (NH), 1676 (C=O), 1649 (C=O), 995 (C–O).

### 3.1.3. 2-ethyl-6,7-dimethoxy-3,1-benzoxazine-4-one (2a)

The white amorphous substance was obtained. Yield: 90%. M.p.: 142–143 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 242, 275. IR spectrum (KBr), ν, $cm^{-1}$: 1736 (C=O), 1026 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.23 (*t, J* = 7.5 Hz, 3H, $CH_3$), 2.67 (*q, J* = 7.4 Hz, 2H, $CH_2$), 3.87 (*s*, 3H, $CH_3$), 3.92 (*s*, 3H, $CH_3$), 7.07 (*s*, 1H, ArH), 7.37 (*s*, 1H, ArH).

### 3.1.4. 2-propyl-6,7-dimethoxy-3,1-benzoxazine-4-one (2b)

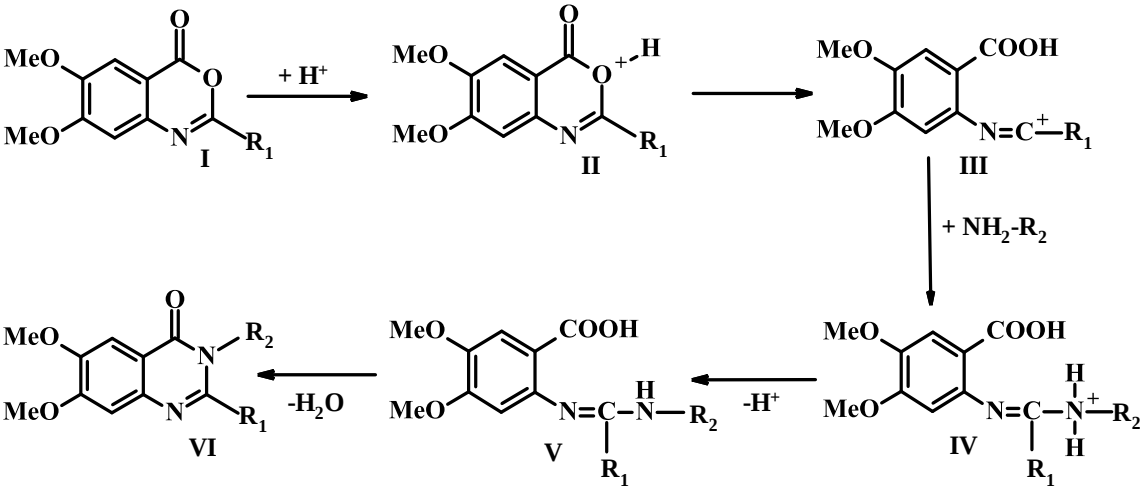
The white amorphous substance was obtained. Yield: 84%. M.p.: 184–185 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 238, 271. IR spectrum (KBr), ν, $cm^{-1}$: 1742 (C=O), 1018 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 0.93 (*t, J* = 7.4 Hz, 3H, $CH_3$), 1.71–1.57 (*m*, 2H, $CH_2$), 2.35 (*t, J* = 7.3 Hz, 2H, $CH_2$), 3.76 (*s*, 3H, $CH_3$), 3.81 (*s*, 3H. $CH_3$), 7.42 (*s*, 1H, ArH), 8.33 (*s*, 1H, ArH). $^{13}$C NMR spectrum (100,6 MHz, DMSO-$d_6$), δ, ppm: 14.00, 18.74, 55.96, 55.99, 103.43, 107.59, 113.13, 137.42, 143.74, 153.61, 169.83, 171.46.

### 3.1.5. 2-isopropyl-6,7-dimethoxy-3,1-benzoxazine-4-one (2c)

The white amorphous substance was obtained. Yield: 75%. M.p.: 214–215 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 237, 271. IR spectrum (KBr), ν, $cm^{-1}$: 1740 (C=O), 1012 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.17 (*dd, J* = 6.9, 1.0 Hz, 6H, $CH_3$), 2.56 (*qd, J* = 6.9, 1.1 Hz, 1H, CH), 3.76 (*s*, 3H, $CH_3$), 3.81 (*s*, 3H, $CH_3$), 7.43 (*d, J* = 1.0 Hz, 1H, ArH), 8.35 (*d, J* = 1.1 Hz, 1H, ArH). $^{13}$C NMR spectrum (100,6 MHz, DMSO-$d_6$), δ, ppm: 19.74, 36.95, 55.95, 55.99, 103.39, 107.61, 113.13, 137.57, 143.73. 153.64, 169.93, 175.33.

### 3.1.6. 2-methyl-6,7-dimethoxy-3,1-benzoxazine-4-one (2d)

The white amorphous substance was obtained. Yield: 86%. M.p.: 226–227 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 236, 271. IR spectrum (KBr), ν, $cm^{-1}$: 1734 (C=O), 1024 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.12 (*t, J* = 7.5 Hz, 3H, $CH_3$), 3.78 (*d, J* = 20.3 Hz, 6H, $CH_3$), 7.42 (*s,* 1H, ArH), 8.33 (*s,* 1H, ArH).




**Scheme 4** Mechanism of formation of target 6,7-dimethoxykinazoline-4(3*H*)-one derivatives.

### 3.1.7. 2-phenyl-6,7-dimethoxy-3,1-benzoxazine-4-one (2e)

The white amorphous substance was obtained. Yield: 96%. M.p.: 194–195 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 202, 225, 263, 320. IR spectrum (KBr), ν, $cm^{-1}$: 1740 (C=O), 1019 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 3.90 (*s,* 3H, $CH_3$), 3.95 (*s,* 3H, $CH_3$), 7.21 (*s,* 1H, ArH), 7.45 (*s,* 1H, ArH), 7.61 (*dq, J* = 14.4, 6.6 Hz, 3H, ArH), 8.15 (*d, J* = 8.1 Hz, 2H, ArH).

### 3.1.8. 2-(6,7-dimethoxy-2-ethyl-4-oxoquinazoline-3-yl)acetic acid (3a)

The white amorphous substance was obtained. Yield: 75%. M.p.: 263–264 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 242. IR spectrum (KBr), ν, $cm^{-1}$: 3437 (OH), 1682 (C=O), 1610 (C=O), 1011 (C-O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.25 (*t, J* = 7.2 Hz, 3H, $CH_3$), 2.75 (*q, J* = 7.3 Hz, 2H, $CH_2$), 3.92 (*s,* 3H, $CH_3$), 3.87 (*s,* 3H, $CH_3$), 4.83 (*s,* 2H, $CH_2$), 7.10 (*s,* 1H, ArH), 7.40 (*s,* 1H, ArH), 13.24 (*s,* 1H, COOH).

### 3.1.9. 2-[[2-(2-ethyl-6,7-dimethoxy-4-oxoquinazoline-3-yl)acetyl]amino]acetic acid (3b)

The white amorphous substance was obtained. Yield: 70%. M.p.: 266–267 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 242. IR spectrum (KBr), ν, $cm^{-1}$: 3410 (OH), 3298 (C=O), 1736 (C=O), 1659 (C=O), 1612 (C=O), 1013 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.24 (*t, J* = 7.2 Hz, 3H, $CH_3$), 2.73 (*q, J* = 7.0 Hz, 2H, $CH_2$), 3.80 (*d, J* = 5.6 Hz, 2H, $CH_2$), 3.86 (*s,* 3H, $CH_3$), 3.91 (*s,* 3H, $CH_3$), 4.82 (*s,* 2H, ArH), 7.08 (*s,* 1H, ArH), 7.39 (*s,* 1H, ArH), 8.63 (*t, J* = 5.7 Hz, 1H, NH), 12,72 (*s,* 1H, COOH).

### 3.1.10. 2-[[2-(2-ethyl-6,7-dimethoxy-4-oxoquinazoline-3-yl)acetyl]amino]-4-methyl-pentanoic acid (3c)

The beige amorphous substance was obtained. Yield: 89%. M.p.: 225–226 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 241. IR spectrum (KBr), ν, $cm^{-1}$: 3419 (OH), 3309 (NH), 1732 (C=O), 1661 (C=O), 1612 (C=O), 1008,65 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 0.86 (*d, J* = 6.6 Hz, 3H, $CH_3$), 0.92 (*d, J* = 6.5 Hz, 3H, $CH_3$), 1.12 (*t, J* = 7.5 Hz, 1H, CH), 1.24 (*t, J* = 7.2 Hz, 3H, $CH_3$), 1.62 – 1.49 (*m,* 2H, $CH_2$), 2.70 (*q, J* = 8.1, 7.0 Hz, 2H, $CH_2$), 3.86 (*s,* 3H, $CH_3$), 3.92 (*s,* 3H, $CH_3$), 4.24 (*td, J* = 8.9, 5.8 Hz, 1H.CH), 4.95 – 4.69 (*m,* 2H, $CH_3$), 7.08 (*s,* 1H, ArH), 7.40 (*s,* 1H, ArH), 8.63 (*d, J* = 7.9 Hz, 1H, NH), 12,65 (*s,* 1H, COOH).

### 3.1.11. 2-[[2-(2-ethyl-6,7-dimethoxy-4-oxoquinazoline-3-yl)acetyl]amino]-3-(1H-indole-3-yl)propanoic acid (3d)

The beige amorphous substance was obtained. Yield: 73%. M.p.: 265–266 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 228, 239, 271. IR spectrum (KBr), ν, $cm^{-1}$: 3383 (NH), 1730 (C=O), 1661 (C=O), 1641 (C=O), 1013 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.14 (*t, J* = 7.2 Hz, 3H, $CH_3$), 3.05 (*dd, J* = 14.7, 8.7 Hz, 2H, $CH_2$), 3.22 (*dd, J* = 14.6, 4.9 Hz, 2H, $CH_2$), 3.85 (*s,* 3H, $CH_3$), 3.91 (*s,* 3H, $CH_3$), 4.53 (*td, J* = 8.3, 4.8 Hz, 1H, CH), 4.91–4.61 (*m,* 2H, $CH_2$), 7.00 (*t, J* = 7.4 Hz, 1H, ArH), 7.07 (*d, J* = 7.6 Hz, 2H, ArH), 7.19 (*d, J* = 2.5 Hz, 1H, ArH), 7.35 (*d, J* = 8.0 Hz, 1H, ArH), 7.39 (*s,* 1H, ArH), 7.56 (*d, J* = 7.7 Hz, 1H, ArH), 8.68 (*d, J* = 8.0 Hz, 1H, NH), 10.91 (*d, J* = 2.5 Hz, 1H, NH), 12,79 (*s,* 1H, COOH).

### 3.1.12. 2-(6,7-dimethoxy-4-oxo-2-propyl-quinazoline-3-yl)acetic acid (3e)

The white amorphous substance was obtained. Yield: 58%. M.p.: 234–235 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 241. IR spectrum (KBr), ν, $cm^{-1}$: 3431 (OH), 1713 (C=O), 1676 (C=O), 1007 (C–O).$^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 0.99 (*t, J* = 7.4 Hz, 3H, $CH_3$), 1.83–1.69 (*m,* 2H, $CH_2$), 2.74–2.66 (*m,* 2H, $CH_2$), 3.87 (*s,* 3H, $CH_3$), 3.92 (*s,* 3H, $CH_3$), 4.82 (*s,* 2H, $CH_2$), 7.09 (*s,* 1H, ArH), 7.40 (*s,* 1H, ArH), 13,25 (*s,* 1H, COOH). $^{13}$C NMR spectrum (100,6 MHz, DMSO-$d_6$), δ, ppm: 14.12, 19,81, 36.17, 45.17, 56.15, 56.44, 105.57, 108.05, 112.96, 143.62, 148.84, 155.21, 155.74, 160.97, 170.16.

### 3.1.13. 2-[[2-(2-propyl-6,7-dimethoxy-4-oxo-quinazoline-3-yl)acetyl]amino]acetic acid (3f)

The white amorphous substance was obtained. Yield: 78%. M.p.: 247–248 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 242. IR spectrum (KBr), ν, $cm^{-1}$: 3439 (OH), 3338 (NH), 1740 (C=O), 1662 (C=O), 1641 (C=O), 1011 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 0.99 (*t, J* = 6.7 Hz, 3H, $CH_3$), 1.84–1.71 (*m,* 2H. $CH_2$), 2.68 (*t, J* = 7.5 Hz, 2H, $CH_2$), 3.83–3.78 (*m,* 2H, $CH_2$), 3.88 (*d, J* = 20.3 Hz, 6H, $CH_3$), 4.82 (*s,* 2H, $CH_2$), 7.07 (*s,* 1H, ArH), 7.39 (*s,* 1H, ArH), 8.65 (*t, J* = 5.8 Hz, 1H, NH), 12,80 (*s,* 1H, COOH). $^{13}$C NMR spectrum (100,6 MHz, DMSO-$d_6$), δ, ppm: 14.12, 19.65, 35.95, 41.26, 45.28, 56.13, 56.41, 105.72, 108.00, 113.10, 143.63, 148.70, 155.08, 156.09, 161.01, 167.86, 171.54.

### 3.1.14. 2-[[2-(2-propyl-6,7-dimethoxy-4-oxoquinazoline-3-yl)acetyl]amino]-4-methyl-pentanoic acid (3g)

The beige amorphous substance was obtained. Yield: 69%. M.p.: 236–237 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 242. IR spectrum (KBr), ν, $cm^{-1}$: 3520 (OH), 3308 (NH), 1678 (C=O), 1655 (C=O), 1614 (C=O), 1007 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 0.86 (*d, J* = 6.5 Hz, 3H, $CH_3$), 0.92 (*d, J* = 6.6 Hz, 3H, $CH_3$), 0.98 (*t, J* = 7.4 Hz, 3H, $CH_3$), 1.61–1.49 (*m,* 2H, $CH_2$), 1.67 (*dt, J* = 12.9, 8.0 Hz, 1H, CH), 1.82–1.72 (*m,* 2H, $CH_2$), 2.63 (*t, J* = 7.6 Hz, 2H, $CH_2$), 3.86 (*s,* 3H, $CH_3$), 3.91 (*s,* 3H, $CH_3$), 4.30–4.20 (*m,* 1H, CH), 4.82 (*q, J* = 17.0 Hz, 2H, $CH_2$), 7.07 (*s,* 1H, ArH), 7.40 (*s,* 1H, ArH), 8.64 (*d, J* = 8.0 Hz, 1H, NH), 12.72 (*s,* 1H, COOH). $^{13}$C NMR spectrum (100,6 MHz, DMSO-$d_6$), δ, ppm: 14.12, 19.64, 21.66, 23.34, 24.80, 36.00, 45.04, 50.91, 56.14, 56.41, 105.74, 108.00, 113.08, 143.62, 148.71, 155.08, 156.04, 160.99, 167.44, 174.31.

### 3.1.15. 2-[[2-(2-propyl-6,7-dimethoxy-4-oxoquinazoline-3-yl)acetyl]amino]-3-(1H-indole-3-yl)propanoic acid (3h)

The beige amorphous substance was obtained. Yield: 57%. M.p.: 272–273 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 229, 239, 271. IR spectrum (KBr), ν, $cm^{-1}$: 3381 (NH), 1730 (C=O), 1662 (C=O), 1641 (C=O), 1009 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 0.91 (*t, J* = 7.4 Hz, 3H, $CH_3$), 1.76–1.60 (*m,* 2H, $CH_2$), 2.47 (*dd, J* = 7.6, 2.7 Hz,

2H, $CH_2$), 3.25–3.01 (*m,* 2H, $CH_2$), 3.86 (*s,* 3H, $CH_3$), 3.91 (*s,* 3H, $CH_3$), 4.52 (*td, J* = 8.2, 5.0 Hz, 1H, CH), 4.77 (*q, J* = 16.9 Hz, 2H, $CH_2$), 7.00 (*t, J* = 6.9 Hz, 1H, ArH), 7.06 (*s,* 1H, ArH), 7.12–7.07 (*m,* 1H, ArH), 7.20 (*d, J* = 2.4 Hz, 1H, ArH), 7.41–7.30 (*m,* 2H, ArH), 7.56 (*d, J* = 7.8 Hz, 1H, ArH), 8.70 (*d, J* = 7.9 Hz, 1H, NH), 10.93 (*s,* 1H, NH), 12.83 (*s,* 1H, COOH). $^{13}$C NMR spectrum (100,6 MHz, DMSO-$d_6$), δ, ppm: 14.07, 19.61, 27.58, 35.86, 45.16, 53.71, 56.14, 56.41, 105.72, 107.99, 110.12, 111.85, 113.06, 118.63, 118.86, 121.39, 124.12, 127.67, 136.54, 143.62, 148.69, 155.07, 156.06, 160.98, 167.26, 173.57.

#### 3.1.16. 2-[[2-(2-isopropyl-6,7-dimethoxy-4-oxoquinazoline-3-yl)acetyl]amino]acetic acid (3i)

The white amorphous substance was obtained. Yield: 61%. M.p.: 219–220 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 239. IR spectrum (KBr), ν, $cm^{-1}$: 3425 (OH), 3298 (NH), 1732 (C=O), 1662 (C=O), 1614 (C=O), 1007 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.24 (*d, J* = 6.6 Hz, 6H, $CH_3$), 3.06–2.92 (*m,* 1H, CH), 3.82 (*d, J* = 5.7 Hz, 2H, $CH_2$), 3.86 (*s,* 3H, $CH_3$), 3.92 (*s,* 3H, $CH_3$), 4.87 (*s,* 2H, $CH_2$), 7.07 (*s,* 1H, ArH), 7.40 (*s,* 1H, ArH), 8.66 (*t, J* = 5.9 Hz, 1H, NH), 12.68 (*s,* 1H, COOH). $^{13}$C NMR spectrum (100,6 MHz, DMSO-$d_6$), δ, ppm: 21.69, 31.88, 41.26, 45.08, 56.15, 56.43, 105.72, 108.05, 113,10, 143.65, 148.76, 155.16, 160.82, 161.09, 167.93, 171.46.

#### 3.1.17. 2-[[2-(2-isopropyl-6,7-dimethoxy-4-oxoquinazoline-3-yl)acetyl]amino]-4-methyl-pentanoic acid (3j)

The white amorphous substance was obtained. Yield: 50%. M.p.: 220–221 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 241. IR spectrum (KBr), ν, $cm^{-1}$: 3414 (OH), 3309 (NH), 1736 (C=O), 1678 (C=O), 1657 (C=O), 1007 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 0.86 (*dd, J* = 6.5, 1.9 Hz, 3H, $CH_3$), 0.92 (*dd, J* = 6.6, 1.9 Hz, 3H, $CH_3$), 1.17 (*dd, J* = 6.9, 2.0 Hz, 3H, $CH_3$), 1.23 (*d, J* = 4.5 Hz, 3H, $CH_3$), 1.63–1.49 (*m,* 2H, $CH_2$), 1.73 – 1.65 (*m,* 1H. CH), 3.02–2.89 (*m,* 1H, CH), 3.86 (*s,* 3H, $CH_3$), 3.92 (*s,* 3H, $CH_3$), 4.31–4.20 (*m,* 1H, CH), 4.89 (*q, J* = 14.0 Hz, 2H, $CH_2$), 7.07 (*d, J* = 1.9 Hz, 1H, ArH), 7.42 (*dd, J* = 13.1, 1.9 Hz, 1H, ArH), 8.64 (*d, J* = 8.0 Hz, 1H, NH), 12.72 (*s,* 1H, COOH).

#### 3.1.18. 2-(6,7-dimethoxy-2-methyl-4-oxoquinazoline-3-yl)acetic acid (3k)

The white amorphous substance was obtained. Yield: 72%. M.p.: 215–216 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 242. IR spectrum (KBr), ν, $cm^{-1}$: 3431 (OH), 16778 (C=O), 1610 (C=O), 1011 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 1.25 (*td, J* = 7.3, 1.6 Hz, 3H, $CH_3$), 3.87 (*s,* 3H, $CH_3$), 3.92 (*s,* 3H, $CH_3$), 4.83 (*s,* 2H, $CH_2$), 7.10 (*t, J* = 1.5 Hz, 1H, ArH), 7.40 (*q, J* = 6.3 Hz, 1H, ArH), 13.26 (*s,* 1H, COOH).

#### 3.1.19. 2-(6,7-dimethoxy-4-oxo-2-phenyl-quinazoline-3-yl)acetic acid (3l)

The white crystals were obtained. Yield: 66%. M.p.: 281–282 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 247, 295. IR spectrum (KBr), ν, $cm^{-1}$: 3437 (OH), 1716 (C=O), 1676 (C=O), 1005 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 3.91 (*d, J* = 2.1 Hz, 6H, $CH_3$), 4.53 (*s,* 2H, $CH_2$), 7.21 (*s,* 1H, ArH), 7.49 (*s,* 1H, ArH), 7.55 (*s,* 5H, ArH), 13.18 (*s,* 1H, COOH).

#### 3.1.20. 2-(6,7-dimethoxy-4-oxo-2-phenyl-quinazoline-3-yl)propanoic acid (3m)

The white crystals were obtained. Yield: 62%. M.p.: 202–203 °C. UV spectrum (ethanol), $\lambda_{max}$, nm: 246, 302. IR spectrum (KBr), ν, $cm^{-1}$: 3419 (OH), 1715 (C=O), 1670 (C=O), 1026 (C–O). $^1$H NMR spectrum (400 MHz, DMSO-$d_6$), δ, ppm: 2.59 (*t, J* = 7.7 Hz, 2H, $CH_2$), 3.90 (*d, J* = 3.7 Hz, 6H, $CH_3$), 4.06 (*t, J* = 7.6 Hz, 2H, $CH_2$), 7.16 (*s,* 1H, ArH), 7.49 (*s,* 1H, ArH), 7.57–7.50 (*m,* 3H, ArH), 7.63 (*dd, J* = 6.8, 2.9 Hz, 2H, ArH), 12.34 (*s,* 1H, COOH).

### 3.2. Pharmacological studies

By assessing the change in cognitive deficits in animals, it was found that in the NC group of rats a pronounced cognitive impairment was observed, as evidenced by a decrease in the percentage of spontaneous alternations of the labyrinth arms in this group of rats by 66.9% ($p$<0.05) in relation to the SO animals (Figure 1). The use of EMHPS contributed to a significant decrease in cognitive deficit in rats by 80.1% ($p$<0.05) in relation to the NC group of animals. The administration of the studied compounds also led to a statistically significant decrease in the symptoms of depression of the higher integrative functions of the brain; however, the indicators of spontaneous movement vector change in the animals that were injected with the studied substances were significantly lower than those in the rats that were treated by EMHPS, with the exception of compounds **3i** and **3j**. The use of these substances contributed to the reduction (relative to the NC group of rats) of cognitive deficit in animals by 60.2% ($p$<0.05) and 57.9% ($p$<0.05), respectively, which did not statistically significantly differ from those in the rats receiving a reference drug.

The size of the brain necrosis zone changed similarly (Figure 2). In the NC group of rats, this parameter was 33.6±0.567%, while the use of the reference drug contributed to a decrease in this indicator by 27.7% ($p$<0.05). Among the studied substances, the most pronounced effect on the change in necrotic processes in the brain tissue during ischemia was exerted by the administration of the compounds **3i**, **3j** and **3k**, against the background of which the necrosis zone decreased relative to the NC group of rats by 23.1% ($p$<0.05), 27.9% ($p$<0.05) and 24.2% ($p$<0.05) and did not statistically significantly differ from the similar one in the group of animals that received the reference drug. The rest of the studied substances did not have a significant effect on the change in the area of brain necrosis.

Currently, cerebrovascular accidents and, in particular, ischemic stroke remain one of the main medical and social health problems. It was established that in 2019 stroke ranked second among the main non-communicable causes of death, second only to coronary heart disease and the terminal stage of this disease – myocardial infarction.

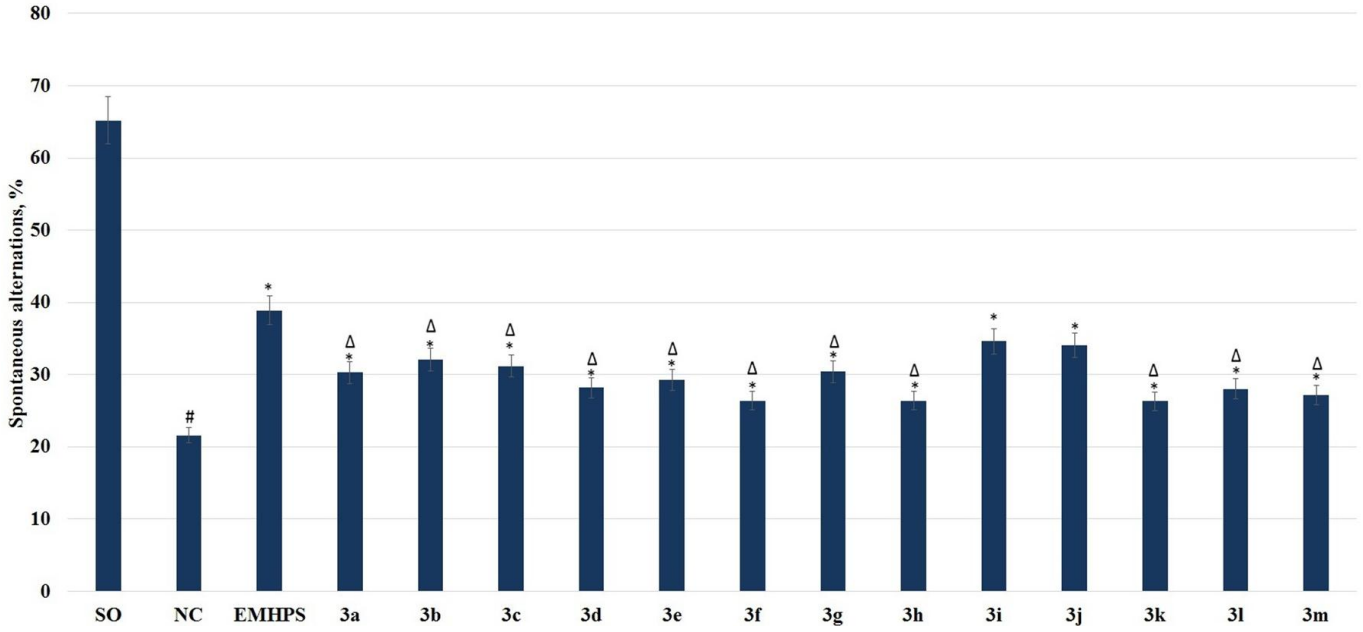



**Figure 1** Influence of the studied compounds and the referent on the change in cognitive deficit in rats under the conditions of cerebral ischemia.
Note: # – significant relative to SO (Tukey's test, $p<0.05$);* – significant relative to NC (Tukey's test, $p<0.05$);Δ – significant relative to EMHPS (Tukey's test, $p<0.05$).

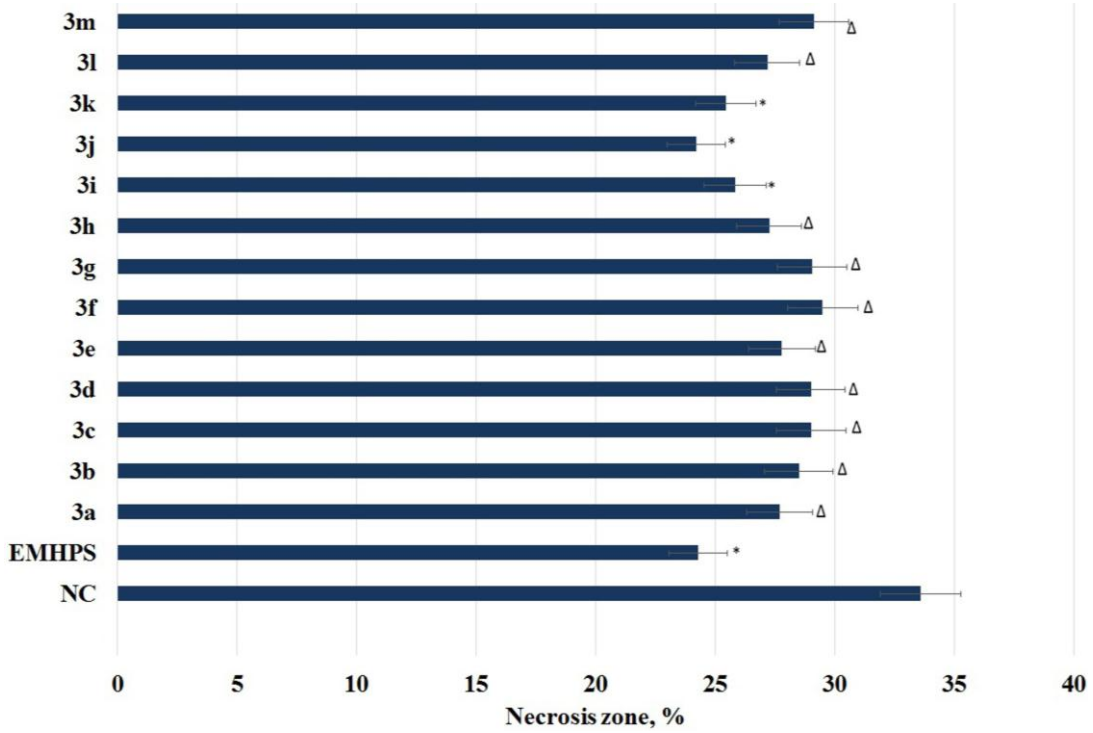



**Figure 2** Influence of the studied compounds and the referent on the change of the brain tissue necrosis zone in rats under the conditions of cerebral ischemia.
Note: in the SO group of rats, the brain necrosis zone was zero; * – significant relative to NC (Tukey's test, $p<0.05$); Δ – significant relative to EMHPS (Tukey's test, $p<0.05$).

More than 6 million deaths from stroke occur annually, while there is a significant increase in cases of primary disability and disability after an ischemic attack, which is more than 50% of the total number of stroke episodes. According to WHO statistical reports, cerebral hemodynamic disorders are more often recorded in countries with middle and middle-high income levels, while with an increase in the well-being of the population and the possibility of obtaining qualified and timely medical care, the incidence of stroke is significantly reduced, which is confirmed by data from high-income countries [40].

The high medical and socioeconomic role of ischemic stroke dictates the need to find new ways to treat this pathological condition. One of such relatively new strategies for adjuvant therapy of stroke may be “freezing” the

activity of neurons in the ischemic penumbra, i.e. cerebroprotection. Cerebroprotectors include agents of various chemical structures: compounds of natural origin, which are most often represented by polyphenols: quercetin, verbascoside, aurapten, thymol, epigallocatechin gallate, curcumin [41], sex hormones, low molecular weight synthetic compounds such as lactic acid salts, acetyl-L-carnitine, angiotensin II receptor antagonists. Several promising neuroprotectors have been discovered in the last decade: succinic acid derivatives, [42], chomones [43] uric acid, otaplimastat (an inhibitor of metalloproteinases) [44], drotrecogin-α (anticoagulant, PAR-1 agonist) [45], verapamil, nerinetide (anti-excitotoxic drug) [46].

The study showed that the investigated quinazolinone derivatives with fragments of amino acids and dipeptides also could be promising cerebroprotective agents. In this respect, especially noteworthy are the compounds **3i**, **3j** and **3k**, the use of which not only led to the recovery of the cognitive functions of animals, but also reduced the size of the brain necrosis zone. Thus, the obtained results will allow expanding the range of potential cerebroprotective agents by three quinazolinone derivatives.

## 4. Conclusions

In the course of the study, a number of novel 6,7-dimethoxyquinazolin-4(3*H*)-one derivatives with alkyl substituents and residues of neuroactive amino acids and dipeptides were synthesized. Among the studied quinazolinone derivatives, compounds **3i**, **3j** and **3k** have the most pronounced cerebrotropic activity and are not inferior to ethylmethylhydroxypyridine succinate in terms of pharmacological activity, which makes them promising objects for further research.

## Supplementary materials

No supplementary materials are available.

## Funding

The reported study was funded by RFBR, project No. 20-315-90060, https://www.rfbr.ru/rffi/eng.

## Acknowledgment

None.

## Author contributions

Conceptualization: I.P.K.
Data curation: I.P.K.
Formal Analysis: A.S.C., D.I.P., I.P.K.
Funding acquisition: A.S.C., I.P.K.
Investigation: A.S.C., I.P.K., D.I.P.
Methodology: A.S.C., D.I.P., I.P.K.
Project administration: I.P.K.
Resources: A.S.C., I.P.K., D.I.P.
Software: A.S.C., D.I.P.
Supervision: I.P.K.
Validation: D.I.P.
Visualization: A.S.C., D.I.P.
Writing – original draft: A.S.C., I.P.K., D.I.P.
Writing – review & editing: A.S.C., D.I.P.

## Conflict of interest

The authors declare no conflict of interest.

## Additional information

Author IDs:
A.S. Chiriapkin, Scopus ID 57218134815;
I.P. Kodonidi, Scopus ID 10240218600;
D.I. Pozdnyakov, Scopus ID 57190954589;

Website:
https://www.pmedpharm.ru/sveden_eng.

## References

1. Lin YF, Smith AV, Aspelund T, Betensky RA, Smoller, JW, Gudnason V, launer LJ, Blacker D. Genetic overlap between vascular pathologies and Alzheimer's dementia and potential causal mechanisms. Alzheimer's Dementia. 2019;15(1):65–75. doi:10.1016/j.jalz.2018.08.002
2. Chiriapkin AS, Kodonidi IP, Pozdnyakov DI, Glushko AA. Synthesis, *in vitro* and docking studies of 2-substituted 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine-4(3H)-one derivatives as agents for the treatment of Alzheimer's disease. Chim Techno Acta. 2022;9(2):20229204. doi:10.15826/chimtech.2022.9.2.04
3. Capizzi A, Woo J, Verduzco-Gutierrez M. Traumatic Brain Injury. Med Clin North Am. 2020;104(2):213–238. doi:10.1016/j.mcna.2019.11.001
4. Danics K, Forrest SL, Kapas I, Erber I, Schmid S, Törő K, Majtenyi K, Kovacs GG. Neurodegenerative proteinopathies associated with neuroinfections. J Neural Transm. 2021;128:1551–1566. doi:10.1007/s00702-021-02371-7
5. Mishchenko O, Palagina N. Experimental research of cerebroprotective activity of the new 4-aminobutatanoic acid derivative. EUREKA Health Sci. 2021;3:95–100. doi:10.21303/2504-5679.2021.001851
6. Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of ischemic brain damage. Neuropharmacol. 2008;55(3):310–318. doi:10.1016/j.neuropharm.2008.01
7. Pasala PK, Shaik RA, Rudrapal |M, Khan J, Alaidarous MA, Khairnar SJ, Bendale AR, Naphade VD, Sahoo RK, Zothantluanga JH, Walode SG. Cerebroprotective effect of Aloe Emodin: In silico and in vivo studies. Saudi J Biol Sci.2022;29(2):998–1005. doi:10.1016/j.sjbs.2021.09.077
8. Sepehri N, Iraji A, Yavari A, Asgari MS, Zamani S, Hosseini S, Bahadorikhalili S, Pirhadi S, Larijani B, Khoshneviszadeh M, Hamedifar H, Mahdavi M, Khoshneviszadeh M. The natural-based optimization of kojic acid conjugated to different thioquinazolinones as potential anti-melanogenesis agents with

tyrosinase inhibitory activity. Bioorg Med Chem. 2021;36:116044. doi:10.1016/j.bmc.2021.116044
9. Soliman AM, Karam HM, Mekkawy MH, Ghorab MM. Antioxidant activity of novel quinazolinones bearing sulfonamide: Potential radiomodulatory effects on liver tissues via NF-κB/PON1 pathway. Eur J Med Chem. 2020;197:112333. doi:10.1016/j.ejmech.2020.112333
10. Soliman AM, Mekkawy MH, Karam HM, Higgins M, Dinkova-Kostova AT, Ghorab MM. Novel iodinated quinazolinones bearing sulfonamide as new scaffold targeting radiation induced oxidative stress. Bioorg Med Chem Lett. 2021;42:128002. doi:10.1016/j.bmcl.2021.128002
11. Anwar A, Shahbaz MS, Saad SM, Kanwal, Khan KM, Siddiqui R, Khan NA. Novel antiacanthamoebic compounds belonging to quinazolinones. Eur J Med Chem. 2019;182:111575. doi:10.1016/j.ejmech.2019.111575
12. Hekal MH, Abu El-Azm FSM. New potential antitumor quinazolinones derived from dynamic 2-undecyl benzoxazinone: Synthesis and cytotoxic evaluation. Synth Commun. 2018;48(18):2391–2402. doi:10.1080/00397911.2018.1490433
13. Ugale VG, Bari SB, Khadse SC, Reddy PN, Bonde CG, Chaudhari PJ. Exploring quinazolinones as anticonvulsants by molecular fragmentation approach: structural optimization, synthesis and pharmacological evaluation studies. Chem Sel. 2020;5(10):2902–2912. doi:10.1002/slct.201904776
14. Akyüz G. Synthesis and urease inhibition studies of some new quinazolinones. J Heterocycl Chem. 2021;58(5):1164–1170. doi:10.1002/jhet.4247
15. Sakr A, Rezq S, Ibrahim SM, Soliman E, Baraka MM, Romero DG, Kothayer H. Design and synthesis of novel quinazolinones conjugated ibuprofen, indole acetamide, or thioacetohydrazide as selective COX-2 inhibitors: anti-inflammatory, analgesic and anticancer activities. J Enzym Inhib Med Chem. 2021;36(1):1810–1828. doi:10.1080/14756366.2021.1956912
16. Sudula SR, Jala R, Siddoju K, Ega JK. One pot synthesis of some new N-allyl and N-benzyl quinazolinones and their anti-inflammatory activity. J Indian Chem Soc. 2021;98(3):100033. doi:10.1016/j.jics.2021.100033
17. Thorat DB, Shivkumar B, Rao N, Mohankumar KM. Synthesis and evaluation of new 4(3H)-quinazolinones derivatives as potential anti-inflammatory agents. Asian J Res Pharmut Sci. 2021;11(3):213–218. doi:10.52711/2231-5659.2021.00034
18. Deshineni RGAC, Banothu J, Velpula R, Chellamella G. Synthesis, anticancer and antibacterial evaluation of novel 1,3-disubstituted-1H-pyrazole-4-yl-methylene embedded fused thiazolo[2,3-b]quinazolinones. Chem Data Collect. 2020;30:100546. doi:10.1016/j.cdc.2020.100546
19. Patel HM, Noolvi MN, Shirkhedkar AA, Kulkarni AD, Pardeshi CV, Surana SJ. Anti-convulsant potential of quinazolinones. RSC Adv. 2016:6(50):44435–44455. doi:10.1039/C6RA01284A
20. Neupane R, Malla S, Abou-Dahech MS, Balaji S, Kumari S, Waiker DK, Moorthy NSHN, Trivedi P, Ashby CR Jr., Karthikeyan C, Tiwari AK. Antiproliferative Efficacy of N-(3-chloro-4-fluorophenyl)-6,7-dimethoxyquinazolin-4-amine, DW-8, in colon cancer cells is mediated by intrinsic apoptosis. Mol. 2021;26(15):4417. doi:10.3390/molecules26154417
21. Saul S, Huang P-T, Einav S, Asquith CRM. Identification and evaluation of 4-anilinoquin(az)online as potent inhibitors of both dengue virus (DENV) and Venezuelan equine encephalitis virus (VEEV). Bioorg Med Chem Lett. 2021;52(15):128407. doi:10.1016/j.bmcl.2021.128407
22. Yao D, Li C, Jiang J, Huang J, Wang J, He Z, Zhang J. Design, synthesis and biological evaluation of novel HDAC Inhibitors with improved pharmacokinetic profile in breast cancer. Eur J Med Chem. 2020;205:112648. doi:10.1016/j.ejmech.2020.112648
23. Nunes PSG, da Silva G, Nascimento S, Mantovani SP, de Andrade P, Bernardes ES, Kawano DF, Leopoldino AM, Carvalho I. Synthesis, biological evaluation and molecular docking studies of novel 1,2,3-triazole-quinazolines as antiproliferative agents displaying ERK inhibitory activity. Bioorg Chem. 2021;113:104982. doi:10.1016/j.bioorg.2021.104982
24. Mizukawa Y, Ikegami-Kawai M, Horiuchi M, Kaiser M, Kojima M, Sakanoue S, Miyagi S, Chick CN, Togashi H, Tsubaki M, Ihara M, Usuki T, Itoh I. Quest for a potent antimalarial drug lead: Synthesis and evaluation of 6,7-dimethoxyquinazoline-2,4-diamines. Bioorg Med Chem. 2021;33:116018. doi:10.1016/j.bioorg.2021.104982
25. Prinsloo IF, Zuma NH, Aucamp J, N'Da DD. Synthesis and in vitro antileishmanial efficacy of novel quinazolinone derivatives. Chem Biol Drug Des. 2021;97:383–398. doi:10.1111/cbdd.13790
26. Chang X, Sun D, Shi D, Wang G, Chen Y, Zhang K, Tan H, Liu J, Liu B, Ouyang L. Design, synthesis, and biological evaluation of quinazolin-4(3H)-one derivatives co-targeting poly(ADP-ribose) polymerase-1 and bromodomain containing protein 4 for breast cancer therapy. Acta Pharm Sinica B. 2021;11(1):156–180. doi:10.1016/j.apsb.2020.06.003
27. Chen J-N, Li T, Chen L, Qin T-S, Sun Y-X, Chen C-T, He Y-Z, Liu G, Yao D, Wei Y, Li Q-Y, Zhang G-J. Synthesis and in vitro anti-bladder cancer activity evaluation of quinazolinyl-arylurea derivatives. Eur J Med Chem. 2020;205:112661. doi:10.1016/j.ejmech.2020.112661
28. Gusev EI, Skvortsova VI, Dambinova SA, Raevskiy KS, Alekseev AA, Bashkatova VG, Kovalenko AV, Kudrin VS, Yakovleva EV. Neuroprotective effects of glycine for therapy of acute ischaemic stroke. Cerebrovasc Dis. 2000;10(1):49–60. doi:10.1159/000016025
29. Kodonidi IP, Chiriapkin AS, Tworowski DE. Molecular design of N-acyl derivatives of 2-(2-oxopyrolidin-1-yl)-acetamide with GABA-ergic and glutamatergic activities. Pharm Pharmacol. 2021;9(1):84–97. doi:10.19163/2307-9266-2021-9-1-84-97
30. Brenneman DE, Gozes I. A femtomolar-acting neuroprotective peptide.J Clin Invest. 1996;97(10):2299–2307. doi:10.1172/JCI118672
31. Gozes I, Perl O, Giladi E, Davidson A, Ashur-Fabian O, Rubinraut S, Fridkin M. Mapping the active site in vasoactive intestinal peptide to a core of four amino acids: Neuroprotective drug design. Proceed Nat Acad Sci. 1999;96(7):4143–4148. doi:10.1073/pnas.96.7.4143
32. Meloni BP, Mastaglia FL, Knuckey NW. Cationic Arginine-Rich Peptides (CARPs): A novel class of neuroprotective agents with a multimodal mechanism of action. Front Neurol. 2020;11:108. doi:10.3389/fneur.2020.00108
33. Edwards AB, Anderton RS, Knuckey NW, Meloni BP. Characterisation of neuroprotective efficacy of modified poly-arginine-9 (R9) peptides using a neuronal glutamic acid excitotoxicity model. Mol Cell Biochem. 2017;426:75–85. doi:10.1007/s11010-016-2882-z
34. Shashoua VE, Adams DS, Boyer-Boiteau A, Cornell-Bell A, Li F, Fisher M. Neuroprotective effects of a new synthetic peptide, CMX-9236, in in vitro and in vivo models of cerebral ischemia. Brain Research. 2003;963(1–2):214–223. doi:10.1016/S0006-8993(02)04058-1
35. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M ,Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol. 2020;18(7):e3000410. doi:10.1371/journal.pbio.3000410
36. Tamura A, Graham DI, McCulloch J, Teasdale GM. Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab. 1981;1(1):53–60. doi:10.1038/jcbfm.1981.6
37. Pozdnyakov DI, Rukovitsina VM, Abaev VT, Oganesyan ET, Larskii MV. Effect of 3-[(e)-3-(3,5-di-tert-butyl-4-hydroxyphenyl)-3-оксопроп-1-енил]-6-methoxy-chrome-4-one on

oxidative status of brain tissue under conditions of cerebral ischemia. Eksperimental'naya i Klinicheskaya Farmakologiya. 2021;84(2):3–7. doi:10.30906/0869-2092-2021-84-3-3-7
38. Amani M, Zolghadrnasab M, Salari AA. NMDA receptor in the hippocampus alters neurobehavioral phenotypes through inflammatory cytokines in rats with sporadic Alzheimer-like disease. Physiol Behav. 2019;202:52–61. doi:10.1016/j.physbeh.2019.01.005
39. Ovakimyan AG, Bicherov AA, Kodonidi IP, Oganesyan ET, Manvelyan EA, Bicherov AV, Tyurin RV, Zaychenko SB, Manvelyan MM. Prediction, synthesis and study of dophaminergic activity of hydroxy- and methoxyphenyl derivatives of 4-(3)quinazolinone. Modern problems of science and education. 2015;2–2:511. https://science-education.ru/ru/article/view?id=23168
40. Rabinstein AA. Update on Treatment of Acute Ischemic Stroke. Continuum (Minneap Minn). 2020;26(2):268–286. doi:10.1212/CON.0000000000000840
41. Angeloni C, Vauzour D. Natural Products and Neuroprotection. Int J Mol Sci. 2019;20(22):5570. doi:10.3390/ijms20225570
42. Pozdnyakov DI, Zolotykh DS, Larsky MV. Correction of mitochondrial dysfunction by succinic acid derivatives under experimental cerebral ischemia conditions. Current Issues in Pharmacy and Medical Sciences. 2021;34(1):42–48. doi:10.2478/cipms-2021-0008
43. Pozdnyakov DI, Voronkov AV, Rukovitsyna VM. Chromon-3-aldehyde derivatives restore mitochondrial function in rat cerebral ischemia. Iran J Basic Med Sci. 2020;23(9):1172–1183. doi:10.22038/ijbms.2020.46369.10710
44. Kim JS, Lee KB, Park J-H, Sung SM, Oh K, Kim E-G, Chang D-i, Hwang YH, Lee E-J, Kim W-K, Ju C, Kim BS, Ryu J-M. Safety and Efficacy of Otaplimastat in Patients with Acute Ischemic Stroke Requiring tPA (SAFE-TPA): A Multicenter, Randomized, Double-Blind, Placebo-Controlled Phase 2 Study. Ann Neurol. 2020;87:233–245. doi:10.1002/ana.25644
45. Lyden P, Pryor KE, Coffey CS, et al. Final results of the RHAPSODY trial: a multicenter, phase 2 trial using a continual reassessment method to determine the safety and tolerability of 3K3A-APC, a recombinant variant of human activated protein C, in combination with tissue plasminogen activator, mechanical thrombectomy or both in moderate to severe acute ischemic stroke. Ann Neurol 2019;85:125–136. doi:10.1002/ana.25383
46. Hill MD, Goyal M, Menon BK, et al. Efficacy and safety of nerinetide for the treatment of acute ischaemic stroke (ESCAPE-NA1): a multicentre, double-blind, randomized controlled trial. The Lancet. 2020;395:878–887. doi:10.1016/S0140-6736(20)30258-0