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Some Aspects of Neuroprotective Action of a New Derivative of 3-Methylxanthine (Compound C-3) Under Conditions of Acute Disorder of Cerebral Circulation (ADCC) Modeling by Ischemic Stroke Type

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Abstract

The purpose of the research was to study the antioxidant and neuroprotective properties of 8-benzilaminotheophyllinil-7-acetic acid (Compound C-3), as well as peculiarities of its action and advantages over the reference drugs. The work was performed on 80 white nonlinear rats of both sexes weighing 160-170 g. Bilateral ligation of the common carotid arteries was performed under thiopental (40 mg/kg) anesthesia. The investigated compound C-3 in a dose of 100 mg/kg and reference drugs in doses recommended by earlier studies (thiotriazolin 50 mg/kg, mexidol 200 mg/kg and 100 mg/kg citicoline) were administered endogastrically with a metal feeding tube for 4 days. Acute disorder of cerebral circulation (ADCC) modeling have led to disturbance in the system NO (increase in NOS activity and NO hyperproduction), deprivation in the activity of antioxidant enzymes (SOD, catalase and glutathione peroxidase) in the brain of experimental animals, and as a result, the activation of oxidative and nitrosative stress: increase in level of carbonylated proteins – aldehyde phenylhydrazone (APH) and ketone phenylhydrazone (KPH), markers of free radicals activation, and nitrotyrosine.

The introduction of 8-benzilaminotheophyllinil-7-acetic acid (Compound 3) in a dose of 100 mg/kg endogastrically for 4 days to rats with bilateral carotid artery occlusion resulted in inhibition of oxidative nitrosative stress: reduction in nitrotyrosine level by 51.1% ($p<0.05$), APH – 59%, KPH – 63%, nitrite anion – 44.1%. The introduction of compound 3 (C-3) to the rats with ADCC led to increase in activity of antioxidant enzymes in the brain of animals on the 4th day of the experiment: SOD by 95.6%, catalase – 78.4%, and glutathione peroxidase (GPx) by 82.3%. Antioxidant and neuroprotective action of C-3 exceeded piracetam and citicoline on its influence on the parameters of the antioxidant system and markers of oxidative and nitrosative stress as well as was superior or comparable to similar effects of thiotriazolin. Mechanism of antioxidant and neuroprotective action of C-3 is connected with the peculiarities of its chemical structure, and hydrazide of 8-benzilaminotheophyllinil-7-acetic acid play the role of "spin trap" interacting with NO.

Keywords: Neuroprotective action, Derivative of 3-Methylxanthine

Introduction

The problem of ischemic stroke is currently becoming increasingly topical. In developed countries stroke occupies the 2-3rd place in the structure of total mortality and is the leading cause of persistent disability [1, 2, 16]. A trigger link for ischemic neuronal death is energy deficit that initiates the glutamate-calcium cascade – release of excitatory aminoacidergic neurotransmitters – aspartate and glutamate, and intracellular accumulation of Ca^{2+} [3]. Processes beginning in the early hours of stroke and underlying glutamate-calcium cascade (changes of glutamate and calcium metabolism, oxidative stress, hyperproduction of $NO^{\bullet}$) induce long-term consequences of ischemia – the reaction of genome with inclusion of genetically-programmed molecular mechanisms, dysfunction of astrocyte and microglia pools, development of immune changes and initiation of neuroapoptosis. The concept of neuroprotection to highlights two main areas of stroke therapy. Primary neuroprotection aims to break fast mechanisms of necrotic cell death – the reactions of calcium-glutamate cascade (antagonists of NMDA and AMPA receptors, and calcium channel blockers – remacemid, relutek, nimotop, et al.) [4]. Secondary neuroprotection is focused on reduction of severity of long-term consequences of ischemia – the blockade of proinflammatory cytokines, cell adhesion molecules, inhibition of oxidative stress, normalization of neurometabolic processes, inhibition of apoptosis (antioxidants, nootropics, neuropeptides: emoxipine, thiotriazolin, glycine, piracetam, tiocetam, cerebrolysin, cortexin, cerebrocurin, et al.) [5].

Despite certain success achieved in the treatment of cerebral stroke, the problem is still quite topical. Modern neuroprotectors have not always demonstrated therapeutic efficacy in clinic, have a number of side effects during long-term use, and due to the lack of reliable medical effect they cannot be adminis-

tered during the acute period of ACCD [6]. Currently the search for newer neuroprotectors is conducted among different azaheterocyclic systems.

Studies conducted over the past 20 years in Zaporizhzhia State Medical University, revealed high antioxidant, anti-ischemic and neuroprotective activity among derivatives of 2-methylxanthine [7]. During experiments in vitro and intracerebral hemorrhage modelling 8-hydrazide benzilaminoteofillinil-7-acetic acid (C-3) was selected [7]. LD_{50} of C-3 is 2100 ± 142.8 mg/kg after endogastric introduction to rats, and the ED50 =100 mg/kg.

The research purpose was study of antioxidant and neuroprotective properties of C-3, particularities of its mechanism of action and advantages over the reference drugs.

Materials and methods. The experimental part was performed on 80 white nonlinear rats weighing 160-170 g collected from the breeding center of Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. Animals were kept on a standard food ration of the vivarium. The experiments were conducted in compliance with Commission on Bioethics and Pharmacy "General ethical animal experimentation" (Kyiv, 2001), consistent with the provisions of the European Convention "On Protection of Vertebrate Animals used for experimental or other scientific purposes" (Strasbourg, 1986; 1998) [8].

Acute cerebral ischemia was modeled by irreversible bilateral common carotid artery ligation under general anaesthesia under sodium thiopental narcosis (40 mg/kg) [8]. By surgical incision the common carotid arteries were separated out, the silk ligatures were drawn under them, and ligated [9].

The aqueous solution of the investigated compound C-3 at a dose of 100 mg/kg body weight, and reference drugs at recommended earlier studies doses: thiotriazolin 50 mg/kg (Public Joint Stock Company "Kievmedpreparat".), mexidol 200 mg/kg ("ZiO-Zdorovie" Joint Stock Company), citicoline 100 mg/kg (Astrafarm TOV) were administered to the animals through the intragastric tube for 4 days. The animals were divided into the following experimental groups: 1, intact (false-operated); 2, control; 3, thiotriazolin; 4, mexidol, 5, citicoline; 6, the investigated compound C-3. There were 10 animals operated in each experimental group, and 30 ones were in the control group.

Physiologic 0.9% sodium chloride solution in the equivalent volume was administered through the intragastric tube to the animals in the control group over a period of experiment. The carotid arteries were separated in false-operated animals in intact group but not ligated. The brain was isolated and examined on the 4th day after the surgery. The blood and brain tunic were immediately removed from the brain, and investigated pieces were placed in liquid nitrogen.

Then the pieces were crushed in liquid nitrogen to powdered state and homogenized in 10-fold volume of medium at (2°C) containing (in mMol): sucrose-250; tris-HCl buffer – 20; EDTA – 1 (pH 7.4) [10].

The cytosol and mitochondrial fractions were isolated at a temperature of (+4°C) by differential centrifugation method (20 minutes at 17000g) on the superspeed refrigerated centrifuge Sigma 3-30k (Germany). The supernatant was decanted and stored at a temperature of (–80°C). To evaluate the intensity of oxidative stress the markers of oxidative modification of protein – aldehydephenylhydrazones (APH) and carboxyphenylhydrazones (CPH) were tested as well as nitrotyrosine. The state of the antioxidative system activity was assessed by superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) activity. Indices of oxidative protein modification (OPM) were measured by the method of Halliwell [11] on interaction of oxidized amino acid residues with 2,4-dinitrophenylhydrazones (2.4-DNPH) and formation of aldehydephenylhydrazones (APH) and carboxyphenylhydrazones (CPH) with absorption spectrum at 274 nm 363 nm respectively []. The measurement of SOD activity was conducted with application of phenazine methosulfate and nitroblue tetrazolium [12].

Catalase activity was measured in the test with hydrogen peroxide and ammonium molybdate [13]. GPx activity was measured by deceleration of NADPH H content (Warburg test) in the sample at a wavelength of 340 nm that corresponds to the speed of oxidized glutathione formation. We also used t-butyl hydroperoxide as a hydroperoxide [14]. Stable metabolites of NO were measured by the level of nitrates and nitrites in the Griess reaction [15], NOS activity was measured by the difference between the rate of oxidation of NADPH, registered by fluorometric method in the two parallel sample that contained NOS inhibitor and did not [16]. Nitrotyrosine was determined in the cytosol fractions of heart homogenate by the solid-phase immunosorbent sandvidge method ELISA, ELISA Kit (Cat # HK 501-02) of the Hycult Biotech company.

The results of the study have been processed with application of Statistical Software Package «STATISTICA® for Windows 6.0» (StatSoftInc., №AXXR712D833214FAN5), as well as «SPSS 16.0», «Microsoft Office Excell 2003». The normalcy of distribution was evaluated on the basis of Shapiro-Wilk criterion. The data are presented as mean values. Statistical significance of the differences between the mean values was determined according to Student's *t*-test at a normal distribution. The criterion U Mann-Whitney was used if the distribution that differed from the normal, as well as for analysis of the ordinal variables. To compare independent variables in more than two samples analysis of variance (ANOVA) was applied for normal distribution and the Kruskal-Wallis criterion was used if distribution differed from normal. The results for all forms of analysis were statistically significant at a significance level of $p<0.05$.

Results and Their Discussion

The bilateral ligation of the common carotid arteries leads to increase in the production of active forms of oxygen (AFO) of the induction of oxidative stress. Primary production of AFO developed as a result of transmitter autocoidosis leads to the oxidative modification of the protein fragments of ion channels, in particu-

lar calcium ones as well as to mitochondrial calcium overload and to the disturbance of their energy-produced function [17]. Subsequently, the mitochondria become a potent source of AFO, initiators of oxidative and nitrosative stresses, and apoptosis []. Free radicals and stable cytotoxic products of oxidative stress under conditions of nervous tissue ischemia leads to desensitization of receptors, mitochondrial dysfunction, and the loss of functional activity of neurons [18].

We have received data (Table 1) suggesting that there has been an increase in the level of products of the oxidative modification of proteins (OPM): APH by 85.6% and KPH by 85.4%, as well as the specific marker of nitrosative stress – nitrotyrosine – by 83.9% in the brain of animals with ADCC on the 4th day after ligation of the arteries.

In our opinion, as well as in the opinion of other authors [19], oxidative modification of proteins leads to a decrease in the function of proteins in the electron transport chain, ATPase activity, and selectivity of transport pore activity. It is believed that oxidative protein modification (OPM) is an early indicator of intracellular damage to functional macromolecules [20]. There is compelling evidence that the increase in OPM products in the cortical neurons leads to significant suppression of cognitive-mnestic functions of the brain [10, 16].

Introduced preparations have produced inhibitory action with regard to the various links of oxidative stress (Tables 1-3). The studied substance C-3 has turned out to be the most effective agent among the studied ones, and its antioxidant action exceeded that of citicoline, mexidol and thiotriazolin.

Table 1

Influence of C-3, thiotriazolin, mexidol and citicoline on the indices of oxidative stress in the brain of arts on the 4th day of ADCC

Groups of animals	**Products of OPM, s.u./g of protein**		**Nitrotyrosine, nmole/g of protein**
	APH (270 nm)	**KPH (363 nm)**	
Intact (False-operated) (n=10)	0,77 ± 0,05	0,35 ± 0,03	23,7±1,12
Ischemia (Control) (n =10)	5,37 ± 0,30	2,41 ± 0,20	147,3±8,3
Ischemia + Thiotriazolin (50 mg/kg) (n = 7)	3,65 ± 0,15*	1,11 ± 0,07*	72,2±4,2*
Ischemia + Mexidol (200 mg/kg) (n = 7)	4,11 ± 0,21*	1,15 ± 0,05*	106,2±9,7*

Table 1 (Continued):
Influence of C-3, thiotriazolin, mexidol and citicoline on the indices of oxidative stress in the brain of arts on the 4th day of ADCC

Ischemia + Citicoline (100 mg/kg) (n = 5)	5,11 ± 0,45	1,81 ± 0,05*	138,6±12,2
Ischemia + C-3 (100 mg/kg) (n = 8)	2,72 ± 0,22*	0,82 ± 0,03*	64,2±5,7*

* – p≤0.05 in relation to the control

Note: the number of animals surviving on the 4th day of the experiment is indicated in brackets.

Introduction of C-3 to animals with occlusion of the common carotid arteries resulted in the reduction of oxidative stress markers – APH by 49% and KPH by 65%, as well as the specific marker of nitrosative stress – nitrotyrosine – by 56.4%. On the influence on these indicators C-3 exceeded citicoline, thiotriazolin and mexidol. Introduction of C-3 to animals with ACCD resulted in increased brain activity of catalase by 147%, SOD by 243% and the activity of GPx increased by 121%. This index in a group of animals taken C-3 for 4 days significantly surpassed those of groups of animals taken mexidol and citicoline.

Table 2
The influence of C-3, thiotriazolin, mexidol and citicoline on the indices of antioxidant system in the brain of rats on the 4th day of ADCC

Groups of animals	**SOD, su/mg of protein/min**	**Catalase, mcab/mg of protein/min**	**GPx, mcmole/mg of protein/min**
Intact (False-operated) (n=10)	288,7 ± 17,2	7,87 ± 0,63	75,5 ± 5,2
Ischemia (Control) (n =10)	93,7 ± 7,11	2,77 ± 0,18	31,5 ± 2,21
Ischemia + Thiotriazolin (50 mg/kg) (n = 7)	298,4 ± 18,8*	7,00 ± 0,11*#0	57,3 ± 3,44*
Ischemia + Mexidol (200 mg/kg) (n = 7)	177,8 ± 9,8*	4,75 ± 0,33*	53,5 ± 4,53*
Ischemia + Citicoline (100 mg/kg) (n = 5)	119,3 ± 10,1*	2,81 ± 2,12	37,6 ± 1,70*
Ischemia + C-3 (100 mg/kg) (n = 8)	322,3 ± 18,7*	6,85 ± 0,52*	69,7 ± 5,11*

* – p≤0.05 in relation to the control

Note: the number of animals surviving on the 4th day of the experiment is indicated in brackets.

Modeling of ADCC by ligation of the both carotid arteries has led to the persistent disorder of the nitroxydergic system in the brain of rats on the 4-th day of the experiment. Thus, we have found that in the brain of animals with ADCC (control) there has been observed an increase in concentration of nitrites by 306% against the background of growth in NOS activity by 224%. (Table 3). The consequence of such damage has been the activation of nitrosative stress – a component of oxidative stress. Increased level of nitrotyrosine indicated on the activation of nitrosative stress (Table 1).

Table 3

The influence of C-3, thiotriazolin, mexidol and citicoline on the content of stable metabolites of nitric oxide and NO synthase activity in the brain of rats on the 4th day of ADCC (M ± m)

Groups of animals	**NO_2^-, mcmol/g of proteine**	**NO-synthase, nmole/g of tissue/min**
Intact (False-operated) (n=10)	4,3 ± 0,32	2,23 ± 0,15
Ischemia (Control) (n =10)	17,5 ± 0,87	7,23 ± 0,57
Ischemia + Thiotriazolin (50 mg/kg) (n = 7)	8,31 ± 0,52*	5,12 ± 0,33*
Ischemia + Mexidol (200 mg/kg) (n = 7)	14,7 ± 1,22*	6,45± 0,57
Ischemia + Citicoline (100 mg/kg) (n = 5)	11,2 ± 1,00*	4,88 ± 0,21*
Ischemia + C-3 (100 mg/kg) (n = 8)	6,43 ± 0,43*	4,14 ± 0,28*

*– p<0,05 по отношению к контролю

The obtained data are consistent with the results of other researchers that have establish a sharp increase in the concentration of NO in brain of animals with both focal and global ischemia [1-3,7,8]. It is known that NO concentration is beginning to rise from the first minutes of ischemia, reaching a maximum on the 1-3th days [12].

Measurement of the activity of NOS showed a sharp increase in the activity of this enzyme both in the ischemic core and in the penumbra, but without regard to affiliation to a particular type of NOS [14]. However, on the more remote dates of observation after ADCC iNOS significantly prevails [15].

It is known that NO forms active derivatives in the target cells, such as nitrosonium (NO +), nitroxyl (NO-) and peroxynitrite (ONOO-) [13]. Studies of

recent years have determined that NO, and especially its transformation products such as peroxynitrite (ONOO-), ion nitrosonia (NO^+), nitroxyl (NO-) and diazo trioxide (N_2O_3) are the main factors in the realization of nitrosative stress, which has resulted to direct interaction of NO with metals (heme iron of hemoglobin, myoglobin, iron-containing enzymes, as well as nonheme iron of iron-sulfur proteins and DNA, copper and zinc of active centers of enzymes), as well as indirect interaction of NO^+ (S-,N-,O-nitrosation) with thiol, phenol, hydroxyl and amino groups of proteins and DNA [10].

This interaction leads to desensitization of receptors, inhibition of mitochondrial enzyme activity and fragmentation of nucleic acids []. In the brain of animals treated with C-3, there has been observed the reduction of nitric oxide synthase hyperenzymemia by 42% and the reduction of nitrite by 63%. By the influence on these indices of nitric oxide production C-3 significantly exceeded the activity of mexidol and citicoline. In our opinion, the mechanism of antioxidant and neuroprotective action of C-3 is connected with the peculiarities of its chemical structure that lets make the assumption that analyzed compound plays the role of "spin trap" when interacting with the NO-radical. The mechanism of interaction of C-3 and NO can be implemented through electron transfer from the highest occupied molecular orbitals of the "spin trap" to the lowest vacant molecular orbital radical with the formation of the more stable radical complex [8].

Apparently, this mechanism of action of C-3 can lead to a decrease in the AFO-damaging effect on active centers of SOD and GPx, as well as promotes the preservation of energy producing mitochondrial function [16]. Thus, the antioxidant mechanism of C-3 is a determinative of its neuroprotective action under conditions of ADCC.

Conclusions

1. ADCC modeling leads to disruption in the NO system (increase in the activity of NOS and hyperproduction of NO), deprivation of antioxidant enzyme activity (SOD, catalase and GPx) in the brain of experimental animals, and as a result, to the activation of oxidative and nitrosative stresses (increase in the level of carbonylated proteins – APH, and KPH, and nitrotyrosine level).
2. The introduction of 8-benzilaminotheophyllinil-7-acetic acid (C-3) at a dose of 100 mg/kg endogastrically to rats with bilateral carotid artery occlusion for 4 days resulted in inhibition of oxidative and nitrosative stresses: decrease in the nitrotyrosine by 51.1%, APH – by 59%, KPH – by 63%, and nitrite anion by 44.1% ($p<0.05$),
3. The introduction of C-3 to rats with ADCC resulted in increased activity of the antioxidant enzymes SOD by 95.6%, catalase – by 78.4%, and GPx by 82.3% in the brain of animals on the 4^{th} day of the experiment.
4. Antioxidant and neuroprotective action of C-3 by the influence on the indices of the antioxidant system and indices of oxidative and nitrosative

stresses exceeded mexidol and citicoline, as well as was comparable to similar effect produced by thiotriazolin.

5. Mechanism of antioxidant and neuroprotective action of C-3 is connected with the peculiarities of its chemical structure that 8-benzilamino theophyllinil-7-acetic acid plays the role of "spin trap" when interacting with NO.

References

[1] I.S. Yevtushenko, Nootrops and neuroprotectors in the modern clinical neuropharmacology, *International Neurological Journal*, **3** (2013), no. 57, 14-20.

[2] M. Ber, *Neuroprotection: Models, Mechanisms, Therapy*, Laboratory of knowledge, Binom, 2014.

[3] J. Mehrholz J. Early, *Rehabilitation after Stroke,* transl. from English, G.E. Ivanova ed., M.: MEDPress-inform., 2014.

[4] I.S. Chekman., I.F. Belenichev, N.V. Bukhtijarova, N.A. Gorchakova, L.I. Kucherenko, *Antioxidants: Clinical and Pharmacological Aspects*, G/A/ Pogotova / Ukrainian Medical Periodical, 2014.

[5] M. V. Djachkov, A.S. Shkoda, O.V. Alexandrova, I.F. Belenichev, S.G Nosach, New derivatives of 3-benzyl xanthine as possible modulators of nitric oxide level, *Bulletin of New Medical Technologies*, **20** (2013), no. 2, 366-368.

[6] O.V. Alexandrova, D.N. Jurchenko, N.I. Romanenko, et al., Study of membrane protective properties and influence on energy metabolism of neurons of a new xanthine derivative C-3 under conditions of total ischemia of the brain, *Ukrainian Biopharmac*, **4** (2013), no. 27, 54-58.

[7] M. A. Belousova, E. A. Korsakova, E. A. Gorodetskaya, E. I. Kalenikova and O. S. Medvedev, New antioxidants as neuroprotective agents for the treatment of ischemic brain injury and neurodegenerative diseases, *Exper. and Clin. Pharmacol.*, **11** (2014), no. 77, 36 – 44.

[8] I.F. Belenichev, I.S. Chekman, N.V. Bukhtijarova, E.P. Nagornaja, N.A. Grchakova, S.V. Gorbacheva, *Preclinical Study of Specific Activity of Potential Medications for Primary and Secondary Neuroprotection*, Methodological guidelines of PE, State Expert Center MoH Ukraine, 2016.

[9] Roger L. Lundblad, Fiona Macdonald, *Handbook of Biochemistry and Molecular Biology*, Fourth Edition, CRC Press, 2010.
https://doi.org/10.1201/b10501

[10] I.F. Belenichev, N.V. Bukhtijarova, The current guidelines of neuroprotection in the therapy of acute stage of brain pathology of various genesis, *International Neurological J.*, **2** (2010), no. 32, 137-147.

[11] I.F. Belenichev, K.V. Alexandrova, O.S. Shkoda, D.M. Yurchenko, O.I. Belenicheva, S.V. Levich, Search neuroprotectors with antioxidant mechanism of action in a number derivatives of 3-methylxanthine, *Conference: XXVI International Symposium on Cerebral Blood Flow, Metabolism and Function & XI International Conference on Quantification of Brain Function with PET Shanghai, China,* (2013), 579-580.

[12] I.F.Belenichev, K. Alexandrova, A. Shkoda, S.V. Levich, Cerebroprotective activity of 3-benzylxanthine derivative – compound Ale-15, in conditions of bilateral common carotid arteries ligation (ischemic stroke), *International Journal of Basic & Clinical Pharmacology*, **2** (2013), no. 6, 705-711. https://doi.org/10.5455/2319-2003.ijbcp20131207

[13] Igor Belenichev, Katherine Aleksandrova, Alexander Shkoda, Sergey Levich, Darja Yurchenko, Nina Buchtiyarova, Research of antioxidant properties of theophyllinyl-7-acetic acid derivatives, *Oxid. Antioxid. Med. Sci.*, **3** (2014), no. 3, 187-194. https://doi.org/10.5455/oams.191214.or.078

[14] I.F. Belenichev, S.V. Levich, S.G. Nosach Influence of the xanthine derivative compound C-3 on morphofunctional indices of the neurons of the sensomotor zone of the cerebral cortex during intracerebral bleeding modeling, *Pharmacology and Medical Toxicology,* **2** (2015), no. 43, 37-43.

[15] Igor Belenichev, Victor Ryzhenko, Olexii Ryzhov, Sergii Levich, Study of Dependence of Xanthine Derivatives NO-Scavenger Properties from Energy Descriptors, *Biological Markers and Guided Therapy*, **5** (2018), no. 1. P. 37 – 46. https://doi.org/10.12988/bmgt.2018.857

[16] I.F. Belenichev, V.I. Cherny, N.V. Bukhtijarova, *Neuroprotection and Neuroplasticity,* Kyiv: Logos, 2015.

[17] I.F. Belenichev, N.V. Bukhtiayrova, S.G. Nosach, S.V. Levich, New xanthine derivative B-YR-2 as antioxidant modulator of post-stroke damage of sensomotor cortex neurons in rats, *Elixir Pharmacy*., **76** (2014), 28286-28292.

[18] I.F. Belenichev, S.G. Nosach, S.V. Levich, V.P. Ryzhenko, Antioxidant mechanism of neuroprotective action of the new 2-methylxanthine (compound C-3) under conditions of intracerebral bleeding, *Pharmacology and Medical Toxicology*, **1** (2016), no. 47, 3139.

[19] A.A. Ryzhov, V.P. Ryzhenko, I.F. Belenichev, *Application of Quantum-Chemical Calculation to Search For Inhibitors of Superoxide Radical Among 3-Arylxanthine Derivatives*, PhD Theses, Ukrainian scientific practical conference with intern. participation in memory of prof. V.V. Dunajev "Fundamental and clinical aspects of pharmacology" 24-25 November, 2016: Zaporizhzhia. P. C.65-66

[20] Igor Belenichev, Victor Ryzhenko, Olexii Ryzhov, Sergii Levich, Analysis of influence of Quantum Chemical Descriptors on NO-Scavenger Properties among Xantine Derivatives, *Biological Markers and Guide Therapy*, **4** (2017), no. 1, 39-48. https://doi.org/10.12988/bmgt.2017.735

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