Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2021, 11(1), 315-318, doi: 10.15421/2021_47

ORIGINAL ARTICLE

Investigation of the acute toxicity of new xanthine xenobiotics with noticeable antioxidant activity

D Vasylyev[1], B. Priimenko[1], K. Aleksandrova[1], Y. Mykhalchenko[1], B. Gutyj[2*], I. Mazur[2], N. Magrelo[2], H. Sus[2], O. Dashkovskyy[2], U. Vus[2], O. Kamratska[2]

[1]Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine
[2]Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine
**Corresponding author E-mail: bvh@ukr.net*
Received: 05.01.2021. Accepted 11.02.2021

Over the last few decades, there was significant interest in applying various xanthine derivatives to treat several diseases as highly potent antioxidant agents. Our study introduced several pharmacophore groups in the xanthine core to improve its antioxidant activity and increase biological fluids' solubility. We synthesized a set of new low-toxic 3-methylxanthine derivatives. Such compounds bear challenging scaffolds for finding effective antioxidant agents with broad application in medicine and pharmacy.
Keywords: reactive oxygen species; 3-methylxanthine derivatives; antioxidant activity.

Introduction

In recent years studies have shown that oxidative stress plays a vital role in the pathogenesis of most diseases (e.g., cardiovascular system, nervous system, liver, lungs, etc.), dramatically impacting mortality and morbidity. Such reactive oxygen and nitrogen species cause damage to cell membranes and even trigger cell death via mechanisms of free radical and peroxide oxidation (Leonarduzzi et al., 2010; Gutyj et al., 2017; Khariv et al., 2017; Mao et al., 2018; Martyshuk & Gutyj, 2019; Grymak et al., 2020; Martyshuk et al., 2020).

The accumulation of reactive oxygen species (RBC) in a cell has a detrimental effect on its components, such as nucleic acids, proteins, and lipids. Such conditions can lead to various diseases like atherosclerosis, diabetes, ischemic stroke, and several age-related conditions.

Thus, the modern pharmacy has a great need for up-to-date strategies and methods to develop antioxidant compounds, which can stop various pathological biochemical processes and, consequently, provide ample therapeutic effects.

Approximately 5% of the is converted into free radicals – superoxide anion radical and its derivatives – hydrogen peroxide, hydroxide radical, fatty acid peroxyl radicals, peroxynitrite, and nitric oxide, which generally referred to as reactive oxygen species (Reyes-Fermín et al., 2020; Pisoschi et al., 2021). Such processes usually occur in the eukaryotic cell's mitochondria because oxygen presence is essential for the electron transfer chain to complete.

However, in most cases cell can abide this problem due to presence of powerful antioxidant cellular defense systems, which includes endogenous antioxidants (bilirubin, glutathione and other thiols, lipoic acid, N-acetyl cysteine, NAD(P)H, ubiquinone, uric acid etc.), enzyme (Cu^{2+}/Zn^{2+}-dependent superoxide dismutase, catalase, thioredoxin, and glutathione peroxidase), exogenous dietary antioxidants (tocopherols, β-carotenes, ascorbate, selenium, flavones, flavonols and proanthocyanidins) and metal-binding proteins (albumin, ceruloplasmin, metallothionein, ferritin, myoglobin, and transferrin) (Ames et al., 1993; Bandyopudya et al., 1999; Krishnamurthy & Wadhwani, 2012; Scotti & Scotti, 2015; Son & Lee, 2019; Delesderrier et al., 2020; Dastmalchi et al., 2020; Harris & DeNicola, 2020; Warraich et al., 2020).

Notwithstanding a large number of experimental investigations, most clinical practice drugs did not possess the desired effects. Therefore, pharmacological agents for metabolic correction of conditions caused by oxidative stress should be intensively developed. Recently, scientists' attention is turned to synthetic drugs with antioxidant properties. Nevertheless, before synthesis, it is better to apply drug accelerating techniques to reduce costs and time by performing *in silico* calculations of bioavailability and toxicity of the novel drug-like candidates. The latter can make a good foundation for building combinatorial libraries.

In this view, the present work aims to develop new antioxidants of 3-methylxanthine derivatives using the following *in vitro* methods: inhibition of oxidative protein modification, initiation of lipid peroxidation, and inhibition of NO• radical. In our work, we apply machine-aided drug design to select some 7,8-disubstituted 3-methylxanthine derivatives with potent antioxidant properties.

Materials and methods

Preliminary computer-aided prediction of the 3-methylxanthine derivatives' biological activities was made by PASS software (Prediction of Activity Spectra for Substances). The most promising compounds were chosen from the set for a more detailed study.

Investigated compounds 1–16 (Fig. 1) were synthesized in our laboratory. Their synthesis, structure, and physicochemical properties were described in our previous works (Vasylyev et al., 2012; Mikhalchenko et al., 2017).

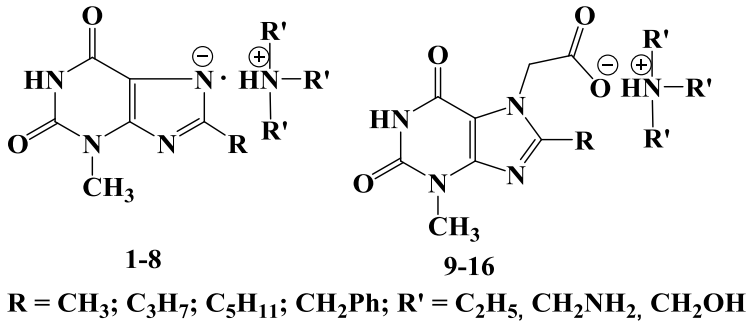

Fig. 1. Water-soluble derivatives of 3-methyl-8-R-xanthine

Table 1. Water-soluble derivatives of 3-methyl-8-R-xanthine

	R	R'	№ comp.	R	R'
1	CH_3	C_2H_5	9	CH_3	C_2H_5
2	C_3H_7	C_2H_5	10	C_3H_7	C_2H_5
3	C_5H_{11}	C_2H_5	11	C_5H_{11}	C_2H_5
4	$CH_2C_6H_5$	C_2H_5	12	$CH_2C_6H_5$	C_2H_5
5	CH_3	CH_2NH_2	13	CH_3	CH_2NH_2
6	C_3H_7	CH_2NH_2	14	C_3H_7	CH_2NH_2
7	C_5H_{11}	CH_2NH_2	15	C_5H_{11}	CH_2NH_2
8	$CH_2C_6H_5$	CH_2NH_2	16	$CH_2C_6H_5$	CH_2NH_2

Preparation of test solutions

Samples of 0.01 mol of the tested compound were dissolved with water in a 10 mL analytical flask (solution with concentration 10^{-3} mol/L). Then 0.1 mL of prepared solutions (10^{-3} mol/L) was placed into a subsequent 10 mL analytical flask and again dissolved with water (for the solution with 10^{-5} mol/L concentration), and the ultimate – 0.1 mL of prepared solutions (10^{-5} mol/L) was placed into subsequent 10 mL analytic flask and again dissolved with water (for 10^{-7} mol/L concentration).

Estimation of AOA at non-enzymatic initiation of free radical lipid peroxidation (LP)

Suspension of egg lipoproteins was used as a substrate (Warraich et al., 2020). It was prepared by homogenization of egg yolk on phosphate buffer (pH = 7.4). Test compounds (in concentrations 10^{-3}, 10^{-5}, or 10^{-7} mol/L) were added to the suspension. The reaction of free radical oxidation was started by the addition of 0.025 M solution of $FeSO_4 \cdot 7H_2O$ with the subsequent incubation of the prepared mixture at 37°C for 60 minutes. The reaction was stopped by adding of 50% solution of trichloroacetic acid with disodium EDTA. After centrifugation (30 minutes, 3,000 rpm), the supernatant was added to the solution of thiobarbituric acid (TBA). Then the mixture was heated in the water bath for 60 minutes. The addition of butane-1-ol extracted a colored complex of malonic dialdehyde with TBA. The concentration of the lipid peroxidation model (MDA), which showed the intensity of free radical oxidation processes, was estimated by the spectroscopic measurement of the absorbance of the sample at 532 nm. Dibunol was used as a reference drug (Son & Lee, 2019). The following equation calculated antioxidant activity (AOA):

$$\text{AOA} = \frac{\text{Ec}-\text{Et}}{\text{Ec}} \cdot 100\%,$$

where Et is the optical density of the test sample; Ec is the control sample's optical density.

The acute toxicity was measured by the method of Prozorovsky (Scotti & Scotti, 2015). Nonlinear rats were used in the experiments. Animals were kept under standard conditions. Researches on live vertebrates conformed to the European Convention's principles on the Protection of Vertebrate Animals, used for research and additional scientific purposes (Strasbourg, 1986). The animals had access to food and water *ad libitum* and were maintained at 24±2 °C with a 12 h light/dark cycle. Experimental groups of two animals per group (dose) were used. Experimental compounds were administered to the animals intraperitoneally (i.p.). The rats were observed for 24 h. Dead animals were examined by gross autopsy.

The statistical data analysis was calculated using Statistica® for Windows 6.0 license program (StatSoft Inc., No. AXXR712 D833214FAN5) (Ames et al., 2019). Represented data are in the form, containing the sample mean ± the standard error of the mean. The fidelity of distinction between experimental groups has been assessed with Student's *t*-test and Fisher's exact test (n=5). The difference on $p<0.05$ was statistically significant.

Results and discussion

In vitro investigation of 16 water-soluble salts of 3-methyl-8-R-xanthine showed that all substances revealed antioxidant activity. Consequently, these results made a good foundation for further investigation of the structure-activity relationship.

All synthesized compounds at concentrations of 10^{-6} mol/L showed a significant antioxidant effect that exceeds the reference drug – dibunol.

Table 2. AOA activity of investigated compounds

Compound	D	AOA,%	Compound	D	AOA,%
1	0.2446	28.55	11	0.2654	47.69
2	0.2126	30.40	12	0.1651	49.18
3	0.2314	29.79	13	0.2021	58.87
4	0.2008	34.14	14	0.1503	51.47
5	0.1992	37.03	15	0.1938	44.23
6	0.1566	58.12	16	0.2078	59.85
7	0.2278	52.48	Control	0.274	–
8	0.1924	45.54	Control1	0.072	–
9	0.2162	53.37	Dibunol	0.185	27.1
10	0.2020	45.64			

As appears from the above table, the elongation of the alkyl chain in the eighth position of xanthine moiety leads to a slight increase in synthesized compounds' antioxidant properties. The introduction of an aromatic radical in xanthine derivatives' C8 position leads to significant antioxidant activity growth.

Since these studies show that the obtained derivatives of xanthine (1-16) show pronounced antioxidant activity and are promising for further research, it might be appropriate also to study their acute toxicity (Table 3).

Table 3. Acute toxicity of investigated compounds

Compound	LD_{50}, mg/kg	Compound	LD_{50}, mg/kg
1	678 (661-697)	9	1180 (1168-1196)
2	588 (569-599)	10	1350 (1327-1372)
3	571 (554-588)	11	980 (962-997)
4	953 (933-978)	12	1400 (1486-1420)
5	792 (775-810)	13	1203 (1187-1218)
6	742 (727-758)	14	1740 (1722-1757)
7	592 (578-608)	15	1575 (1561-1589)
8	877 (861-887)	16	1870 (1856-1891)

According to acute toxicity, the LD_{50} of this class lies in the range from 571 to 1870 mg/kg. Moreover, according to K. K. Sidorov's classification, we established that all of them belong to IV and V toxicity class – low-toxic compounds (Bandyopudya et al., 1999). During the analysis of obtained data, some patterns were revealed between the structure and toxicological characteristics of the 3-methylxanthine derivatives.

Elongation of the alkyl chain in the eighth position of the 3-methylxanthine core leads to a slight increase in toxicity. The highest toxicity amongst C7-nonsubstitued xanthine derivatives showed compound 3 (571 mg/kg), a pentyl residue at C8.

The introduction of substituents in the C7 position of the xanthine moiety leads to a small reduction in acute toxicity. However, compound 11 with C8-pentyl radical is characterized by the highest toxicity in its class (980 mg/kg). The most low-toxic 7-substitued 3-methylxanthine derivative was compound 16 containing an aromatic residue in the eighth position (1870 mg/kg). Consequently, xanthine core substituted also at the N7 had less pronounced acute toxicity. The synthesis above of new N7- and C8-substituted 3-methylxanthines can create new low-toxic compounds with various biological effects.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ames, B.N., Shigenaga, M.K., & Hagen, T.M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA., 90, 7915-7922.

Bandyopudya, U., Das, D., & Banerjee, R.K. (1999). ROS: oxidative damage and pathogenesis. Curr. Sci., 77, 658-666.

Dastmalchi, N., Baradaran, B., Latifi-Navid, S., Safaralizadeh, R., Khojasteh, S.M.B., Amini, M., Roshani, E., & Lotfinejad, P. (2020). Antioxidants with two faces toward cancer. Life Sciences, 258, 118186. doi: 10.1016/j.lfs.2020.118186.

Delesderrier, E., Curioni, C., Omena, J., Macedo, C.R., Cople-Rodrigues, C., & Citelli, M. (2020). Antioxidant nutrients and hemolysis in sickle cell disease. Clinica Chimica Acta, 510, 381-390. doi: 10.1016/j.cca.2020.07.020.

Gerard-Monnier, D, Erdelmeir, I, Chaudiere, J, & Yadan, J. (1998). Pat. 5726063 USA, G01N 33/52. Method of colorimetric analysis of malonic dialdehydes and 4-hydroxy-2-enaldehydes as indexes of lipid peroxidation, kits for use in said mathod and their preparation. № 08/702197; Pub. date.10.03.98.

Grymak, Y., Skoromna, O., Stadnytska, O., Sobolev, O., Gutyj, B., Shalovylo, S., Hachak, Y., Grabovska, O., Bushueva, I., Denys, G., Hudyma, V., Pakholkiv, N., Jarochovich, I., Nahirniak, T., Pavliv, O., Farionik, T., Bratyuk, V. (2020). Influence of "Thireomagnile" and "Thyrioton" preparations on the antioxidant status of pregnant cows. Ukrainian Journal of Ecology, 10(1), 122-126. doi: 10.15421/2020_19

Gutyj, B., Stybel, V., Darmohray, L., Lavryshyn, Y., Turko, I., Hachak, Y., Shcherbatyy, A., Bushueva, I., Parchenko, V., Kaplaushenko, A., Krushelnytska, O. (2017). Prooxidant-antioxidant balance in the organism of bulls (young cattle) after using cadmium load. Ukrainian Journal of Ecology, 7(4), 589–596

Harris, I.S., & DeNicola, G.M. (2020). The Complex Interplay between Antioxidants and ROS in Cancer. Trends in Cell Biology, 30(6), 440-451. doi: 10.1016/j.tcb.2020.03.002.

Khariv, M., Gutyj, B., Ohorodnyk, N., Vishchur, O., Khariv, I., Solovodzinska, I., Mudrak, D., Grymak, C., & Bodnar, P. (2017). Activity of the T- and B-system of the cell immunity of animals under conditions of oxidation stress and effects of the liposomal drug. Ukrainian Journal of Ecology, 7(4), 536–541.

Krishnamurthy, P., & Wadhwani, A (2012). Antioxidant enzymes and human health. Antioxidant Enzyme. InTech.

Leonarduzzi, G., Sottero, B., & Poli, G. (2010). Targeting tissue oxidative damage by means of cell signaling modulators: The antioxidant concept revisited. Pharmacology & Therapeutics, 128(2), 336-374. doi: 10.1016/j.pharmthera.2010.08.003.

Mao, X.Y., Jin, M.Z., Chen, J.F., Zhou, H.H., & Jin, W.L. (2018). Live or let die: Neuroprotective and anti-cancer effects of nutraceutical antioxidants. Pharmacology & Therapeutics, 183, 137-151. doi: 10.1016/j.pharmthera.2017.10.012.

Martyshuk, T.V., & Gutyj, B.V. (2019). Influence of feed additive "Butaselmevit-Plus" on antioxidant status of rats in conditions of oxidative stress. Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Agricultural sciences, 21(90), 76–81. doi: 10.32718/nvlvet-a9013

Martyshuk, T.V., Gutyj, B.V., Zhelavskyi, M.M., Midyk. S.V., Fedorchenko, A.M., Todoriuk, V.B., Nahirniak, T.B., Kisera, Ya.V., Sus, H.V., Chemerys, V.A. , Levkivska, N.D., Iglitskej, I.I. (2020). Effect of Butaselmevit-Plus on the immune system of piglets during and after weaning. Ukrainian Journal of Ecology, 10(2), 347-352. doi: 10.15421/2020_106

Mikhalchenko, E.K., Aleksandrova, K.V., Levich, S.V., & Sinchenko, D.M. (2017). Synthesis and physical-chemical properties of 3-benzyl-8-propylxanthinyl-7-acetic acid and its derivatives. Actual questions of pharmaceutical and medical science and practice, 1(23), 14-19.

Pisoschi, A.M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A.I. (2021). Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry, 209, 112891. doi: 10.1016/j.ejmech.2020.112891.

Prozorovsky, V.B., & Prozorovskaya, V.M. (1978) Express-method for determining mean effective dose and its error. Farmakol i toksikol, 4, 497–502 (in Russian).

Ramis-Ramos, G. (2003). Synthetic antioxidants. In: Caballero B (ed.). Encyclopaedia of food sciences and nutrition. 2nd edition, Springer, Dordrecht, Netherlands, 265–275.

Reyes-Fermín, L.M., Aparicio-Trejo, O.E., Avila-Rojas, S.H., Gómez-Sierra, T., Martínez-Klimova, E., & Pedraza-Chaverri, J. (2020). Natural antioxidants' effects on endoplasmic reticulum stress-related diseases. Food and Chemical Toxicology, 138, 111229. doi: 10.1016/j.fct.2020.111229.

Scotti, L., & Scotti, M.T. (2015). Computer aided drug design studies in the discovery of secondary metabolites targeted against age-related neurodegenerative diseases. Curr. Top. Med. Chem., 15(21), 2239-2252. doi: 10.2174/1568026615666150610143510.

Sidorov, K.K (1973). On the classification of toxicity of poisons in parenteral methods of administration. Toxicology of new industrial chemicals. Moscow, 13, 47-57.

Son, J.M., & Lee, C. (2019). Mitochondria: multifaceted regulators of aging. BMB Rep., 52(1), 13-23. doi: 10.5483/BMBRep.2019.52.1.300.

Vasylyev, D.A., Kazunin, M.S., Priimenko, A.O., Achkasova, E.N., & Priimenko, B.O. (2012). Synthesis and antimicrobial estimation of some new substituted purine derivatives. Actual questions of experimental, clinical medicine and pharmacy, 1, 53.

Warraich, U.E.A., Hussain, F., & Kayani, H.U.R. (2020). Aging - Oxidative stress, antioxidants and computational modeling. Heliyon, 6(5), e04107. doi: 10.1016/j.heliyon.2020.e04107.

Zaycev, V.M., Liflyandskiy, V.G., & Marinkin, V.I. (2006). Applied medical statistics: textbook. Foliant, Saint Petersburg.

Citation:

Vasylyev, D., Priimenko, B., Aleksandrova, K., Mykhalchenko, Y., Gutyj, B., Mazur, I., Magrelo, N., Sus, H., Dashkovskyy, O., Vus, U., Kamratska, O. (2021). Investigation of the acute toxicity of new xanthine xenobiotics with noticeable antioxidant activity.
Ukrainian Journal of Ecology, 11 (1), 315-318.