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Study of Dependence of Xanthine Derivatives NO-Scavenger Properties from Energy Descriptors

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Abstract

Objective: Apart from ROS, reactive nitrogen species also play a complex role in different pathological processes. The interaction of NO with ROS causes the production of several reactive nitrogen species that potentiate cellular damage. Nowadays is actual the search of potential antioxidants, that are capable to interrupt the pathological biochemical processes at various steps of oxidative and nitrosative stress. In this article are represented results of our research of 3-benzyl(4-methylphenyl)xanthine derivatives as potential NO-scavengers.

Materials and methods: For our research we made quantum mechanical calculations of next energy descriptors of molecular orbitals:

E(LUMO), E(HOMO) and reactivity index. Investigation of antioxidant properties of xanthine derivatives was carried out using *in vitro* method by inhibition of NO•-radical.

Results: In vitro study of xanthine derivatives have been shown that almost all compounds exhibit antioxidant properties that are in direct dependence from reactivity index of compound.

Conclusions: Obtained results could be used for further search of NO-scavengers among xanthine derivatives and use their reactivity index as marker of antioxidant properties.

Keywords: Xanthine derivatives, NO-scavengers, energy descriptors

Introduction

Reactive oxygen species (ROS, also called oxygen free radicals) are a side-product of sites on mitochondrial complexes I and III of the electron transmitter chain [8, 9]. They also play a role of mediators of important intracellular signaling pathways [1]. Increased production of ROS leads to development of oxidative and nitrosative stresses In excess, ROS contribute to membrane damage by lipid peroxide formation and are part of the signaling sequence leading to apoptosis. Excess ROS are also derived from many complex sources besides damaged mitochondria, such as from uncoupled nitric oxide synthase in heart and endothelial cells, from xanthine oxidase and stimulation of membrane NADPH oxidase [6, 14, 16].

Apart from ROS, reactive nitrogen species also play a complex role in different pathological processes. Nitric oxide (NO) (produced from sources such as nitric oxide synthase) released due to stimuli such as shear stress, has become an important subject of research [12, 17, 19]. The interaction of NO with ROS causes the production of several RNS that potentiate cellular damage [15, 19].

Thus, search of compounds that could be used as NO-scavengers is actual task of modern pharmacology and biochemistry.

Xanthine derivatives are natural heterocyclic compound that had wide spectrum of biological properties with high antioxidant potential [3-5, 10]. The aim of our research was study of NO-scavenger properties of 3-benzyl-(4-methylphenyl)xanthinyl-7-acetic acids derivatives from several energy descriptors

Materials and Methods

Quantum mechanical calculations

For our research we made quantum mechanical calculations of next energy descriptors of molecular orbitals [11]:

– energy of the lowest unoccupied molecular orbital – E(LUMO);

– energy of the highest occupied molecular orbital – E(HOMO);
– reactivity index, that was calculated by the formula 1:

$$\omega = \chi^2/2\eta \quad (1)$$

Calculations were provided at program complex WinMopac (ver 7.2). The optimization of the structure was achieved using the semiempirical method AM1 (descriptors – HOMOEnergy, LUMOEnergy) with such parameters: Calculation = SinglePoint, WaveFunction = ClosedShell (RHF).

Estimation of antioxidant activity (AOA) by inhibition of NO•-radical

The method is based on photoinduction of Sodium nitroprusside, which is accompanied by the accumulation of NO•-radical [18]. The strength of AOA was determined by the rate of ascorbic acid oxidation via the spectroscopic measurement of the absorbance of the sample at 265 nm. As a reference standard we used N-acetylcysteine (NAC) [7].

At first were prepared water solutions of ascorbic acid and Sodium nitroprusside. Then, to the 0.01 ml of solution of Sodium nitroprusside (0.08 %) 0.01 ml of solution of ascorbic acid (0.6 %), 0.1 ml solution of examined compounds (in concentrations 10^{-3} mol/l, 10^{-5} mol/l or 10^{-7} mol/l) and 3 ml of distilled water were added. After stirring reaction were started by immersion of the light source (300 W with λ = 425 nm) for 30 min. AOA were estimated by conservation of ascorbic acid concentrations.

AOA was calculated by formula 2:

$$AOA = \frac{\mathbf{Et - Ec}}{\mathbf{Ec}} \times \mathbf{100\%} \quad (2)$$

where Et – optical density of test sample; Ec – optical density of control sample.

Statistical analysis.

The statistical data analysis was carried out with the help of the software STATISTICA® for Windows 6.0 [20]. The data is presented as the sample mean ± the standard error of the mean. The fidelity of differences between experimental groups was estimated with the help of Student's t-test and Fisher's exact test.

Results

Quantum mechanical calculations

Provided calculations showed, that reactivity index of studied compounds was within -1.9605–-2.9398 and the highest index has ammonium 3-benzyl-8-methylxanthinyl-7-acetate **5**. This compound also had the highest LUMO energy.

It should be noted that calculated parameters of initial acids and their amides were almost equal (Table 2). Usage of ammonium and N,N-diethylammonium as bases for salts obtaining led to increasing of both LUMO and HOMO energy with reactivity index.

Estimation of AOA by inhibition of NO•-radical

All studied compounds (**1-11)** showed high antioxidant properties and their values and in the most cases exceed the standard - NAC (Table 1).

AOA of studied compounds was within 49.43 %-82.16% (at concentration 10^{-3} mol/l). The most active among all studied xanthine derivatives was ammonium 3-benzyl-8-methylxanthinyl-7-acetate **5**, that exceeded index of AOA of NAC at 79.60%. At concentration 10^{-5} mol/l activity of almost all compounds decreased (except compound **11**), but most of compounds exceeded the effect of standart. Decreasing of concentration to 10^{-7} mol/l led to decreasing of antioxidant properties but still all compounds showed activity, that was higher then effect of N-acetylcysteine.

Table 1. Antioxidant activity of test compounds (n = 5) by inhibition of NO•-radical (M±m).

Compound	10^{-3} mol/l		10^{-5} mol/l		10^{-7} mol/l	
	E, M±m	%	E, M±m	%	E, M±m	%
1	1,514 ± 0,066[2]	56,57	1,115 ± 0,040[2]	15,30	1,532 ± 0,057[2]	58,43
2	1,564 ± 0,054[2]	61,73	1,233 ± 0,084[1]	27,50	1,585 ± 0,081[2]	63,91
3	1,525 ± 0,055[2]	57,70	1,323 ± 0,059[2]	36,81	1,357 ± 0,079[2]	40,33
4	1,54 ± 0,074[2]	59,25	1,133 ± 0,089	17,16	1,464 ± 0,08[2]	51,39
Control			0,967 ± 0,054			
5	1,603 ± 0,089[1]	82,16	1,408 ± 0,079[1]	60,00	1,385 ± 0,051[1]	57,38
6	1,562 ± 0,082[1]	77,50	1,506 ± 0,091[1]	71,13	1,356 ± 0,107[1]	54,09
7	1,439 ± 0,077[1]	63,52	1,376 ± 0,076[1]	56,36	1,371 ± 0,074[1]	55,79
8	1,532 ± 0,065[1]	74,09	1,383 ± 0,067[1]	57,16	1,396 ± 0,083[1]	58,63
Control			0,880 ± 0,024			
9	1,532 ± 0,055[2]	58,43	1,072 ± 0,084	10,86	1,565 ± 0,088[2]	61,84
10	1,445 ± 0,123[2]	49,43	0,987 ± 0,094	2,07	1,544 ± 0,093[2]	59,67
Control			0,967 ± 0,054			
11	1,417 ± 0,056[1]	61,02	1,515 ± 0,074[1]	72,16	1,369 ± 0,045[1]	55,57
Control			0,880 ± 0,024			
NAC	0,901 ± 0,092	2,46	1,042 ± 0,087	18,47	0,981 ± 0,074	11,53
Control			0,880 ± 0,024			

Remark: [1] – $p<0.05$ relative to control; [2] – $p<0.01$ relative to control.

Table 2. Quantum mechanical calculations of xanthinyl-7-acetic acids derivatives

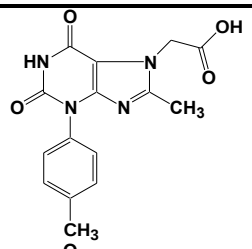
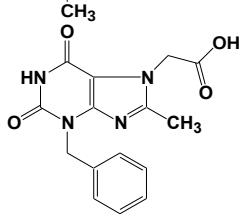
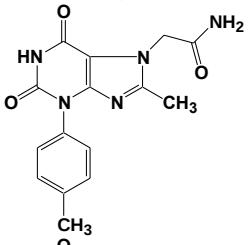
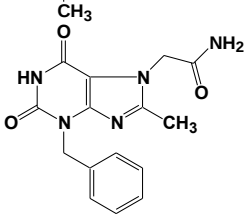
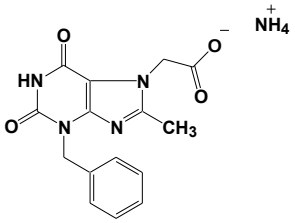
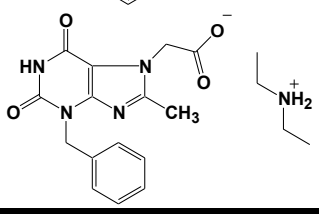
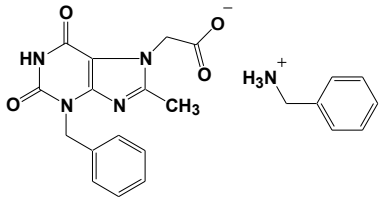
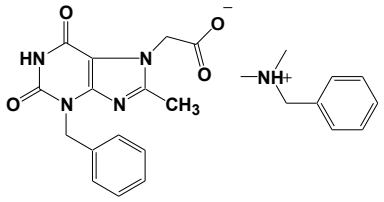
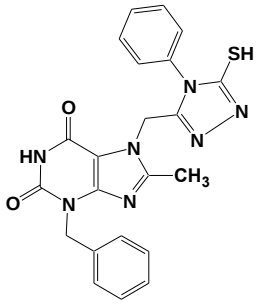
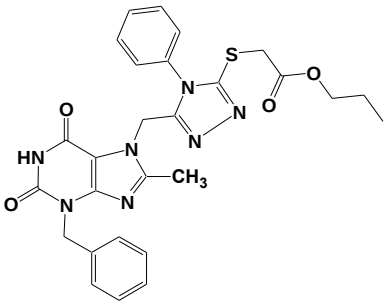
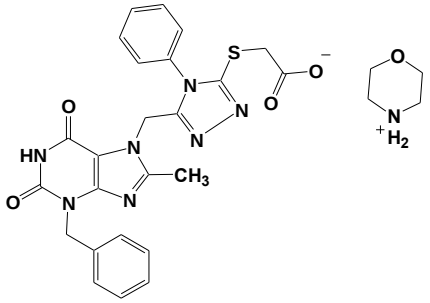
Compound	Structure	E (LUMO), eV	E (HOMO), eV	ω, eV
1		-0,662058	-8,85597	-2,7640
2		-0,621876	-9,09988	-2,7869
3		-0,664048	-8,88114	-2,7719
4		-0,617553	-9,10999	-2,7855
5		0,175988	-8,35553	-1,9605
6		-0,166582	-8,11534	-2,1572

Table 2. (Continued): Quantum mechanical calculations of xanthinyl-7-acetic acids derivatives

7		-0,825065	-8,38973	-2,8062
8		-0,683878	-8,14727	-2,6123
9		-0,551883	-8,55349	-2,5903
10		-0,848165	-8,85555	-2,9398
11		-0,738576	-8,81243	-2,8246

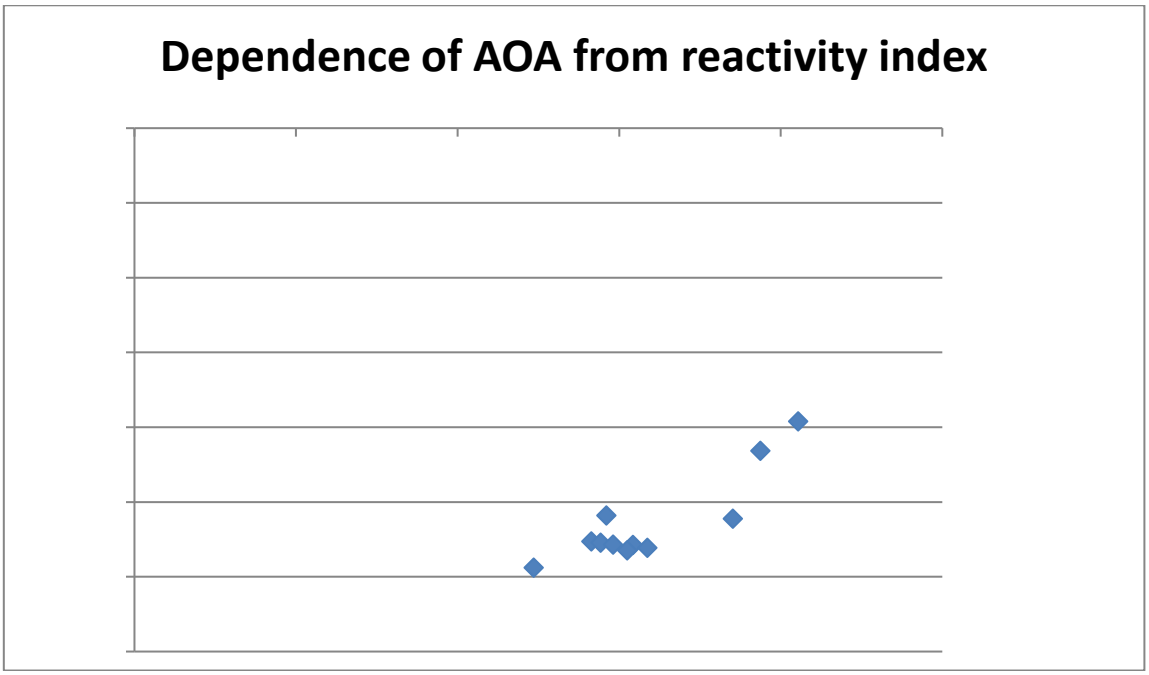


Figure 1. Dependence of AOA from reactivity index

Discussion

In vitro study of 11 derivatives of xanthinyl-7-acetic acids have been shown that almost all compounds exhibit antioxidant properties in vitro on the model of nitroprusside photoinduced oxidation, due to their NO scavenger properties. Obtained results also help us to establish some patterns of structure-activity relationship and some dependence from energy descriptors.

Thus, obtained results showed, that AOA is in direct dependence with reactivity index (Figure 1). The most pronounced properties has ammonium 3-benzyl-8-methylxanthinyl-7-acetate **5** and this compound also has the highest reactivity index.

Substitution of ammonium on N,N-diethylammonium (compound **6**) as base led to the decreasing of reactivity index at 10.03 %. At the same time AOA decreased at 5.6 %. Benzylammonium salts had less pronounced NO-inhibition properties, then salts without aromatic fragment at cation (compound **7** and **8**).

Basic structures – 8-methylxanthinyl-7-acetic acids **1** and **2,** showed medium level of action. Addition of amide groups to their structures decreased antioxidant properties of initial compounds. Thus, 3-(4-methylphenyl)-8-methylxanthinyl-7-acetamide **3** showed less pronounced effect (on 1.99%) in comparison with initial acid **1**.

Combination of triazole ring with xanthine heterocycle doesn't have pronounced effect on antioxidant properties and reactive index. AOA of triazolyl xanthines **9-11** was within 49.43-61.02% and reactivity index was -2.5903– -2.9398.

Obtained results could be used for further search of NO-scavengers among xanthine derivatives and use their reactivity index as marker of antioxidant properties.

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