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Analysis of Influence of Quantum Chemical Descriptors on NO-Scavenger Properties among Xanthine Derivatives

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Abstract

Objective: Accumulation of reactive oxygen species and NO derivatives in the cell exerts damaging effects on its constituents, such as carbohydrates, proteins, lipids, and nucleic acids.. The search for antioxidant compounds capable of interrupting the pathological biochemical processes at various steps of oxidative and nitrosative stress development, and thus exerting prophylactic and therapeutic effects, is a priority for medical and pharmaceutical sciences. In this article we investigated the dependence of NO-scavenger properties of 3-benzyl(4-methylphenyl)xanthine derivatives from energy descriptors.

Materials and methods: For our research we made quantum mechanical calculations of next energy descriptors of molecular orbitals: E(LUMO), E(HOMO), HOMO–LUMO gap, absolute hardness of molecule, absolute electron

negativity, reactivity index. Investigation of antioxidant properties of xanthine derivatives was carried out using *in vitro* method by inhibition of $NO^{\bullet}$-radical.

Results: In vitro study of xanthine derivatives have been shown that almost all compounds exhibit antioxidant properties. Obtained results also help us to establish some patterns of structure-activity relationship and dependence from LUMO energy..

Conclusions: Obtained results could be used for further search of NO-scavengers among xanthine derivatives.

Keywords: Xanthine derivatives, NO-scavengers, energy descriptors

Introduction

Reactive oxygen species (ROS) are continuously made in a living cell as products of its normal metabolism [8]. 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 [6, 12, 14].

In biological systems, NO is generated by catalytic action of nitric oxide synthase and the role of NO has become an important subject of research [11, 15, 17]. However, in pathological situations NO injure cells and tissues [13].

The consequences of interactions of ROS and NO derivatives with their targets manifest in formation of oxidative and nitrosative stress products, undesirable for normal cell metabolism. Advanced oxidized plasma proteins (especially albumin) are produced during nitrosative stress, as well as advanced glycation ends products, the results of carbohydrate oxidation [17].

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

Quantitative structure-activity relationship (QSAR) represents the depending between the structure and biological (pharmaceutical or toxicological) activity of chemical substances [10].QSAR-methods are very important for prediction of the pharmacological potency of structurally-related compounds [10].

Xanthine derivatives are valuable class of natural organic compounds with wide spectrum of biological properties and pronounced antioxidant activity [3-5, 9]. In the previous work we described the ability of xanthine derivatives to NO-inhibition [2]. In continuation of previous research aim of this work was study dependence of NO-scavenger properties of 3-benzyl(4-methylphenyl)xanthine derivatives from energy descriptors.

Materials and Methods

Quantum mechanical calculations

For our research we made quantum mechanical calculations of next energy descriptors of molecular orbitals [10]:

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

– energy of the highest occupied molecular orbital – E(HOMO);
– HOMO–LUMO gap – the energy difference between the HOMO and LUMO;
– absolute hardness of molecule, that was calculated by the formula 1:

$$\eta = \frac{E(LUMO) - E(HOMO)}{2} \quad (1)$$

– absolute electron negativity, that was calculated by the formula 2:

$$\chi = -\frac{E(LUMO) + E(HOMO)}{2} \quad (2)$$

– reactivity index, that was calculated by the formula 3:

$$\omega = \chi^2/2\eta \quad (3)$$

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 [16]. 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 4:

$$AOA = \frac{\mathbf{Et - Ec}}{\mathbf{Ec}} \times \mathbf{100\%} \quad (4)$$

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 [18]. 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 lowest HOMO energy had 3-benzylxanthinyl-8-propionic acid (compound **2**) (Table 2). It should be noted, that usage of ammonium, monoethanoleamine, morpholine and piperazine as bases increased of HOMO energy in comparison with initial acids. In the same time salts of acids had higher LOMO energy and higher energy gap. Addition of ester groups didn't have much effect on these energy descriptors.

Reactivity index of studied compounds was within -2.59686– -2,72951. An it should be noted that initial acids had almost equal ω parameter and their further chemical modification decreased reactivity index of initial compounds.

Estimation of AOA by inhibition of NO•-radical

Compound **1-12** showed relatively high antioxidant properties and their values in some cases exceed the standard - NAC (Table 1).

Thus, among all compounds, AOA was within 49.43%-98.45% (at concentration 10^{-3} mol/l). The most active compound in this group was 3-(4-methylphenyl)xanthinyl-8-propionic acid **1**, which exceeded index of AOA of NAC at 95.99%. At concentration 10^{-5} mol/l activity of almost all compounds decreased (except compound **4**), but most of compounds exceeded the effect of standart. At concentration 10^{-7} mol/l, all of substances still 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	1,919 ± 0,027[2]	98,45	1,747 ± 0,059[2]	80,66	1,815 ± 0,053[2]	87,69
2	1,592 ± 0,052[2]	64,63	1,251 ± 0,056[2]	29,37	1,565 ± 0,071[2]	61,84
Control			0,967 ± 0,054			
3	1,559 ± 0,045[1]	77,16	1,326 ± 0,055[1]	50,68	1,351 ± 0,067[1]	53,52
4	1,315 ± 0,105[1]	49,43	1,523 ± 0,093[1]	73,07	1,363 ± 0,098[1]	54,89
5	1,485 ± 0,147[1]	68,75	1,369 ± 0,120[1]	55,57	1,394 ± 0,118[1]	58,41
6	1,631 ± 0,073[1]	85,34	1,434 ± 0,067[1]	62,95	1,377 ± 0,041[1]	56,48
7	1,500 ± 0,061[1]	70,45	1,497 ± 0,051[1]	70,11	1,349 ± 0,049[1]	53,29
8	1,431 ± 0,065[1]	62,61	1,343 ± 0,071[1]	52,61	1,388 ± 0,061[1]	57,73
Control			0,880 ± 0,024			
9	1,637 ± 0,075[2]	69,29	1,137 ± 0,084	17,58	1,45 ± 0,066[2]	49,95
10	1,593 ± 0,064[2]	64,73	1,029 ± 0,037	6,41	1,446 ± 0,068[2]	49,53
11	1,629 ± 0,081[2]	68,46	1,381 ± 0,084[2]	42,81	1,697 ± 0,092[2]	75,49
12	1,552 ± 0,065[2]	60,49	1,081 ± 0,064	11,79	1,344 ± 0,116[1]	38,98
Control			0,967 ± 0,054			
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-8-propionic acid derivatives

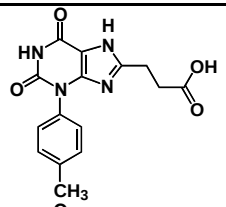
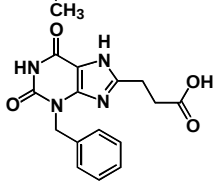
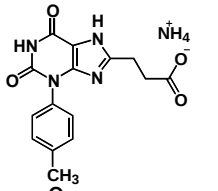
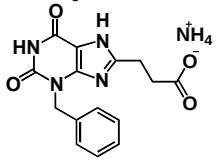
№	Structure	E (HOMO), eV	E (LUMO), eV	Energy gap, eV	η, eV	χ_0, eV	ω, eV
1		-8.90037	-0.598091	-8,302279	-4.1511395	4,7492305	-2,71675
2		-9.16228	-0.540142	-9,702422	-4.311069	4,851211	-2,72951
3		-8.84915	-0.529164	-9,378314	-4.159993	4,689157	-2,64282
4		-9.04179	-0.421121	-9,462911	-4.3103345	4,7314555	-2,59686

Table 2. (Continued): Quantum mechanical calculations of xanthinyl-8-propionic acid derivatives

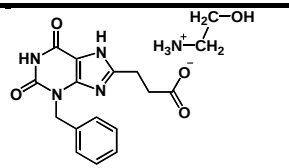
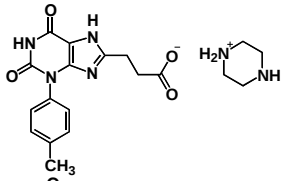
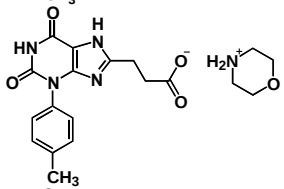
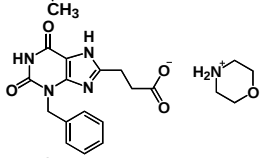
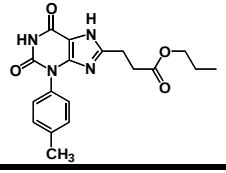
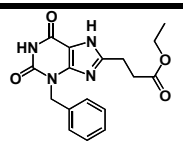
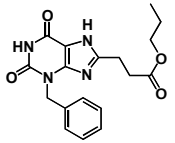
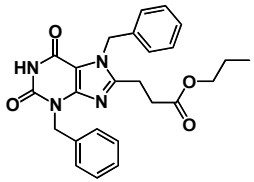
5		-9.09904	-0.466491	-9,565531	-4.3162745	4,782766	-2,64984
6		-8.84159	-0.519298	-9,360888	-4.161146	4,680444	-2,63227
7		-8.86439	-0.547332	-9,411722	-4.158529	4,705861	-2,66262
8		-9.11205	-0.4881	-9,60015	-4.311975	4,800075	-2,67171
9		-8.86955	-0.549139	-9,418689	-4.1602055	4,7093445	-2,66548

Table 2. (Continued): Quantum mechanical calculations of xanthinyl-8-propionic acid derivatives

10		-9.12129	-0.497693	-9,618983	-4.3117985	4,8094915	-2,68232
11		-9.12161	-0.497966	-9,619576	-4.311822	4,809788	-2,68263
12		-8.99969	-0.44096	-9,44065	-4.279365	4,720325	-2,60336

Discussion

In vitro study of 12 derivatives of xanthinyl-8-propionic acids have been shown that almost all compounds exhibit antioxidant properties. Obtained results also help us to establish some patterns of structure-activity relationship and some dependence from energy descriptors.

Basic structures – xanthinyl-8-propionic acids **1** and **2,** showed antioxidant action in vitro on the model of nitroprusside photoinduced oxidation, due to their NO scavenger properties. Addition of ester groups to their structures mostly decreased antioxidant properties of initial compounds. Thus, propyl 3-(4-methylphenyl)xanthinyl-8-propionate **9** showed less pronounced effect in comparison with initial acid **1**. The same effect had addition of ethoxy group to the structure of acid **2**. Insertion of benzyl residue to the position 7 of xanthine molecule led to the decreasing of antioxidant properties.

Water-soluble salts of initial acids **3-8** in most cases had less pronounced antioxidant properties than acids **1** and **2**. Thus, usage of ammonium as cation decreased activity of acids on 29.23 % and 15.2% respectively. Usage of secondary amines as bases also had negative effect: piperazine (compound 6) and morpholine (compounds 7 and 8) salts decreased antioxidant properties on 13.11%, 28% and 2.02% respectively.

During comparison of calculated energy descriptors with data of antioxidant properties of studied compounds we found that antioxidant properties mostly depended from LUMO energy. Thus, 3-(4-methylphenyl)xanthinylpropionic acid **1**, that showed the most pronounced antioxidant properties, had the lowest LUMO energy between studied compounds. In the same time the highest LUMO energy had ammonium **3-**(4-methylphenyl)xanthinylpropionate **4**, that showed the lowest antioxidant activity among studied compounds.

Obtained results could be used for further search of NO-scavengers among xanthine derivatives.

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