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Antiradical activity of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide-thermodynamic DFT study†

Marko Antonijević [1]*, Jelena Đorović Jovanović [1], Žiko Milanović [2], Dejan Milenković [1], Edina Avdović [1], Dušica Simijonović[1], Zorica Petrović[2] and Zoran Marković [1]

[1] University of Kragujevac, Institute for Information Technologies, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia; e-mail: mantonijevic@uni.kg.ac.rs, jelena.djorovic@uni.kg.ac.rs, deki82@kg.ac.rs, edina.avdovic@pmf.kg.ac.rs, dusica.simijonovic@pmf.kg.ac.rs, zmarkovic@uni.kg.ac.rs

[2] University of Kragujevac, Faculty of Science, Department of Chemistry, Radoja Domanovića 12, 34000 Kragujevac, Serbia; e-mail: ziko.milanovic@pmf.kg.ac.rs, zorica@pmf.kg.ac.rs

* Correspondence: mantonijevic@uni.kg.ac.rs; Tel.: +381 34 61 00 195

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Abstract: In the plant world, coumarins are highly represented heterocyclic compounds. This large class of compounds have found application in the treatment of numerous diseases due to one of their most important properties, such as the strong antioxidative activity. In this paper, the antioxidative properties of newly synthesized compound (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide against the six selected reactive oxygen species (ROS) were investigated. To estimate antioxidative activity, DFT calculations were performed at M06-2X/6-311++G(d,p) level of theory. All calculations were accomplished in the gas phase, and the *Gaussian09* software package was utilized. Analysis of the obtained thermodynamic parameters indicates SET-PT (single electron transfer - proton transfer) as a non-operative mechanism of free radical scavenging. On the other hand, the operative mechanistic pathways for free radical scavenging of all investigated radical species are HAT (hydrogen atom transfer) and SPLET (sequential proton loss electron transfer). One fact is interesting to note, and that is that inactivation of investigated radical species are more favourable when –NH group participates inactivating of mentioned radicals, in regard to –OH group. The radical scavenging capacity of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide decreases in the following order $^{\bullet}OH > {}^{\bullet}OC(CH_3)_3 > {}^{\bullet}OCH_3 > {}^{\bullet}OOH > {}^{\bullet}OOCH_3 > {}^{\bullet}OOCH_2CH_3$.

Keywords: Antiradical activity, DFT, Thermodynamic approach

1. Introduction

Molecular oxygen is an indispensable element for the life of aerobic organisms because it enables different metabolic processes. This molecule is the most important electron acceptor in process of oxidative phosphorylation in the living organisms, but, by nature of its bi-radical properties, it also enables a formation of partially reduced chemical species known as reactive oxygen species (ROS). In small quantities, they are essential for cell signalling and other vital physiological functions, but in high doses, these chemical species are very toxic. Free radicals can be involved in starting a radical-chain reaction that could be potentially dangerous for the cell [1]. They are constantly forming within the cells upon exposure to drugs, pollutants, ultraviolet rays, smoke, and some endogenous metabolites of the redox and respiratory chain during the transfer of electrons [2]. Excessive exposure to free radicals can lead to the state known as oxidative stress [3].

Scientists believe that, if the organism is often found in the state of oxidative stress, it is more likely to impair the proper functioning of metabolism, tissue damage, fibrous blood vessel changes, or even mutations on the hereditary material leading to various diseases. When free radicals interact with protein/enzyme molecules, they can cause changes in the structure of those molecules. Considering that the function of enzymes depends on their structure, exposure to free radicals often leads to the inactivation of given enzymes and the abolition of their physiological function [4].

Consequently, agents with the ability to protect against reactive chemical species, for example, free radicals, may be therapeutically useful. In recent years, numerous phenolic compounds have gained particular interests as beneficial and health-promoting agents due to their high antioxidant activity [5-8].

Coumarins are a large class of heterocyclic compounds that are highly represented in the plant world. They can be found in different plants, as well as in different parts of the plant, in various concentrations [9-11]. They are involved in different metabolic processes in a plant, such as growth regulation, control of respiration, photosynthesis, etc. As secondary metabolites, these compounds in higher concentrations protect the plant against the different pathogens [12-14]. Coumarin derivates have found use in the treatment of numerous diseases, thanks to a wide range of their biological and physiological activities [15]. Antiviral, antibacterial, anticoagulant, antitumor, anti-inflammatory, antioxidative, and other important properties of coumarin derivates are not originating from their simple coumarin base but mainly from substituents in different positions. Substituting hydrogen atoms from coumarin molecule with hydroxy, phenol, benzoyl, alkyl, aryl, and other similar group, can increase and even widen the spectrum of physiological, pharmacological and biological activities of those compounds. For example, coumarin derivates with hydroxy, phenyl or benzyl groups as substituents have better spin-delocalization abilities, which makes them better antioxidants [16-19]. All of above-mentioned makes the coumarin derivates good potential drugs, and some of them, like warfarin, are already in use today.

In this paper, based on above-presented literature findings, and our interest in coumarins as potential antioxidants [20,21] as well as other properties of these compounds, i*n silico* investigation of antioxidative action of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide (Figure 1) [22,23] was carried out.

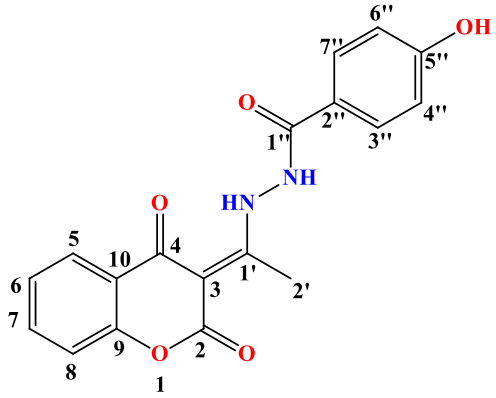

Figure 1. Structure and labelling of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide

2. Materials and Methods

All quantum chemical calculations were carried out by using the *Gaussian 09* software package [24]. The geometry optimization and harmonic vibrational frequencies were obtained using the Density Functional Theory (DFT) method M06-2X with basis set 6-311G++(d, p) [25,26]. The first

part of investigating of the antioxidative activity is calculating the thermodynamic parameters using the following equations:

$$\text{HAT:} \quad \text{Ar-OH} \rightarrow \text{Ar-O}^{\bullet} + \text{H}^{+} \tag{1}$$

$$\text{SET-PT:} \quad \text{Ar-OH} \rightarrow \text{Ar-OH}^{\bullet+} + e^{-} \tag{2}$$

$$\text{Ar-OH}^{\bullet+} \rightarrow \text{Ar-O}^{\bullet} + \text{H}^{+} \tag{3}$$

$$\text{SPLET:} \quad \text{Ar-OH} \rightarrow \text{Ar-O}^{-} + \text{H}^{+} \tag{4}$$

$$\text{Ar-O}^{-} \rightarrow \text{Ar-O}^{\bullet} + e^{-} \tag{5}$$

The values of thermodynamic parameters that describe the antioxidative activity of investigated compounds (BDE, IP, PDE, PA and ETE) are calculated from total enthalpies by applying the following equations:

$$\text{BDE} = H(\text{Ar-O}^{\bullet}) + H(\text{H}^{+}) - H(\text{Ar-OH}) \tag{6}$$

$$\text{IP} = H(\text{Ar-OH}^{\bullet+}) + H(e^{-}) - H(\text{Ar-OH}) \tag{7}$$

$$\text{PDE} = H(\text{Ar-O}^{\bullet}) + H(\text{H}^{+}) - H(\text{Ar-OH}^{\bullet+}) \tag{8}$$

$$\text{PA} = H(\text{Ar-O}^{-}) + H(\text{H}^{+}) - H(\text{Ar-OH}) \tag{9}$$

$$\text{ETE} = H(\text{Ar-O}^{\bullet}) + H(e^{-}) - H(\text{Ar-O}^{-}) \tag{10}$$

Antioxidant properties of the investigated compound were also investigated against the various radicals such as hydroxy ($^{\bullet}$OH), methoxy ($^{\bullet}$OCH$_3$), *terc*-butoxy ($^{\bullet}$OC(CH$_3$)$_3$), peroxy ($^{\bullet}$OOH), methyl-peroxy ($^{\bullet}$OOCH$_3$), ethyl-peroxy ($^{\bullet}$OOCH$_2$CH$_3$). Mechanisms of radical scavenging activity are described by the following equations:

$$\text{HAT:} \quad \text{Ar-OH} + \text{RO}^{\bullet} \rightarrow \text{Ar-O}^{\bullet} + \text{ROH} \tag{11}$$

$$\text{SET-PT:} \quad \text{Ar-OH} + \text{RO}^{\bullet} \rightarrow \text{Ar-OH}^{\bullet+} + \text{RO}^{-} \tag{12}$$

$$\text{Ar-OH}^{\bullet+} + \text{RO}^{-} \rightarrow \text{Ar-OH}^{\bullet} + \text{ROH} \tag{13}$$

$$\text{SPLET:} \quad \text{Ar-OH} + \text{RO}^{-} \rightarrow \text{Ar-O}^{-} + \text{ROH} \tag{14}$$

$$\text{Ar-O}^{-} + \text{RO}^{\bullet} \rightarrow \text{Ar-O}^{\bullet} + \text{RO}^{-} \tag{15}$$

where RO$^{\bullet}$ denotes reactive oxygen species and where Ar-OH, Ar-O$^{\bullet}$, Ar-OH$^{\bullet+}$, and Ar-O^{-} denote antioxidant, its radical, radical-cation and anion, respectively.

Thermodynamic parameters that describe radical scavenging activity of investigated compound are calculated from total Gibbs energies by using the following equations:

$$\Delta_r G_{BDE} = G(\text{Ar-O}^{\bullet}) + G(\text{ROH}) - G(\text{Ar-OH}) - G(\text{RO}^{\bullet}) \tag{16}$$

$$\Delta_r G_{IP} = G(\text{Ar-OH}^{\bullet+}) + G(RO^{-}) - G(\text{Ar-OH}) - G(\text{RO}^{\bullet}) \tag{17}$$

$$\Delta_r G_{PDE} = G(\text{Ar-O}^{\bullet}) + G(\text{ROH}) - G(\text{Ar-OH}^{\bullet+}) - G(\text{RO}^{-}) \tag{18}$$

$$\Delta_r G_{PA} = G(\text{Ar-O}^{-}) + G(\text{ROH}) - G(\text{Ar-OH}) - G(\text{RO}^{-}) \tag{19}$$

$$\Delta_r G_{ETE} = G(\text{Ar-O}^{\bullet}) + G(RO^{-}) - G(\text{Ar-O}^{-}) - G(\text{RO}^{\bullet}) \tag{20}$$

Besides parameters that describe antioxidative properties of the investigated compound in this paper were discussed global reactive parameters that were calculated according to the literature [27].

3. Results and Discussion

3.1. Frontier molecular orbitals and global reactive parameters

The optimized structure of the investigated compound is presented in Figure 2. The structure is based on the coumarin on which is bonded to *p*-hydroxybenzoic acid residue by hydrazide bridge.

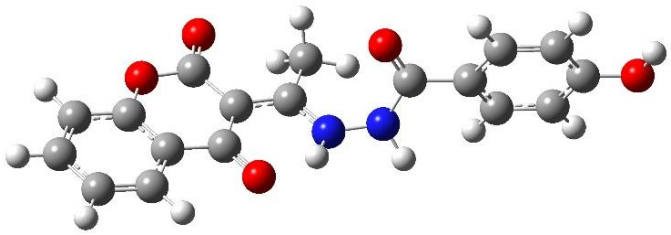

Figure 2. The optimized structure of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide

The difference in energy of HOMO and LUMO orbitals can indicate the relative stability of the investigated compound [28, 29]. The higher value of the HOMO-LUMO energy gap indicates a more stable molecule [30]. Having in mind that polycyclic aromatic hydrocarbons that have HOMO-LUMO gap that is higher than 1.30 eV are considered stable [31], the values of the HOMO-LUMO gap of 6.72 eV is0 indicating that investigated compound is relatively stable.

Table 1. Global descriptive parameters for (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide

HOMO (eV)	LUMO (eV)	H-L GAP	I	A	μ=-(I+A)/2	χ=-μ	η=(I-A)/2	ω=μ²/² η	S=1/2 η	Δnmax=-μ/η
-7.88	-1.16	6.72	7.89	1.16	-4.52	4.52	3.36	1.52	1.68	1.35

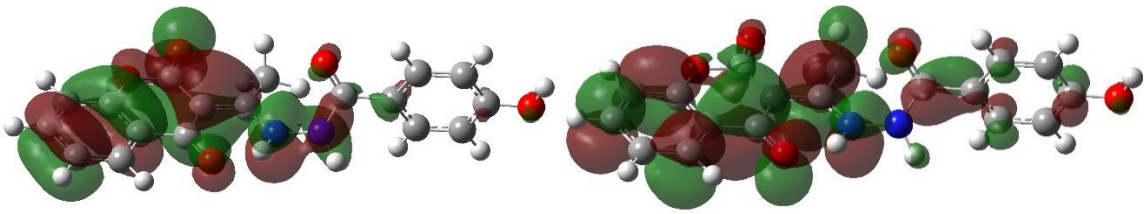

Figure 3. HOMO (left) and LUMO (right) orbitals of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide

3.2. Radicals, anions, and radical-cation of the investigated compound

The hemolytic and heterolytic cleavage of the bond between hetero atom and hydrogen leads to the formation of radicals and anions retrospectively. Values of thermodynamic parameters that describe these reactions are given in Table 2. Favouring –NH over –OH group in reactions of radical scavenging ability can be explained through the better delocalization of unpaired electron. As can be seen on spin density maps of investigated radicals (Figure 4.), when radical is formed on the oxygen of -OH group, spin delocalization is concentrated on the *p*-hydroxybenzoyl group. In case of 1'–NH group being radical scavenging actor, spin delocalize over the whole molecule of the investigated antioxidant. Furthermore, by investigating ESP maps (Figure. 5) it is evident that the change in electrostatic potential is better distributed over the molecule in case of –NH group being involved in radical scavenging activity, as oppose to –OH where majority of ESP is localized over the *p*-hydroxybenzoyl group.

A similar situation is found by investigating anions of tested antioxidant. Delocalization of negative charge is better in a case where anion originates from N-H bond cleavage. (Figure. 6)

All of these facts can be explained by analyzing the structure of the investigated compound. As it can be seen, from one side the diazo group is attached to the C-1′ atom from carbonyl group, and the other nitrogen atom is bonded to other sp^2 hybridized carbon atom in position C-1″. This allows good delocalization of unpaired electron, as well as delocalization of charge through the whole molecule.

It is very important to mention that dihedral angle C′-N-N-C″ is about 90° when the molecule is in the ground state, as well as radical and anion when radical scavenging activity is going over –OH group. In a case where radical scavenging includes –NH group, the molecule becomes planar. That allows delocalization of spin/charge to happen on coumarin and *p*-hydroxybenzoyl part of the molecule simultaneously, and that makes this position thermodynamically favorable.

Besides that, it is important to emphasize that hydrogen loss/proton loss always happens on the nitrogen atom that is closer to the *p*-hydroxybenzoyl group because hydrogen, bonded to the nitrogen atom in position C-1`, is interacting with oxygen from coumarin base (position C-2), building strong hydrogen bond. That makes this hydrogen less reactive towards the ROS (Figure 2).

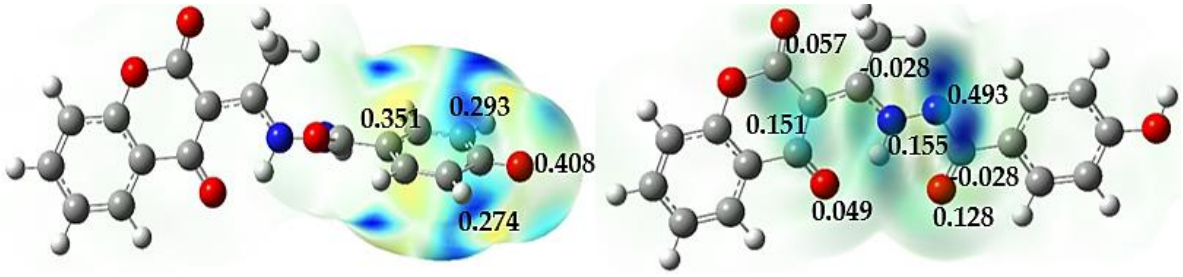

Figure 4. Spin density maps of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide radical: when hydrogen is abstracted from -OH group (left) and when is abstracted from -NH group (right)

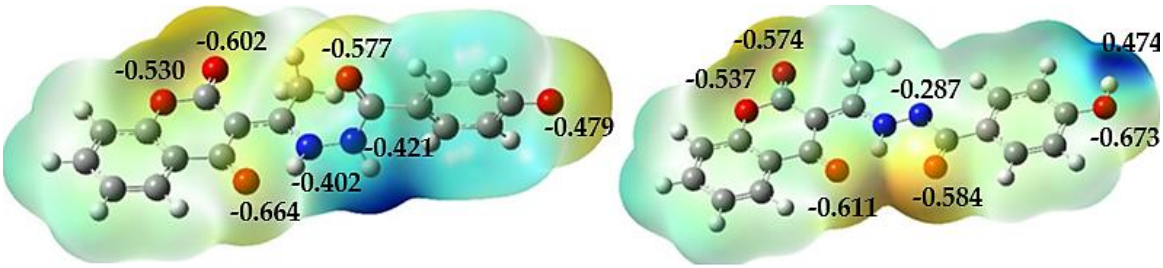

Figure 5. ESP maps of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide radical: when hydrogen is abstracted from -OH group (left) and when is abstracted from -NH group (right)

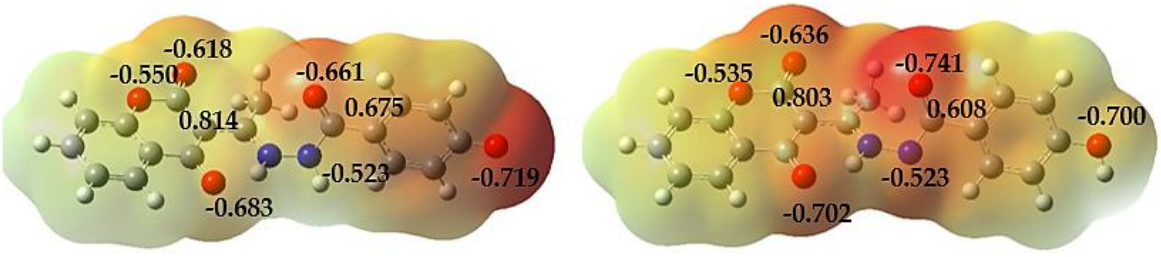

Figure 6. ESP maps of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide anion: when hydrogen is abstracted from -OH group (left) and when is abstracted from -NH group (right)

Besides those chemical species, there is one more intermedier that occurs in SET-PT mechanism, and it is described by IP. That is radical-cation. As can be seen from maps of the electrostatic potential (ESP) of investigated radical-cation, in regard to ESP map of molecule in ground state (Figure 7)

delocalisation of positive charge is quite good, which indicates relatively good stability of investigated radical-cation.

The similar conclusion arises from the spin density map (Figure 8), that shows good spin delocalisation across the whole molecule.

An interesting consequence of losing an electron is N-H bond deformation and intermolecular proton transfer from N-H group to sp^2 hybridized oxygen of coumarin base.

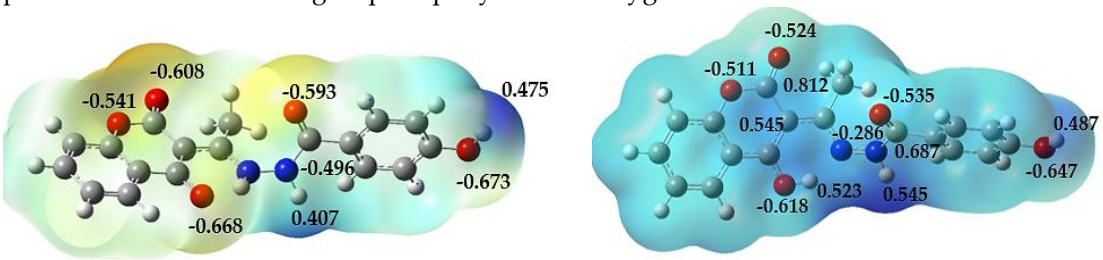

Figure 7. ESP map of molecule in the ground state (left) and radical-cation (right) of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide

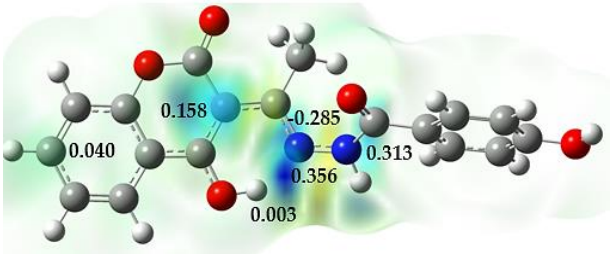

Figure 8. Spin density map of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide radical-cation

3.3. Thermodynamic parameters

Reaction enthalpies related to investigated mechanisms of antioxidative action (HAT, SET-PT and SPLET) were calculated according to the equations (5-10). The preferable mechanistic pathway of antioxidative action was estimated based on the BDE, IP, and PA values.

BDE describes the process of homolytic breaking of O-H bond and donation of a hydrogen atom to a radical species. In the case of heterolytic cleavage of O-H bond anion and proton are formed, and the thermodynamics of this process is described by IP value. According to the values presented in Table 2, the investigated compound show slightly lower enthalpies for N-H cleavage, in regard to enthalpies of cleavage the O-H group.

Table 2. Calculated parameters of antioxidant mechanisms of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide in kJ mol^{-1}

Gas-phase					
Positions	***HAT***	***SET-PT***		***SPLET***	
	BDE	**IP**	**PDE**	**PA**	**ETE**
5''-OH	380	760	931	1358	333
1'-NH	339		891	1344	307

3.4. Free radical scavenging activity of investigated compounds with different reactive oxygen species

There are two main groups of radical species chosen to be investigated in this paper. The first group consist of alkoxy radicals: •OH, •OCH_3, and •$OC(CH_3)_3$. The second group includes peroxy radicals: •OOH, •$OOCH_3$, and •$OOCH_2CH_3$. There is a tight correlation between these two groups of

free radical species. Most of them are being formed during biological redox processes or in the biosynthesis of natural products. Alkoxy and peroxy radicals are included in the complex process of innate immunity and phagocytosis [32]. The harmful effects of these radicals are the result of some autoimmune diseases. This is the case when the immune cells become excessively activated and toxic to neighboring healthy cells [33]. The peroxy radicals are usually less reactive than analogue alkoxy radicals are. The damage initiated on the human body by peroxy radicals is usually the consequence of producing the respective alkoxy radical, rather than damage induced by them in the first place.

Gibbs free energies of reaction between investigated compound and different radical species related to different mechanisms of antioxidative activity (HAT, SET-PT, and SPLET) are presented in Table 3. The dominant mechanism of radical scavenging activity of investigated antioxidant is estimated from $\Delta_r G_{BDE}$, $\Delta_r G_{PA}$, and $\Delta_r G_{IP}$ values, where the lowest of these values indicates the preferred mechanism.

Table 3. Calculated parameters of antioxidant mechanisms of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide with radicals in kJ mol^{-1}

Gas-phase					
Positions	***HAT***	***SET-PT***		***SPLET***	
	$\Delta_r G_{BDE}$	$\Delta_r G_{IP}$	$\Delta_r G_{PDE}$	$\Delta_r G_{PA}$	$\Delta_r G_{ETE}$
$^{\bullet}$OH					
5′′-OH	-108	606	-715	-288	180
1′-NH	-149		-756	-302	153
$^{\bullet}$OOH					
5′′-OH	24	666	-641	-214	239
1′-NH	-16		-682	-229	212
$^{\bullet}$OCH$_3$					
5′′-OH	-44	627	-672	-245	200
1′-NH	-85		-712	-259	174
$^{\bullet}$OOCH$_3$					
5′′-OH	30	659	-629	-202	232
1′-NH	-11		-669	-216	205
$^{\bullet}$OOCH$_2$CH$_3$					
5′′-OH	31	655	-623	-196	228
1′-NH	-9		-664	-211	201
$^{\bullet}$OC(CH$_3$)$_3$					
1′′-OH	-57	586	-644	-217	380
1′-NH	-98		-684	-231	404

As results obtained and presented in Table 3 are suggesting, the SET-PT mechanistic pathway is non-operative, but SPLET is the most preferable pathway of radical scavenging. Even though HAT mechanism is still operative, $\Delta_r G_{PA}$ values, that are sometimes a couple of times lower than $\Delta_r G_{BDE}$, are indicating that SPLET is the most favourable mechanism of radical scavenging. This is the most pronounced in the case of the ethylperoxy radical, where $\Delta_r G_{BDE}$ has value around -9 kJ mol^{-1}, while $\Delta_r G_{PA}$ is -211 kJ mol^{-1}. It is interesting to notice that the order of radical reactivity slightly varies when the reaction follows HAT in regard to SPLET mechanism. While radical scavenging capacity by the HAT mechanism decreases in following order $^{\bullet}$OH > $^{\bullet}$OC(CH$_3$)$_3$ > $^{\bullet}$OCH$_3$ > $^{\bullet}$OOH > $^{\bullet}$OOCH$_3$ > $^{\bullet}$OOCH$_2$CH$_3$, enthalpies from Table 3 suggest that radical scavenging capacity in SPLET mechanism

decreases in slightly different order $^{\bullet}OH > {}^{\bullet}OCH_3 > {}^{\bullet}OC(CH_3)_3 > {}^{\bullet}OOH > {}^{\bullet}OOCH_3 > {}^{\bullet}OOCH_2CH_3$. Increased reactivity of *terc*-butoxy radical (HAT) is a consequence of the electron-donor effect of methyl groups. In case of SPLET mechanism, reactive species in the first step are not radicals but anions. Due to the effect of hyperconjugation, the *terc*-butoxy anion is more stable than methoxy anion, and that is why reactivity order for HAT and SPLET mechanisms are not the same.

Another interesting observation is that difference in Δ_rG_{PA} values between the –OH and –NH position of the investigated antioxidant, are around 15kJ mol^{-1}, and that is a lot lower than 40 kJ mol^{-1} which is the case with ΔrG_{BDE} values, regardless of radical that is being inactivated.

4. Conclusion

In this paper, the antioxidative properties of newly synthesized coumarin hybrid (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide against the six selected reactive oxygen species (ROS) were investigated.

Analysis of the obtained thermodynamic parameters indicate SET-PT as non-operative mechanism of free radical scavenging. On the other hand, the operative mechanistic pathways for free radical scavenging of all investigated radical species are HAT and SPLET, with SPLET as the dominant one.

Inactivation of investigated radical species is more favourable when –NH group participates inactivating of mentioned radicals, in regard to –OH group, as consequence of achieving planar conformation of radical molecule in this form, which leads to better spin delocalisation. The difference in ΔG_{BDE} values between the –NH and –OH group is around 40 kJ, and the difference in ΔG_{PA} values is around 15 kJ in favour of –NH group, regardless of radical that is being inactivated. The radical scavenging capacity of (*E*)-N'-(1-(2,4-dioxochroman-3-yl)ethyl)-4-hydroxybenzohydrazide decreases in the following order $^{\bullet}OH > {}^{\bullet}OCH_3 > {}^{\bullet}OC(CH_3)_3 > {}^{\bullet}OOH > {}^{\bullet}OOCH_3 > {}^{\bullet}OOCH_2CH_3$.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, Marko Antonijević and Zoran Marković; methodology, Žiko Milanović.; software, Marko Antonijević.; validation, Jelena Đorović Jovanović, Dejan Milenković, Zorica Petrović and Dušica Simijonović; formal analysis, Dejan Milenković; investigation, Marko Antonijević; resources, Zoran Marković; data curation, Jelena Đorović Jovanović; writing—original draft preparation, Edina Avdović; writing—review and editing, Zoran Marković; visualization, Edina Avdović; supervision, Zoran Marković; project administration, Zoran Marković; funding acquisition, Zoran Marković. All authors have read and agreed to the published version of the manuscript.

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References:

1. Martinez-Cayuela, M. Oxygen free radicals and human disease. *Biochimie*, 1995, *77*(3), 147-161.
2. Borg, J., Reeber A. *Biochimie Metabolique´*, Ellipses, Paris, France, 2008.
3.] Maritim, A. C., Sanders, A., & Watkins Iii, J. B. Diabetes, oxidative stress, and antioxidants: a review. *Journal of biochemical and molecular toxicology,* **2003,** *17*(1), 24-38.
4. Droge, W. Free radicals in the physiological control of cell function. *Physiological reviews*, **2002,** *82*(1), 47-95.
5. Dangles, O., Dufour, C., Tonnelé, C., & Trouillas, P. The physical chemistry of polyphenols: insights into the activity of polyphenols in humans at the molecular level. *Recent advances in polyphenol research*, **2017,** *5*, 1-35.

6. Galano, A., Raúl Alvarez-Idaboy, J. Computational strategies for predicting free radical scavengers' protection against oxidative stress: Where are we and what might follow? *International Journal of Quantum Chemistry*, **2018**, *119*(2), e25665.
7. Pisoschi, A. M., & Pop, A. The role of antioxidants in the chemistry of oxidative stress: A review. *European journal of medicinal chemistry*, **2015,** *97*, 55-74.
8. Yao, H., Zhang, X., Wei, G., Wu, W., Duan, J., Li, Y., ... & Liu, Y. Protective effects of thevetiaflavone on H_2O_2-induced pc12 cells injury via improving oxidative stress and mitochondrial dysfunction. *Lat Am J Pharm*, **2017,** *36*, 1821-1826 mitochondria. *Phytochemistry*, 2007, *68(7)*, 1075-1080.
9. Erdelmeier, C. A. J., & Sticher, O. Coumarin Derivatives from Eryngium campestre1. *Planta medica*, **1985,** *51*(05), 407-409.
10. Dandriyal, J., Singla, R., Kumar, M., & Jaitak, V. Recent developments of C-4 substituted coumarin derivatives as anticancer agents. *European journal of medicinal chemistry*, **2016,** *119*, 141-168.
11. Magadula, J. J., Masimba, P. J., Tarimo, R. B., Msengwa, Z., Mbwambo, Z. H., Heydenreich, M., ... & Richomme, P. Mammea-type coumarins from Mammea usambarensis Verdc. *Biochemical Systematics and Ecology*, **2014,** *56*, 65-67.
12. Ojala, T., Remes, S., Haansuu, P., Vuorela, H., Hiltunen, R., Haahtela, K., & Vuorela, P. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. *Journal of ethnopharmacology*, **2000**, *73*(1-2), 299-305.
13. Cottigli, F., Loy, G., Garau, D., Floris, C. O. S. T. A. N. T. I. N. O., Caus, M., Pompei, R. A. F. F. A. E. L. L. O., & Bonsignore, L. Antimicrobial evaluation of coumarins and flavonoids from the stems of Daphne gnidium L. *Phytomedicine*, **2001**, *8*(4), 302-305.
14. Rosselli, S., Maggio, A. M., Faraone, N., Spadaro, V., Morris-Natschke, S. L., Bastow, K. F., ... & Bruno, M. The cytotoxic properties of natural coumarins isolated from roots of Ferulago campestris (Apiaceae) and of synthetic ester derivatives of aegelinol. *Natural product communications*, **2009,** *4*(12), 1701-1706
15. Kontogiorgis, C., Detsi, A., & Hadjipavlou-Litina, D. Coumarin-based drugs: a patent review (2008–present). *Expert opinion on therapeutic patents*, **2012,** *22*(4), 437-454.
16. Cravotto, G., Nano, G. M., Palmisano, G., & Tagliapietra, S. An asymmetric approach to coumarin anticoagulants via hetero-Diels–Alder cycloaddition. *Tetrahedron: Asymmetry*, **2001,** *12*(5), 707-709.
17. Hodak, K., Jakesová, V., & Dadák, V. On the antibiotic effects of natural coumarins. VI. The relation of structure to the antibacterial effects of some natural coumarins and the neutralization of such effects. *Ceskoslovenska farmacie*, **1967,** *16*(2), 86-91.
18. Velasco-Velázquez, M. A., Agramonte-Hevia, J., Barrera, D., Jiménez-Orozco, A., García-Mondragón, M. J., Mendoza-Patiño, N., ... & Mandoki, J. 4-Hydroxycoumarin disorganizes the actin cytoskeleton in B16–F10 melanoma cells but not in B82 fibroblasts, decreasing their adhesion to extracellular matrix proteins and motility. *Cancer letters*, **2003**, *198*(2), 179-186.
19. Al-Ayed, A. S., & Hamdi, N. A new and efficient method for the synthesis of novel 3-acetyl coumarins oxadiazoles derivatives with expected biological activity. *Molecules*, **2014**,*19*(1), 911-924.
20. Avdović, E. H., Milanović, Ž. B., Živanović, M. N., Šeklić, D. S., Radojević, I. D., Čomić, L. R., ... & Marković, Z. S. Synthesis, spectroscopic characterization, biological activity, DFT and molecular docking study of novel 4-hydroxycoumarine derivatives and corresponding palladium (II) complexes. *Inorganica Chimica Acta*, **2020,** *504*, 119465.
21. Milanović, Ž. B., Dimić, D. S., Avdović, E. H., Milenković, D. A., Marković, J. D., Klisurić, O. R., ... & Marković, Z. S. Synthesis and comprehensive spectroscopic (X-ray, NMR, FTIR, UV–Vis), quantum chemical and molecular docking investigation of 3-acetyl-4-hydroxy-2-oxo-2H-chromen-7-yl acetate. *Journal of Molecular Structure*, **2020,** *1225*, 129256.

22. Kotali, A., Nasiopoulou, D. A., Harris, P. A., Helliwell, M., & Joule, J. A. Transformation of a hydroxyl into an acyl group on α-pyrone ring: a novel route to 3, 4-diacylcoumarins. *Tetrahedron*, **2012**, *68*(2), 761-766.
23. Kotali, A., Nasiopoulou, D. A., Tsoleridis, C. A., Harris, P. A., Kontogiorgis, C. A., & Hadjipavlou-Litina, D. J.. Antioxidant Activity of 3-[N-(Acylhydrazono) ethyl]-4-hydroxy-coumarins. *Molecules*, **2016**, *21*(2), 138.
24. Frisch, M. J.; Trucks, G. W.; Schlegel, H.B.; Scuseria, G. W.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. **2009**, *Gaussian 09*.
25. Zhao, Y., & Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theoretical Chemistry Accounts*, **2008** *120*(1-3), 215-241.
26. McLean, A. D., Chandler, G. S. Contracted Gaussian basis sets for molecular calculations. I. Second row atoms, Z= 11–18. *The Journal of Chemical Physics*, **1980**, *72*(10), 5639-5648.
27. Karelson, M., Lobanov, V. S., & Katritzky, A. R. Quantum-chemical descriptors in QSAR/QSPR studies. *Chemical reviews*, **1996,** *96*(3), 1027-1044.
28. Dimić D, Milenković D, Dimitrić Marković J, Marković Z. Antiradical activity of catecholamines and metabolites of dopamine: Theoretical and experimental study. Physical Chemistry Chemical Physics, **2017,** 19(20), 12970-12980.
29. Wang L, Yang F, Zhao X, Li Y. Effects of nitro- and amino-group on the antioxidant activity of genistein: A theoretical study. Food Chemistry, **2019**, 275, 339–345.
30. Karelson M, Lobanov V. S, Katritzky A. R. Quantum-Chemical Descriptors in QSAR/QSPR Studies. Chemical Reviews, **1996**, 96(3), 1027–1044.
31. Aihara, J. I. Reduced HOMO− LUMO gap as an index of kinetic stability for polycyclic aromatic hydrocarbons. *The Journal of Physical Chemistry A*, **1999**, *103*(37), 7487-7495.
32. Knight, J. A. (2000). Free radicals, antioxidants, and the immune system. *Annals of Clinical & Laboratory Science*, **2000,** *30*(2), 145-158.
33. Cooper, G. M., & Hausman, R. E. A molecular approach. *The Cell. 2nd ed. Sunderland, MA: Sinauer Associates*. **2000.**