

Comparative Antiradical Activity and Molecular Docking Study of Bergaptol and Xanthotoxol

Žiko Milanović^{1*}, Marko Antonijević², Jelena Đorović², Dejan Milenković²

¹ University of Kragujevac, Faculty of Science, Department of Chemistry, 12 Radoja Domanovića Street, 34000 Kragujevac, Serbia
e-mail: ziko.milanovic@pmf.kg.ac.rs

² University of Kragujevac, Institute of Information Technologies, Department of Science, Jovana Cvijića bb, 34000 Kragujevac, Serbia
e-mail: mantonijevic@uni.kg.ac.rs, jelena.djorovic@uni.kg.ac.rs, dejanm@uni.kg.ac.rs

**corresponding author*

Abstract

The antioxidant activity of bergaptol (4-hydroxyfuro[3,2-g]chromen-7-one, BER) and xanthotoxol (9-hydroxyfuro[3,2-g]chromen-7-one, XAN) was investigated in water and benzene, as solvents. For this purpose, the density functional theory (DFT) was used. The free radical scavenging potency of investigated compounds towards different reactive oxygen species (ROS) was performed. Antioxidative mechanism of investigated compounds – hydrogen atom transfer (HAT), single-electron transfer–proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET) were examined using M06-2X/6-311++G(d,p) theoretical model. The important thermodynamic parameters (BDE, IP, PDE, PA, ETE) and Gibbs free energies of reactions, were used to determine the most probable antioxidant mechanism of action. The obtained thermodynamic parameters suggested that Hydrogen Atom Transfer (HAT) is the most probable reaction pathway in benzene, while Sequential Proton Loss Electron Transfer (SPLET) was favored in water. The obtained results indicate that the favorable mechanism of antiradical activity depends on the polarity of medium and the nature of free radical species. By comparing the antioxidant activity of investigated compounds, it can be concluded that bergaptol exhibits better antioxidant properties. Molecular docking study of neutral and anionic species of investigated compounds was performed according to Estrogen receptor alpha (ER α). In both cases, bergaptol showed better inhibitory potency. All the anionic species showed a higher inhibition constant, indicating lower inhibition potency than corresponding parent molecules.

Keywords: bergaptol, xanthotoxol, DFT, antioxidant, estrogen receptor alpha

1. Introduction

Reactive oxygen species (ROS) are generated in cells metabolism of aerobic organisms. At physiological concentrations, they have an important role in normal cell function as mediators of intracellular signaling cascades (Turrens, 2003). Excessive production and accumulation of

ROS may lead to oxidative stress which causes loss of normal cell function. Consequently, oxidative damage of cells significantly contributes to multiple pathologies such as inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, autism, and aging (Liou et al., 2010, Apel et al., 2004). To prevent or reduce the ROS induced oxidative damage, the aerobic organisms have developed an antioxidant defense system that includes specific intracellular enzymes. On the other hand, specific molecules-antioxidants can neutralize free radicals via different mechanisms of antioxidant activity (Bian et al., 2009).

Coumarins (known as 1,2-benzopyrones), consisting of fused pyrone and benzene rings, are an important group of low-molecular weight compounds and have been widely used for the prevention and treatment of many diseases (Kostova et al., 2011). The specific class of these compounds is furanocoumarins who are consist of a furan ring fused with a coumarin base. A handful of plant families such as Apiaceae and Rutaceae contain the largest numbers of different furanocoumarins. Several reviews summarize advances in the application of coumarins, especially concerning their antioxidant properties (Pathak et al., 1962).

Several recently published review articles show advances in the application of coumarins, especially concerning their antiradical properties (Pathak et al., 1962). The different bioactive compounds isolated from grapefruit juice and grapefruit peel oil have shown significant antioxidant capacity. One of the structures was elucidated and identified as bergaptol (4-hydroxyfuro[3,2-g]chromen-7-one, BER). The isolated compound was tested for their radical scavenging activity using 2,2-azobis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazil (DPPH) methods at different concentrations. Bergaptol showed very good radical scavenging activity at all the tested concentrations (Girenavar et al., 2007).

A variety of furanocoumarin isolated from the Chinese herbs were tested for antiradical activity and the ability to inhibit lipid peroxidation in rat brain and kidney homogenates as well as rat erythrocyte hemolysis. One of the isolated compounds xanthotoxol (9-hydroxyfuro[3,2-g]chromen-7-one, XAN) was exhibited potent antioxidative activity in both, lipid peroxidation and hemolysis assays (Devienne et al., 2007).

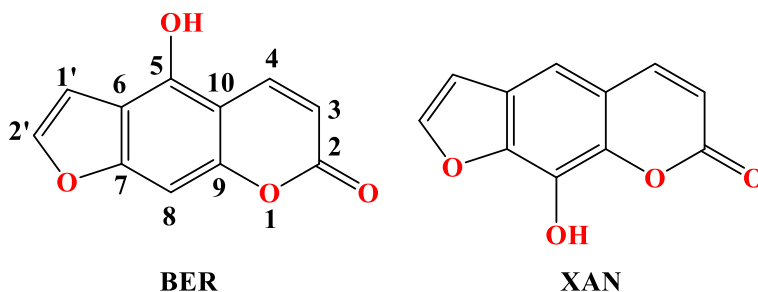
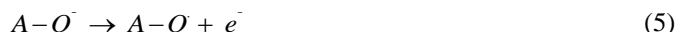


Fig. 1. Structures of bergaptol (BER) and xanthotoxol (XAN) with atomic numbering

This study aims to examine the antioxidant properties of bergaptol and xanthotoxol in benzene and water and to determine how the OH group in a different position contributes to antioxidant capacity. The reaction of H atom transfer from A–OH can obey at least three different mechanisms: hydrogen atom transfer (**HAT(1)**), single-electron transfer followed by proton transfer (**SET-PT(2, 3)**), and sequential proton loss electron transfer (**SPLET(4, 5)**).





All present mechanisms are competitive and depend on the reaction conditions, as well as on the properties of solvents and free radicals (Klein et al., 2007). In addition, antioxidant properties were investigated in reaction with various reactive oxygen species such as hydroxyl ($\cdot OH$), hydroperoxy ($\cdot OOH$), methoxy ($\cdot OCH_3$), methyl peroxy ($\cdot OOOCH_3$), vinyl peroxy ($\cdot OOOCHCH_2$), iso-propoxy ($\cdot OCH(CH_3)_2$) following equation:



In Eqs (1) – (10) $A-O\cdot$, $A-O^-$, and $A-O^+$ represent the free radicals, anions, and radical cations issued from the corresponding phenolic (A-OH) compounds. In addition, a molecular docking study was used to investigate the ability and binding mode of investigated compounds to biological important estrogen receptor alpha (**ER α**). A detailed description of the interactions between investigated compounds and protein can help in a better understanding of the biological effects of these components. Special attention is paid to the effect of a hydroxyl group in different positions attached to an aromatic ring of furanocoumarin bases on the stability of the protein-ligand complex.

2. Methodology

2.1. Thermodynamic parameters of antiradical activity

All quantum chemical calculations were performed using the Gaussian 09 software package (Frisch et al., 2010). The hybrid meta M06-2X functional was used for geometry optimization and computation of harmonic vibrational frequencies using the 6-311++G(d,p) basis set (unrestricted M06-2X) for the resulting radicals. The applied level of theory was used for the optimization of each neutral molecule and the related radicals, radical cations, and anions. Previous studies have shown that the M06-2X method is suitable for thermochemical and kinetic research (Zhao et al., 2008). To determine stationary points on the potential energy surface for all optimized structures the harmonic vibrational frequencies were calculated. All structures were optimized in two different solvents: benzene ($\epsilon = 2.2706$), water ($\epsilon = 78.3553$), without any geometrical constraints using the CPCM solvation model (Takano et al., 2005). These solvents were selected to mimic the polar and nonpolar surroundings as well as conditions of experimental measurements. It is well established that the antioxidant activity of a compound rather depends on a microenvironment of the reaction medium, and not just of its structure and nature of the involved ROS.

The thermodynamical parameters relevant for the investigated antioxidative mechanisms (BDE, IP, PDE, PA, and ETE) were calculated from total enthalpies of the optimized species using the following equations:

$$BDE = H(A-O) + H(H^\cdot) - H(A-OH) \quad (11)$$

$$IP = H(A-OH^+) + H(e^-) - H(A-OH) \quad (12)$$

$$PDE = H(A-O) + H(H^+) - H(A-OH^+) \quad (13)$$

$$PA = H(A-O^-) + H(H^+) - H(A-OH) \quad (14)$$

$$ETE = H(A-O) + H(e^-) - H(A-O^\cdot) \quad (15)$$

On the other hand, the thermodynamical parameters relevant to the investigated antioxidative mechanisms with different radical species were calculated from the difference between free energies of the optimized products and reactants using the following equations:

$$\Delta_r G_{BDE} = G(A-O) + G(ROH) - G(A-OH) - G(\cdot OR) \quad (16)$$

$$\Delta_r G_{IP} = G(A-OH^+) + G(\cdot OR) - G(A-OH) - G(\cdot OR) \quad (17)$$

$$\Delta_r G_{PDE} = G(A-O) + G(ROH) - G(A-OH^+) - G(\cdot OR) \quad (18)$$

$$\Delta_r G_{PA} = G(A-O^-) + G(ROH) - G(A-OH) - G(\cdot OR) \quad (19)$$

$$\Delta_r G_{ETE} = G(A-O) + G(\cdot OR) - G(A-O^\cdot) - G(OR) \quad (20)$$

where **RO[•]** denotes the reactive oxygen species (Đorović et al, 2014). The Gibbs free energies of the examined reactions were determined at standard conditions ($T=298.15$ K and $p=101325$ Pa) as the difference between the sum of the Gibbs energy of products and the reactants. If the reaction of the exergonic, $\Delta_r G < 0$, or isoergonic, $\Delta_r G \leq 0$, this means that the newly formed radical is more stable than the initial, indicating that the reactive path is favored (Tošović et al., 2018)

2.2. Molecular docking

AutoDock 4.2. program package was utilized for molecular docking simulations (Morris et al., 2009). The search for possible target proteins was performed using online server SwissTargetPrediction. This strategy is based on accurately identify and quantify the similarity between the investigated molecule and the known ligands (Gfeller et al., 2014). The crystal structure of Estrogen receptor alpha (**ER α**) was taken from RCSB Protein Data Bank with PDB code 3ERT (Shiau et al., 1998). To prepare the receptor file (to select the needed chains, delete multiple ligands and non-protein parts) the Discovery Studio 4.0 (BIOVIA Discovery Studio 2016) was used. For simulations, the optimized structures of investigated compounds (Fig.2) were taken. Before the docking simulations, Autodock tools (ADT) was used to add Kollman united atom charges and polar hydrogen atoms to the prepared selected receptor. The grid box of dimensions $60 \times 60 \times 60$ Å along the XYZ directions with a grid spacing of 0.375 Å was created using the AutoGrid module. For the search, the conformers with the lowest energy Lamarckian Genetic Algorithm (LGA) was selected.

3. Results and discussion

3.1. Frontier molecular orbitals and global reactive parameters

The optimized structures of the investigated compounds are presented in Fig.2. Both structures have planar psoralen bases with a free OH group attached to the aromatic ring. The structure of xanthotoxol is stabilized with very weak intramolecular hydrogen bond between OH group and the oxygen atom of pyrone ring (2.23Å).

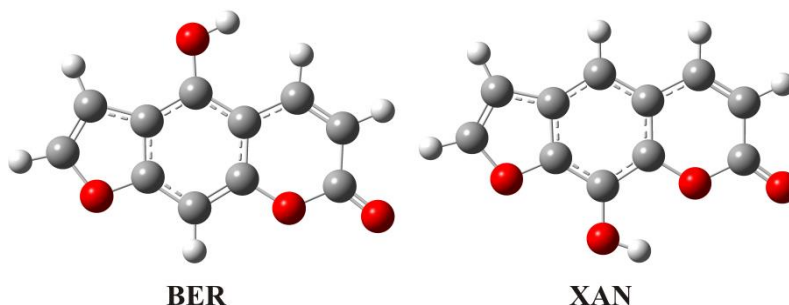


Fig. 2. The optimized structure of bergaptol (left) and xanthotoxol (right) in water obtained at the M06-2X/6-311++G(d,p) level of theory

The HOMO and LUMO orbitals and their difference in energy, are important parameters for the prediction of the general reactivity of molecules (Dimić et al., 2017; Wang, et al.,2019). The higher energy of HOMO orbital implies that the molecule is a better electron donor. The lowest difference in energy or HOMO-LUMO gap signifies the most reactive molecules, while the large energy gap value implies high stability (Karelson, et al., 1996). If we compare the results presented in Table 1, it can be concluded that the bergaptol molecule is more reactive than xanthotoxol with an energy gap of 6.26 eV.

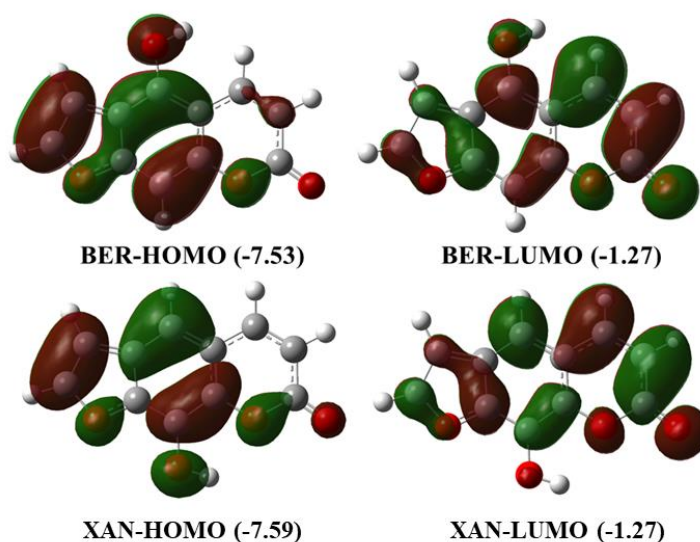


Fig.3. The HOMO and LUMO orbitals of bergaptol (**BER**) and xanthotoxol (**XAN**) with orbital energies in eV

On the other hand, general reactivity parameters can be a useful tool for the theoretical comparison of a group of molecules. Ionization potential (I), vertical electron affinity (A), chemical potential (μ), electronegativity (χ), chemical hardness (η), electrophilicity (ω), and softness (S) were calculated according to the literature (Karelson et al., 1996; Rajan et al., 2017) and the results are given in Table 1. Bergaptol has lower values for ionization potential than xanthotoxol. Hardness describes the stability of the electron cloud of a molecule and therefore the lower value of this parameter would be present in more reactive species. This is also proven in this study, as the lowest η is calculated for bergaptol. Electronegativity is the lower for bergaptol and it is important because this parameter explains the reactivity of a molecule. The chemical potential has the same value as electronegativity just without a minus sign, therefore it describes the resistivity of the molecule towards free radicals. Electrophilicity of a molecule is also a measure of reactivity because it describes the capacity of the system to acquire an electron (Wang et al., 2019). This value is the same for both molecules. The overall conclusion from the analysis of reactivity parameters is that bergaptol can be considered as a more reactive molecule than xanthotoxol.

Compounds	Gap (eV)	I	A	μ (eV)	χ (eV)	η (eV)	ω (eV)	S (eV ⁻¹)
BER	6.26	7.53	1.27	-4.40	4.40	3.13	1.55	1.57
XAN	6.32	7.59	1.27	-4.43	4.43	3.16	1.55	1.58

Table 1. Global reactivity parameters for the investigated compounds (calculated at M06-2X/6-311++G(d,p) level of theory)

3.2. Radicals, radical cations, and anions of investigated compounds

The homolytic cleavage of the O-H bonds in the investigated compounds leads to the formation of two radicals (Fig. S1). The corresponding BDE values are given in Table 2. The BDE values for bergaptol, in both solvents, are lower than xanthotoxol.

The stability and reactivity of both formed radicals were examined by the natural spin densities as they successfully represent electron delocalization (Marković et al., 2011). The unpaired electron is mainly delocalized over the oxygen which suffered hydrogen abstraction, and *ortho* and *para* carbon atoms (O5, C6, C8, C10 and in BER-R; O9, C8, C10, and C5 in XAN-R) and that is shown on the Fig 4. The spin density value on O5 in BER-R is smaller than the values on O8 in the XAN-R. In addition, the unpaired electron in both radicals was delocalized via the carbon of the furan ring (C2' in BER-R and C1' in XAN-R radical).

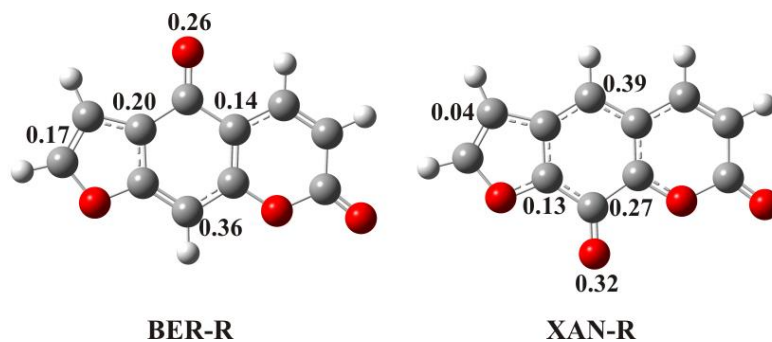


Fig. 4. The optimized structure of bergaptol (left) and xanthotoxol (right) radicals in water with natural spin densities values

Compounds	Position	Benzene, $\epsilon=2.27$				
		HAT	SET-PT		SPLET	
		BDE	IP	PDE	PA	ETE
BER	5-OH	332	666	99	375	390
XAN	8-OH	357	671	119	427	363
Compounds	Position	Water, $\epsilon=78.35$				
		HAT	SET-PT		SPLET	
		BDE	IP	PDE	PA	ETE
BER	5-OH	334	490	44	169	365
XAN	8-OH	347	497	50	198	349

Table 2. Calculated thermodynamic parameters of mechanisms of antioxidant activity (kJ mol^{-1}) of BER and XAN in benzene and water

By an electron loss investigation, compounds yield the corresponding radical cations. The IP values for both compounds in all solvents are presented in Table 2. The IP values of BER and XAN in benzene are higher, while in water are lower than to those of caffeic acid (572.2 and $664.2 \text{ kJ mol}^{-1}$), cyanidin (583.7 and $797.7 \text{ kJ mol}^{-1}$), epicatechin (540.9 and $634.6 \text{ kJ mol}^{-1}$), and resveratrol (519.6 and $592.0 \text{ kJ mol}^{-1}$) (Leopoldini et. al., 2004).

The IP values of investigated compounds in the polar solvent are significantly lower than the value in the nonpolar solvent. This finding is a consequence of the stabilization of the radical cation in the polar solvents. Our investigation shows that the spin density and the positive charge of the radical cations of investigated compounds are delocalized over the benzene ring and carbon atoms of furan rings (Fig. 5). The highest spin density and positive charge values of **BER** and **XAN** are located on C5 and C8, respectively. This suggests that proton transfer is slightly easier from the 5-OH of bergaptole than from 8-OH of xanthoxol. This fact is supported by the PDE values (Table 2), since the smallest PDE value, in both solvents, corresponds to bergaptole.

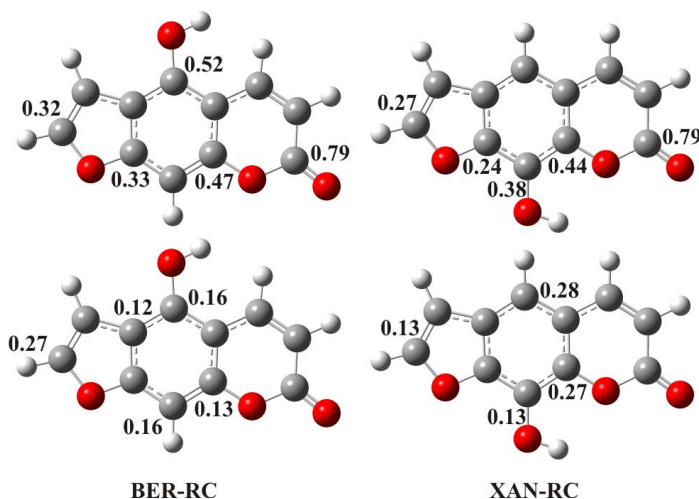


Fig. 5. The optimized structure of bergaptole (left) and xanthoxol (right) radical cations in water with natural spin (above) and charge (down) distribution

The calculated PA values of both OH groups of investigated compounds are given in Table 2. In both solvents, the PA values are lower for the 5-OH group of bergaptole, indicating that proton transfer from this group is easier than from the 8-OH of xanthoxol. Significantly lower

PA values in polar solvents are a consequence of the interactions of OH groups and anions with the solvent molecules. The electron charge density of all anions is presented in Fig. 6. In BER-A the negative charge is delocalized over O5, C10, C6, C8 on benzene ring and C1' atoms of the furan ring, which contributes to the stability of the anion. On the other hand, in XAN-A the negative charge is delocalized over O8, C10, C5, C6 of the benzene ring and C1' atom of the furan ring. The spin density value indicates that the negative charge on O8 ($-0.84 e$) atom in the XAN-A higher than O5 ($-0.80 e$) atom in BER-A.

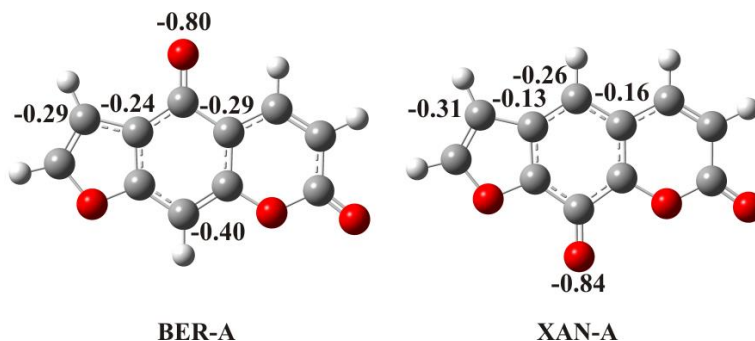


Fig. 6. The optimized structure of bergaptol (left) and xanthoxol (right) anions with values of natural charge distribution

3.3. Free radical scavenging mechanisms of investigated compounds

Reaction enthalpies related to three mechanisms of free radical scavenging activity of bergaptol and xanthoxol (HAT, SET-PT, and SPLET) were calculated according to equations listed above (11-15). The privileged mechanism of the antioxidant activity is estimated based on the BDE, IP, and PA values. The lowest of these values shows which mechanism of antiradical action is thermodynamically more favorable. One of the prerequisites for an investigated compound to be an antioxidant is stable radical that is formed after scavenging. From results presented in Table 2, it is clear that in water, PAs values of examined compounds are significantly lower than the corresponding BDE and IP values. This indicates that the SPLET mechanism represents the most probable reaction pathway of antioxidant action in polar media. In nonpolar solvent, benzene, the estimated BDE and PA values are significantly lower than than the corresponding BDE and IP values. However, the ETE values describing the second step of the SPLET mechanism are significantly large. This indicates that the HAT mechanism represents the most probable reaction pathway in the nonpolar solvent. From the significantly higher values for IP, in both solvents, it can be concluded that SET-PT is not an operational mechanism. If we compared the reactivity of the investigated compounds, it can be concluded that a higher free radical scavenging potency has BER.

3.4. Antioxidant activity of investigated compounds with different reactive oxygen species

Gibbs free energies of BER and XAN with the different reactive oxygen species: hydroxy ($\cdot\text{OH}$), hydroperoxy ($\cdot\text{OOH}$), methoxy ($\cdot\text{OCH}_3$), methyl peroxy ($\cdot\text{OOCH}_3$), vinyl peroxy ($\cdot\text{OOCHCH}_2$), iso-propoxy ($\cdot\text{OCH}(\text{CH}_3)_2$), who are related to three mechanisms of free radical scavenging activity (HAT, SET-PT, and SPLET) are presented in Table 3.

The preferred mechanism of the antiradical activity of investigated compounds can be estimated from $\Delta_r G_{\text{BDE}}$, $\Delta_r G_{\text{IP}}$, and $\Delta_r G_{\text{PA}}$ values. Namely, the lowest of these values indicate which mechanism of antiradical action is favorable.

Radicals	Compounds	Position	Benzene, $\epsilon=2.27$					Water, $\epsilon=78.35$				
			HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
			BDE	IP	PDE	PA	ETE	BDE	IP	PDE	PA	ETE
·OH	BER	5-OH	-162	334	-495	-221	59	-163	112	-276	-150	-14
	XAN	8-OH	-137	340	-478	-170	32	-150	118	-269	-121	-30
·OOH	BER	5-OH	-28	409	-437	-162	135	-28	200	-228	-102	74
	XAN	8-OH	-4	416	-419	-111	108	-15	206	-221	-73	58
·OCH ₃	BER	5-OH	-100	377	-477	-202	102	-100	173	-273	-147	47
	XAN	8-OH	-75	383	-459	-151	75	-87	179	-266	-118	31
·OOCH ₃	BER	5-OH	-19	414	-433	-158	139	-18	209	-228	-102	83
	XAN	8-OH	5	420	-415	-107	112	-6	215	-221	-73	67
·OOCHCH ₂	BER	5-OH	-26	365	-391	-116	90	-26	172	-199	-73	46
	XAN	8-OH	-2	371	-373	-65	63	-14	178	-192	-44	30
·OCH(CH ₃) ₂	BER	5-OH	-104	364	-468	-193	90	-103	170	-273	-147	44
	XAN	8-OH	-79	371	-450	-142	63	-93	176	-266	-118	28

Table 3. Calculated thermodynamic parameters of mechanisms of antioxidant activity (kJ mol^{-1}) of BER and XAN with different reactive oxygen species in benzene and water

In benzene, the $\Delta_r G_{\text{BDE}}$ and $\Delta_r G_{\text{PA}}$ values indicate that reactions with all radicals are exergonic, except in the case of $\Delta_r G_{\text{BDE}}$ for reaction with a ·OOCH₃ radical that is slightly endergonic (5 kJ mol^{-1}). Although the $\Delta_r G_{\text{PA}}$ values are exergonic, the energies $\Delta_r G_{\text{ETE}}$ who describing the second step of SPLET is significantly endergonic. For this reason, it can be concluded that in benzene the HAT is a preferred mechanism of the antiradical activity.

In the aqueous medium, the $\Delta_r G_{\text{BDE}}$ and $\Delta_r G_{\text{PA}}$ values indicate that reactions with all radicals are exergonic. While the $\Delta_r G_{\text{BDE}}$ values are similar to those in benzene, the $\Delta_r G_{\text{PA}}$ values in water are significantly lower. It is well known that when reactants or products are ionic species, the polarity of the solvent has a significant effect on the reaction course. Consequently, it is clear why the $\Delta_r G_{\text{BDE}}$ values are almost constant for certain radicals in all solvents under investigation, while $\Delta_r G_{\text{PA}}$ is not. The first step of the SPLET mechanism decreases the solvent polarity results in less stability of the reactants and leads to the $\Delta_r G_{\text{PA}}$ decrease. The negative values of $\Delta_r G_{\text{ETE}}$ for the reaction BER and XAN with the ·OH radical (-14 and -30 kJ mol^{-1} , respectively), as well as $\Delta_r G_{\text{ETE}} \leq 30 \text{ kJ mol}^{-1}$ in the reaction XAN with ·OCH₃, ·OOCHCH₂, ·OCH(CH₃)₂ radicals (31 , 30 and 28 kJ mol^{-1} respectively), indicate that SPLET and HAT mechanisms, in this case, are competitive. In reaction with other radicals in water, the HAT mechanism of antiradical action is dominant.

Expectedly, based on a previous discussion of thermodynamic parameters, BER shows a better ability to purify reactive radical species. Based on the results from Table 2, it can be concluded that in both solvents, the reactivity of the examined compounds to the reactive oxygen species decreases in sequence ·OH > ·OCH₃ > ·OCH(CH₃)₂ > ·OOH > ·OOCHCH₂ > ·OOCH₃. The $\Delta_r G_{\text{IP}}$ values reveal that SET-PT is not an operative antioxidant mechanism under these conditions. Moreover, the stability of the radical cation decreases with the decreasing solvent polarity.

3.5. Molecular docking simulation

Estrogen receptor alpha (**ER α**) is nuclear of the estrogen receptor that is activated by the sex hormone estrogen. **ER α** has important physiological functions in different organ systems including the reproductive, central nervous, skeletal, and cardiovascular systems (Katzenellenbogen et al., 2000). For this reason, this receptor is widely distributed in the human body, including the female reproductive organs (uterus and ovary), male reproductive organs, mammary glands, hypothalamus, heart, kidney, spleen, pituitary gland, liver, lung, bone, and adipose tissue. In addition to the wide role and prevalence in important physiological processes, estrogen is also involved in the development and progression of various diseases, including various cancers (breast, ovarian, colorectal), prostate, endometrium, osteoporosis, neurodegenerative diseases, cardiovascular diseases, insulin resistance, lupus erythematosus, endometriosis, and obesity. In these abnormalities, estrogen mediates its effects through the estrogen receptor, which is the basis for many therapeutic interventions (Jia et al., 2015).

To predict the inhibitory effect of the investigated compounds according to the **ER α** receptor the molecular docking study was applied. Based on the previously discussed values, it was concluded that the first step of the SPLET mechanism, the deprotonation process, is thermodynamically favorable. For this reason, a molecular docking study was also performed with the corresponding anionic species.

The potential ligand binding sites, as well as important thermodynamic parameters of protein-ligand complex, were determined from this study. The thermodynamic parameters obtained after the molecular docking simulation are free energy of binding (ΔG_{bind}) and constant of inhibition (K_i). Additionally, the free energy of binding involves different contributions such as final total internal energy (ΔG_{total}), torsional free energy (ΔG_{tor}), unbound system's energy (ΔG_{unb}), electrostatic energy (ΔG_{elec}) and the sum of dispersion and repulsion (ΔG_{vdw}), hydrogen bond (ΔG_{hbond}) and desolvation (ΔG_{desolv}). These values can help in a better understanding of the binding mode of the investigated compound in the active site of the receptor. The importance of these parameters is described in detail in the previous research (Avdović et al., 2020).

Conformation s	ΔG_{bind} kJ mol ⁻¹	K_i (μM)	ΔG_{inter} kJ mol ⁻¹	$\Delta G_{\text{vdw+hbond+desolv}}$ kJ mol ⁻¹	ΔG_{elec} kJ mol ⁻¹	ΔG_{total} kJ mol ⁻¹	ΔG_{tor} kJ mol ⁻¹	ΔG_{unb} kJ mol ⁻¹
ERα-BER	-29.7	6.4	-30.9	-29.5	-1.3	0.3	1.3	0.3
ERα-BER-A	-28.7	9.6	-28.7	-27.9	-0.7	0.0	0.0	0.0
ERα-XAN	-27.8	13.4	-29.1	-27.7	-1.4	-3.0	1.3	-3.0
ERα-XAN-A	-27.6	6.6	-29.1	-27.8	-1.8	0.0	0.0	0.0

Table 4. Important thermodynamic parameters obtained after molecular docking simulation

If one compared the most stable conformations of investigated species in the active site of the receptor, the binding affinity decreases in sequence **ER α -BER** > **ER α -BER-A** > **ER α -XAN** > **ER α -XAN-A**. The best confirmation of the investigated compound, **ER α -BER**, have the most negative value for the free energy of binding (-29.7 kJ mol⁻¹) and the lowest value for the constant of inhibition (6.4 μM). Both compounds in their neutral form show a better inhibitory effect than the corresponding anionic species. This means that the OH group contributes to the stability of the protein-ligand complex. The neutral and anionic forms of BER show a better inhibitory effect against the **ER α** receptor. This means that the OH group at position 5 of the psoralen ring contributes to a better inhibitory effect. Besides, results presented in Table 4 shows clearly that the greatest contribution to ΔG_{bind} , for all structures, comes from the sum of $\Delta G_{\text{vdw+hbond+desolv}}$. In addition, the values for ΔG_{total} , ΔG_{tor} , ΔG_{unb} in complexes **ER α -BER-A** and **ER α -XAN-A** are 0.0 kJ mol⁻¹ because of the rigidity of anionic species.

The most stable docking structures of neutral and anionic species into **ER α** receptor are presented at Figure 7, while results collected in Table 5 represent the type of interactions and corresponding distances.

ERα-BER			ERα-BER-A		
Interactions:	Types of interactions:	Å	Interactions:	Types of interactions:	Å
LEU327:HN-BER:O	Conventional Hydrogen Bond	1.90	LEU327:HN – BER-A:O	Conventional Hydrogen Bond	1.96
BER:H - GLU353:O	Conventional Hydrogen Bond	1.71	ILE326:C – BER-A:O	Carbon Hydrogen Bond	3.17
ILE326:C - BER:O	Carbon Hydrogen Bond	3.10	LYS449:H – BER-A	Pi-Cation	2.78
GLU353:O - BER	Pi-Anion	2.97	GLU353:O – BER-A	Pi-Anion	3.04
A:TRP360 - BER	Pi-Pi T-shaped	5.09	TRP360 – BER-A	Pi-Pi T-shaped	5.16
BER - TRP360	Pi-Pi T-shaped	5.05	BER-A - TRP360	Pi-Pi T-shaped	5.12
BER - PRO324	Pi-Alkyl	3.63	BER-A - PRO324	Pi-Alkyl	3.84
BER - PRO324	Pi-Alkyl	3.83	BER-A - PRO324	Pi-Alkyl	3.62
BER - PRO324	Pi-Alkyl	4.74	BER-A - PRO324	Pi-Alkyl	4.17
BER - MET357	Pi-Alkyl	4.66	BER-A - MET357	Pi-Alkyl	4.43
ERα-XAN			ERα-XAN-A		
LEU327:HN - XAN:O	Conventional Hydrogen Bond	2.02	LYS449:H – XAN-A:O	Conventional Hydrogen Bond	1.90
LYS449:H - XAN:O	Conventional Hydrogen Bond	1.88	LYS449:H – XAN-A:O	Conventional Hydrogen Bond	2.19
ILE326:C - XAN:O	Carbon Hydrogen Bond	3.24	GLY390:C – XAN-A:O	Carbon Hydrogen Bond	3.67
GLU353:O - :XAN	Pi-Anion	3.15	ARG394:NH – XAN-A	Pi-Cation	3.30
TRP360 - :XAN	Pi-Pi T-shaped	4.74	TRP360-XAN-A	Pi-Pi T-shaped	5.73
XAN - TRP360	Pi-Pi T-shaped	4.69	TRP360-XAN-A	Pi-Pi T-shaped	5.88
XAN - PRO324	Pi-Alkyl	3.49	XAN-A - PRO324	Alkyl	4.08
XAN - PRO324	Pi-Alkyl	3.80	XAN-A - PRO324	Pi-Alkyl	3.57
XAN - PRO324	Pi-Alkyl	4.52	XAN-A - ILE326	Pi-Alkyl	4.60
XAN - MET357	Pi-Alkyl	3.15			

Table 5. Types of interactions and corresponding distances obtained after molecular docking simulation

By analyzing the most stable conformations of **BER** and **BER-A** in the **ER α** receptor, it can be concluded that the tested species are surrounded by identical amino acid residues. Despite the polar oxygen atoms, there is a small number of hydrogen bonds that are most significant in the interpretation of protein-ligand interactions.

In the **ER α -BER** protein-ligand complex, the amino acid GLU 353 establishes a conventional hydrogen bond via an oxygen atom of the carbonyl group to the hydrogen atom of the OH group of BER (1.71Å). This contact in the **ER α -BER-A** complex was missing. In addition, in **ER α -BER** and **ER α -BER-A** complexes the amino acid LEU 327, via the NH group, forms a hydrogen bond with the carbonyl oxygen of the lactone ring with different distances. An interesting carbon hydrogen bond is formed between amino acid ILE 326 and the oxygen atom of the lactone ring of **BER** and **XAN**. In addition, the amino acid GLU 353 forms an electrostatic π -anion interaction, through a partial negative oxygen atom (O^-) of the carbonyl group with the chroman ring of investigated compounds. Additionally, in the **ER α -BER-A** complex, amino acid LYS 449, via a partially positive NH^+ group, establishes an electrostatic π -cation interaction with the aromatic furan ring. In both complexes, the most common type of interactions are hydrophobic interactions. Furan ring of investigated compounds establishes weak π - π -T contacts with the aromatic indole ring of the amino acid TRP 360. Shorter and

slightly stronger domain π -alkyl contacts are established between aromatic and partial aromatic rings of investigated compounds and amino acid PRO 324.

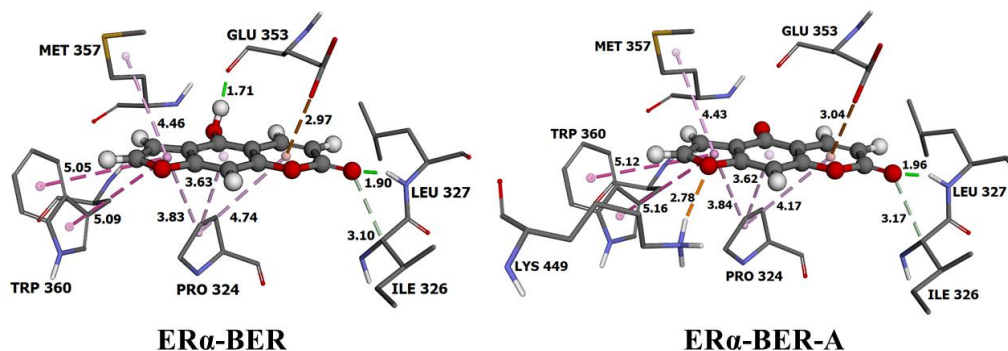


Fig.7. The most stable docking structures of **BER** and **BER-A** into **ER α**

On the other hand, in **ER α -XAN** and **ER α -XAN-A** complexes (Fig.8) amino acids LEU 327 and LYS 449, via hydrogen atom of NH group, establish hydrogen bond with the oxygen atom of chroman and furan ring, respectively. Hydrogen bond furcation is a ubiquitous phenomenon in macromolecular structures. A donor can interact with several acceptors simultaneously or an acceptor can interact simultaneously with many donors. The terms bifurcated and trifurcated are commonly used to describe these arrangements. The amino acid LYS 449 across hydrogen atoms of $-\text{NH}_2$ groups, build bifurcated geometry with oxygen atoms O1 (1.90 Å) and O9 (2.90 Å) of pyron ring of XAN-A. The amino acid ILE 326 in **ER α -XAN** and GLY 390 in **ER α -XAN-A** establish carbon hydrogen bond with O1 atom of chroman ring and O9 atom of the aromatic ring, respectively. In this case, electrostatic interactions contribute to the total binding energy. In **ER α -XAN** complex amino acid GLU 353 establish π -cation interaction with partial aromatic lactone ring, while amino acid ARG 394 in **ER α -XAN-A** formed π -anion interaction with aromatic furan ring. Besides, aromatic rings of investigated compounds in both complexes are included in the stabilization by hydrophobic π -alkyl and π - π -T interactions with amino acids PRO 324, TRP 326, MET 357, LYS 449.

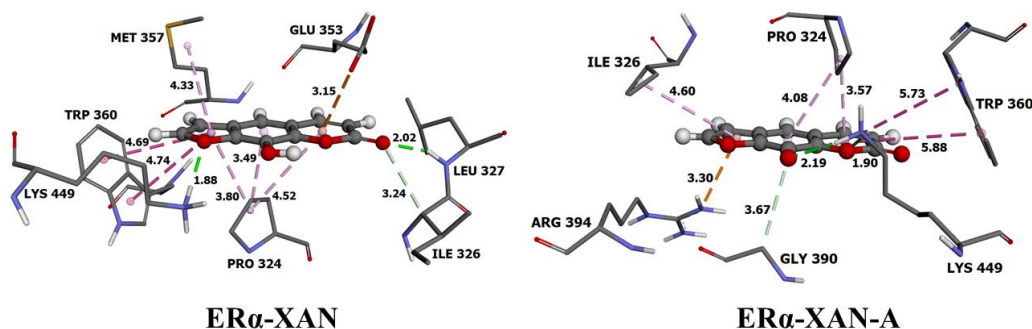


Fig. 8. The most stable docking structures of **XAN** and **XAN-A** into **ER α**

4. Conclusions

Based on the calculated thermodynamic parameters, we conclude the examined compounds exhibit antioxidant properties through the HAT mechanism that in benzene. In water, a competition between the HAT and SPLET mechanisms of antioxidant action is present in both investigated compounds. Inactivation of ROS with reactive oxygen species (ROS), the possibility and manner of elimination depend on the reactivity of the oxygen species and of the type of solvent. The ability to remove radicals has been shown to decrease in the series $\cdot\text{OH} > \cdot\text{OCH}_3 > \cdot\text{OCH}(\text{CH}_3)_2 > \cdot\text{OOH} > \cdot\text{OOCHCH}_2 > \cdot\text{OOCH}_3$. It also can be concluded that, in both solvents, bergaptol exhibits better antioxidant properties than xanthotoxol. This is due to the stabilization by hydrogen bond in the xanthotoxol structure. The molecular docking simulation results indicate that the neutral forms of both compounds show a better inhibitory effect than the corresponding anionic species. On the other hand, both investigation bergaptol forms (neutral and anionic) show slightly higher inhibitory activity toward the ER α receptor than xanthotoxol. In summary, the OH group at position 5 has a more significant effect on the antioxidant and biological properties of bergaptol than OH group at position 8 of xanthotoxol.

Acknowledgments: This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreements No. 451-03-68/2020-14/200122; 451-03-68/2020-14/200378).

References

- Apel K., Hirt H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, 55, 373-399.
- Avdović E. H, Milanović Ž. B, Živanović M. N, Šeklić D. S, Radojević I. D, Čomić L. R, Marković Z. S. (2020). Synthesis, spectroscopic characterization, biological activity, DFT and molecular docking study of novel 4-hydroxycoumarine derivatives and corresponding palladium (II) complexes. *Inorganica Chimica Acta*, 504, 119465.
- Bian S, Jiang Y. (2009). Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. *Scientia Horticulturae*, 120(2), 264-270.
- Devienne K. F, Cálgaro-Helena A. F, Dorta D J., Prado I. M, Raddi M. S. G, Vilegas W, Curti C. (2007). Antioxidant activity of isocoumarins isolated from *Paepalanthus bromelioides* on mitochondria. *Phytochemistry*, 68(7), 1075-1080.
- Dimić D, Milenković D, Dimitrić Marković J, Marković Z. (2017). Antiradical activity of catecholamines and metabolites of dopamine: Theoretical and experimental study. *Physical Chemistry Chemical Physics*, 19(20), 12970-12980.
- Đorović J, Marković J. M. D, Stepanić V, Begović N, Amić D, Marković Z. (2014). Influence of different free radicals on scavenging potency of gallic acid. *Journal of molecular modeling*, 20(7), 2345.
- Frisch M. J, Trucks G. W, Schlegel H. B, Scuseria G. E, Robb M. A, Cheeseman J. R, Millam J. M. (2003). Revision B. Gaussian. Inc., Pittsburgh PA.
- Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. (2014). SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic acids research*, 42(W1), W32-W38.
- Girenavar B, Jayaprakasha G. K, Jadegoud Y, Gowda G. N, Patil B. S. (2007). Radical scavenging and cytochrome P450 3A4 inhibitory activity of bergaptol and geranylcoumarin from grapefruit. *Bioorganic & medicinal chemistry*, 15(11), 3684-3691.

- Jia M, Dahlman-Wright K, Gustafsson J Å. (2015). Estrogen receptor alpha and beta in health and disease. *Best practice & research Clinical endocrinology & metabolism*, 29 (4), 557-568.
- Karelson M, Lobanov V. S, Katritzky A. R. (1996). Quantum-Chemical Descriptors in QSAR/QSPR Studies. *Chemical Reviews*, 96(3), 1027–1044.
- Katzenellenbogen B. S, Katzenellenbogen J. A. (2000). Estrogen receptor transcription and transactivation Estrogen receptor alpha and estrogen receptor beta: regulation by selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Research*, 2(5), 335.
- Klein E, Lukeš V, Ilčin M. (2007). DFT/B3LYP study of tocopherols and chromans antioxidant action energetics. *Chemical physics*, 336(1), 51-57.
- Kostova I, Bhatia S, Grigorov P, Balkansky S. S, Parmar V, K Prasad, A, Saso L. (2011). Coumarins as antioxidants. *Current medicinal chemistry*, 18(25), 3929-3951.
- Leopoldini M, Marino T, Russo N, Toscano M. (2004). Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. *The Journal of Physical Chemistry A*, 108(22), 4916-4922.
- Liou G. Y, Storz P. (2010). Reactive oxygen species in cancer. *Free radical research*, 44(5), 479-496.
- Marković Z. S, Dimitrić-Marković J. M, Milenković D, Filipović N. (2011). Structural and electronic features of baicalein and its radicals. *Monatshefte für Chemie-Chemical Monthly*, 142(2), 145-152.
- Morris G. M, Huey R, Lindstrom W, Sanner M. F, Belew R. K, Goodsell D. S, Olson A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*, 30(16), 2785-2791.
- Pathak M. A, Daniels Jr F, Fitzpatrick T. B. (1962). The presently known distribution of furocoumarins (psoralens) in plants. *Journal of investigative Dermatology*, 39(3), 225-239.
- Rajan V. K, Muraleedharan K. (2017). A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol, Gallic acid. *Food Chemistry*, 220, 93–99.
- Shahidi F, Janitha P. K, Wanasundara P. D. (1992). Phenolic antioxidants. *Critical reviews in food science & nutrition*, 32(1), 67-103.
- Shiau A. K, Barstad D, Loria P. M, Cheng L, Kushner P. J, Agard D. A, Greene, G. L. (1998). The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell*, 95(7), 927-937.
- Takano Y, Houk K. N. (2005). Benchmarking the conductor-like polarizable continuum model (CPCM) for aqueous solvation free energies of neutral and ionic organic molecules. *Journal of Chemical Theory and Computation*, 1(1), 70-77.
- Tošović J, Marković S. (2018). Reactivity of chlorogenic acid toward hydroxyl and methyl peroxy radicals relative to trolox in nonpolar media. *Theoretical Chemistry Accounts*, 137(6), 76.
- Turrens J. F. (2003). Mitochondrial formation of reactive oxygen species. *The Journal of physiology*, 552(2), 335-344.
- Wang L, Yang F, Zhao X, Li Y. (2019). Effects of nitro- and amino-group on the antioxidant activity of genistein: A theoretical study. *Food Chemistry*, 275, 339–345.
- Zhao Y, Truhlar D. G. (2008). 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*, 120(1-3), 215-241.