

Theoretical study on the antioxidant properties of 2'-hydroxychalcones: H-atom vs. electron transfer mechanism

Yunsheng Xue · Youguang Zheng · Ling Zhang ·
Wenya Wu · Ding Yu · Yi Liu

Received: 25 April 2013 / Accepted: 12 June 2013 / Published online: 26 June 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract The free radical scavenging activity of six 2'-hydroxychalcones has been studied in gas phase and solvents using the density functional theory (DFT) method. The three main working mechanisms, hydrogen atom transfer (HAT), stepwise electron-transfer-proton-transfer (ET-PT) and sequential-proton-loss-electron-transfer (SPLET) have been considered. The O-H bond dissociation enthalpy (BDE), ionization potential (IP), proton affinity (PA) and electron transfer energy (ETE) parameters have been computed in gas phase and solvents. The theoretical results confirmed the important role of the B ring in the antioxidant properties of hydroxychalcones. In addition, the calculated results matched well with experimental values. The results suggested that HAT would be the most favorable mechanism for explaining the radical-scavenging activity of hydroxychalcone in gas phase, whereas SPLET mechanism is thermodynamically preferred pathway in aqueous solution.

Keywords DFT · Hydrogen atom transfer · Hydroxychalcones · Radical scavenging · Sequential-proton-loss-electron-transfer · Stepwise electron-transfer-proton-transfer

Electronic supplementary material The online version of this article (doi:10.1007/s00894-013-1921-x) contains supplementary material, which is available to authorized users.

Y. Xue · Y. Zheng · L. Zhang · Y. Liu
Chemical and Biological Pharmaceutical Engineering Research
Center, Xuzhou Medical College, No.209, Tongshan Road,
Xuzhou, Jiangsu 221004, China

Y. Xue (✉) · Y. Zheng · L. Zhang · W. Wu · D. Yu · Y. Liu (✉)
School of Pharmacy, Xuzhou Medical College, No.209,
Tongshan Road, Xuzhou, Jiangsu 221004, China
e-mail: xueyunsheng@xzmc.edu.cn
e-mail: njuliuyi2003@gmail.com

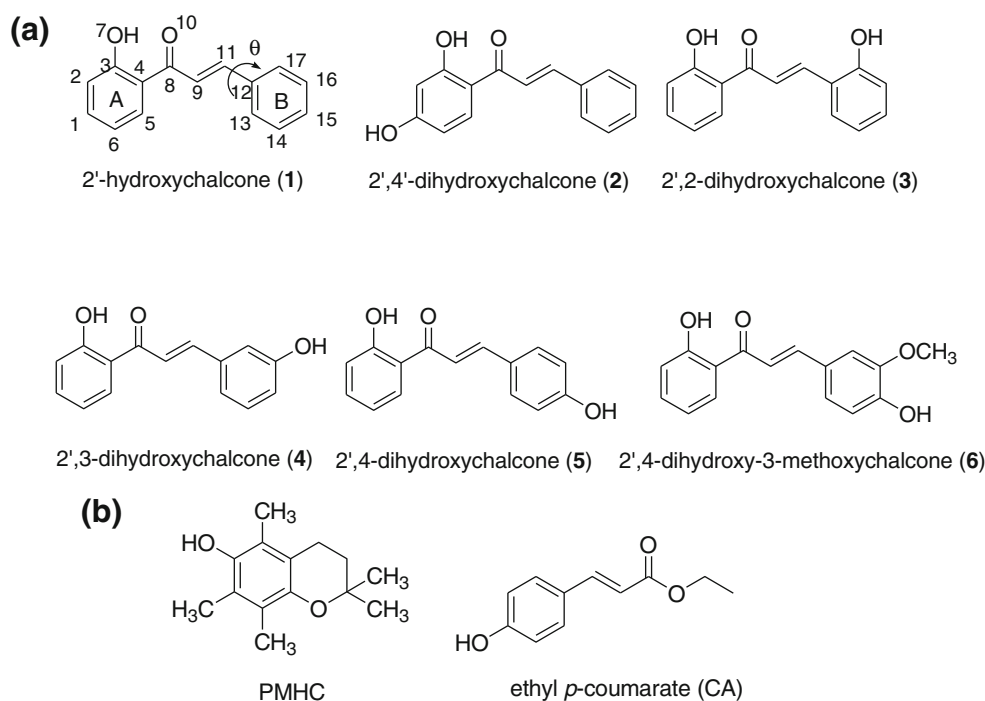
Introduction

Oxidative stress induced by free radicals can cause damage to cellular proteins, membrane lipids and nucleic acids. This process has been implicated in the pathogenesis of aging and other diseases such as cancer, atherosclerosis, and Alzheimer's disease [1, 2]. Therefore, free radicals and related species have attracted a great deal of attention in recent years. Phenolic antioxidants are a potent compound applied in the fields of commerce and biology to inhibit the material oxidation. The antioxidant capacity of phenolic compounds has long been recognized for their strong chain-breaking actions and ability to scavenge radicals, thereby protecting cells against the detrimental effects of reactive oxygen species [3]. Structure activity relationships of their antioxidant capacity have been experimentally established and rationalized on the basis of quantum chemical studies over the past years [4–13]. It was universally considered that the radical scavenging abilities of these phenolic compounds depend greatly on the number and arrangement of phenolic hydroxyl groups.

Chalcones (*trans*-1,3-diaryl-2-propen-1-ones, Fig. 1), belonging to flavanoid family are both biosynthetic precursors of flavonoids and end products associated with a variety of biological activities [14]. Chalcones continue to attract considerable interest due to their broad spectrum of pharmacological activities, including antimalarial [15, 16], anticancer [17], anti-inflammatory [18, 19], antibacterial [20], antifilarial [21], antifungal [22], antimicrobial [23], larvicidal [24], anticonvulsant [25] and antioxidant [26, 27] activities. In addition, they have also shown inhibition of the enzymes, especially mammalian alpha-amylase [28], cyclo-oxygenase [29] and monoamine oxidase [30].

Chalcones have a diverse array of substituents on the two aromatic rings. Among the various substituents, the hydroxyl substituent is widespread among natural and synthesised chalcones. In the last decades, a large number of reports have been published on the beneficial effects of hydroxychalcones,

Fig. 1 Molecular structures of the studied hydroxychalcones **1–6** (a) and related compounds (b)



especially the antioxidant activity [31–37]. These experimental studies have demonstrated that hydroxychalcones are efficient for the scavenging of various radicals. Moreover, some of them, such as 2,3,4,6-tetrahydroxychalcone, exhibit more potent radical-scavenging activity than vitamin C and α -tocopherol [35].

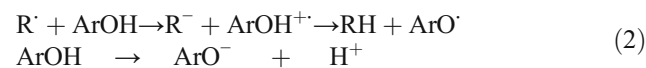
To elucidate the radical-scavenging activity of chalcones, a few theoretical studies mainly based on DFT have also been performed. Mosquera et al. [38] calculated O–H bond dissociation energies, ionization potentials and quantum theory of atoms in molecules (QTAIM) atomic and bond properties of butein and isoliquiritigenin at the B3LYP/6-311++G(2d,2p) level. It was found that the two hydroxychalcones bear a significant radical-scavenging activity similar to those of natural hydroxybenzoic and cinnamic acids. The redox properties of a series of hydroxychalcones have been investigated by Cotelle et al. [39] using cyclic voltammetry method and theoretical calculation. Kozłowski and co-workers [40] evaluated the O–H bond dissociation enthalpy (BDE) and the ionization potential (IP) in a series of natural hydroxychalcones by B3P86/6-31+G(d,p) calculations. Their results indicated the importance of the H atom transfer mechanism to explain their capacity to scavenge the free radicals. Furthermore, the active sites were identified as the 6'-OH group and the 3,4-dihydroxy-catechol. The theoretical study by Chen et al. [41] showed that the 2,4,3',4'-tetrahydroxychalcone (butein) can serve as a powerful antioxidant against DPPH radical.

Recently, a series of 2'-hydroxychalcones were synthesized by Ohkatsu and Satoh [42], and the effects of hydroxyl group(s) on the antioxidant activity were investigated. To gain

a deeper insight into the structure and antioxidant activity relationship and as a continuation of our previous works concerning the SARs of chalcones [43, 44], the objective of this study is to investigate the structure–radical scavenging activity relationship of these hydroxychalcones at DFT level, in order to provide new clue for antioxidant development. The thermodynamic descriptors such as O–H bond dissociation enthalpy (BDE), adiabatic ionization potential (IP), proton affinity (PA), and electron transfer enthalpy (ETE) were obtained to examine the HAT, ET-PT, and SPLET mechanisms. In addition, the influence of the polarity of solvent on the reactivity is also studied.

Theoretical and computational methods

Three mechanisms are commonly proposed to explain radical scavenging processes of phenolic antioxidants [6, 10, 45, 46]: (1) a direct H atom transfer (HAT); (2) stepwise electron-transfer-proton-transfer (ET-PT); and (3) sequential-proton-loss-electron-transfer (SPLET).



In HAT, the antioxidant, ArOH, reacts with the free radical, R^{\bullet} , by transferring to it a hydrogen atom, through

homolytic rupture of the O–H bond. The reactivity of an ArOH can be estimated by calculating the O–H BDE, where the lower the BDE value the higher the expected activity. The ET-PT mechanism provides for an electron to be donated to the R \cdot . In this case, the IP is the most significant energetic factor for the scavenging activity evaluation. Molecules with the low IP values are expected to have high activity. The PA is involved in mechanism 3, where the ETE of ArO $^-$ is another controlling parameter. Thus, in the present study BDE, IP, PA and ETE values were used as the main molecular descriptors to elucidate the radical scavenging activity of the investigated compounds.

The molecular enthalpy at 298 K is consisted of B3LYP/6-311++G(2d,2p) calculated single point energy (SPE) values and B3LYP/6-31G(d) thermal contributions to enthalpy (TCE, in which the vibrational contributions include zero-point vibrational energy). Then, the BDE values were calculated according to the formula $BDE = H_r + H_h - H_p$, where H_r is the enthalpy of the radical generated by H-abstraction, H_h is the enthalpy of the H-atom, and H_p is the enthalpy of the parent molecule. The exact value of the electronic energy of the H atom (0.5 hartree) was used instead of the prediction by DFT method. The enthalpy of the H-atom at 298.15 K, including the translational and PV corrections, was 0.49764 hartree [10]. The IP values of neutral and anion forms were obtained according to the equation $IP = E_{cr} - E_p$ and $ETE = E_r - E_{an}$, respectively, where cr and an indicate the cation and anion radical. Zero-point energy (ZPE) was added to the electronic energy to obtain E_0 at 0 K. The PA of ArOH was calculated from the following equation, $PA = H_{an} + H_H^+ - H_p$, where H_{an} and H_H^+ are the enthalpy of anion radical and proton, respectively. As previously proposed [47] gas-phase corrections were employed to calculate these parameters in the aqueous phase, since the vibrational frequencies calculation with polarized continuum model (PCM) is computationally very demanding (and not very accurate) and thermal corrections are expected to be rather similar in the gas and solvated phases.

All calculations were performed by using Gaussian03 software package [48]. Density functional theory (DFT) method combining with B3LYP hybrid density functional [49, 50] has been applied because of its successful application in the studies of radical scavenging mechanisms [51–56]. To find the starting geometries and structures with minimum energy, a conformational analysis was performed. A fully relaxed potential energy scan was carried out against the dihedral angle C9–C11–C12–C13 at B3LYP/6-31G(d) level. After getting the minimum energy conformations, a further geometry optimization was performed at the same level of theory. Vibrational frequencies of the optimized structures were computed using the same level of theory and thermodynamic corrections were obtained at 298 K and 1 atm, and added to electronic energies. Single point energy (SPE) calculations were evaluated at B3LYP/6-

311++G(2d,2p) level using the B3LYP/6-31G(d) geometries and used to refine electronic energies.

The distribution and energy of HOMO orbitals and the spin density of the radicals were determined by using the (U)B3LYP/6-31G(d) level of theory for the fully optimized structure of the compounds. As the physiological medium of human living cells is water, the influence of the solvation effects on the antioxidant activity was taken into account by using the polarized continuum model (PCM) [57] at B3LYP/6-311++G(2d,2p) level of theory. Since in biological conditions antioxidants may also act in lipid membranes, this influence was also estimated using the benzene dielectric constant ($\epsilon = 2.27$). The spin contamination of the UHF wave functions of the radical species ArO \cdot and ArOH $^{+\cdot}$ was generally small ($\langle S^2 \rangle = 0.76$ – 0.79) regardless of phase.

Results and discussion

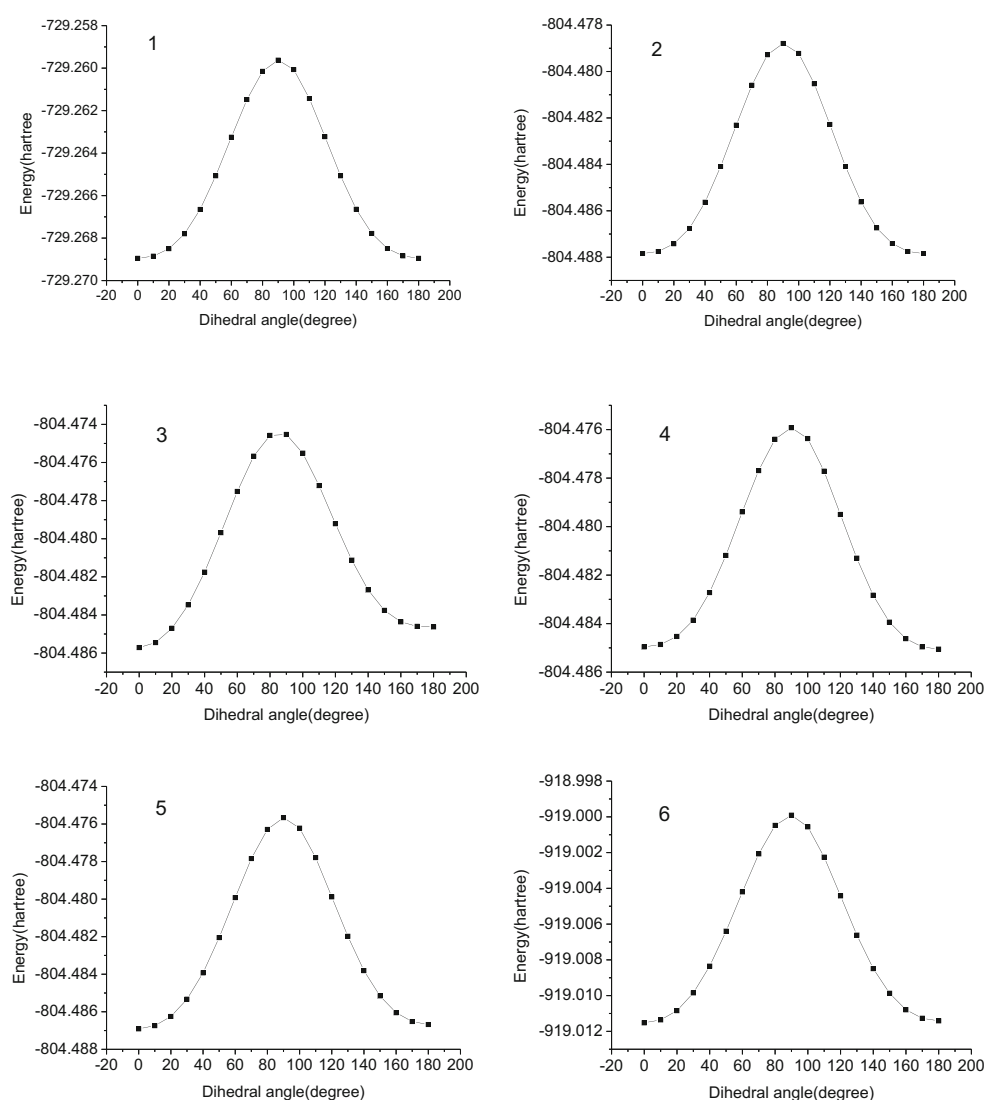
Conformational analysis and equilibrium geometries

The knowledge of the conformational and geometrical features of the studied compounds is of crucial importance to understand the relationship between the molecular structure and antioxidant activity. Chalcones exist as either the *trans*- or the *cis*-isomers, the *trans*-isomer being in most cases the thermodynamically most stable form and consequently, the majority of chalcones is isolated as the *trans*-isomer [58]. *S-cis* conformation for the O=C–C α =C β has also been found as the most stable conformers from our previous studies [43, 44, 59, 60]. Thus, in this study, we decided to focus on the *trans-s-cis* conformer for compounds 1–6.

Potential energy curves for the 2'-hydroxychalcones as a function of dihedral angle θ around the C11–C12 bond have been characterized exploring in steps of 10 $^\circ$ values from 0 $^\circ$ –180 $^\circ$ at B3LYP/6-31G(d) level of theory in vacuo. The plots of the potential energy profile are shown in Fig. 2. The energies of the main stationary point (M1, M2 and TS) are listed in Table S1 in Supporting information. As can be seen from Fig. 2 and Table S1, the most stable structures of 1–6 are formed at $\theta = 0^\circ$ followed by a relative minimum at $\theta = 180^\circ$ with average energy difference (ΔE_1) of 0.18 kcal mol $^{-1}$ after removing the torsion angle constraint. The potential energy (ΔE_2) is in the range of 5.73–7.28 kcal mol $^{-1}$. These results indicate that all the molecules can exist in two conformations that are practically isoenergetic.

Based on the minimum energy conformations obtained from the energy scan, further geometry optimization was performed with the B3LYP/6-31G(d) method. The optimized structures of hydroxychalcones 1–6 are presented in Fig. 3. All the conformers obtained here present *trans* arrangement around the C9=C11 bond (Fig. 1) and in the most stable conformer of each molecule dihedral angles C4–C8–

Fig. 2 Potential energy curves of hydroxychalcones **1–6** calculated at the B3LYP/6-31G(d) level in gas phase



C9=C11 and C8-C9=C11-C12 are antiperiplanar. An intramolecular hydrogen bond (IHB) between the carbonylic oxygen and the 2'-hydroxylic hydrogen was found in each molecule (Fig. 3). The resulting hydrogen bond lengths for **1–6** are about 1.64 Å. These IHBs are expected to favor coplanarity among the A ring, enone system and the B ring.

The optimized geometrical parameters for neutral molecules and their radicals were given in Tables S2-S4 in Supporting information. From the data of bond distances and bond angles, it can be seen that no significant geometrical change has been observed when going from the neutral molecule to the phenoxy (ArO^\bullet) and cation (ArOH^{++}) radicals and the anion forms (ArO^-). Most of the bond distances are exhibiting double bond character and at the same time are shorter than single bonds. From the data of dihedral angle (Table S4), it can also be seen that the neutral hydroxychalcones **1–6** are all coplanar. When a hydrogen atom or electron was removed from the neutral form, all the phenoxy and cation radicals retained planarity and consequently conjugation is

similar to the parent molecule. As far as the anion forms are concerned, only the anions generated from the A ring of **2**, **5** and **6** are the nonplanar geometries, while other anions still retain planarity.

BDE and solvent effect

In order to obtain a classification of the radical-scavenging activity of each OH group, BDE was calculated for every radical species obtained after the removal of the H atom from each hydroxyl group. The calculated BDE values in gas phase, benzene and water are presented in Table 1.

As discussed above, the 2'-hydroxyl group in each compound forms IHB with the carbonylic oxygen. This in turn causes a higher BDE for the 2'-hydroxyl group. Table 1 clearly shows that the BDE of 2'-hydroxyl group is in the 91.9–105.3 kcal mol⁻¹ range, whereas the OH in ring B is in the 82.3–88.5 kcal mol⁻¹ range. Thus, the 2'-hydroxyl group can be neglected with respect to the H-atom donating

Fig. 3 Optimized ground state structures of hydroxychalcones **1–6** and the related compounds calculated at the B3LYP/6-31G(d) level in the gas phase

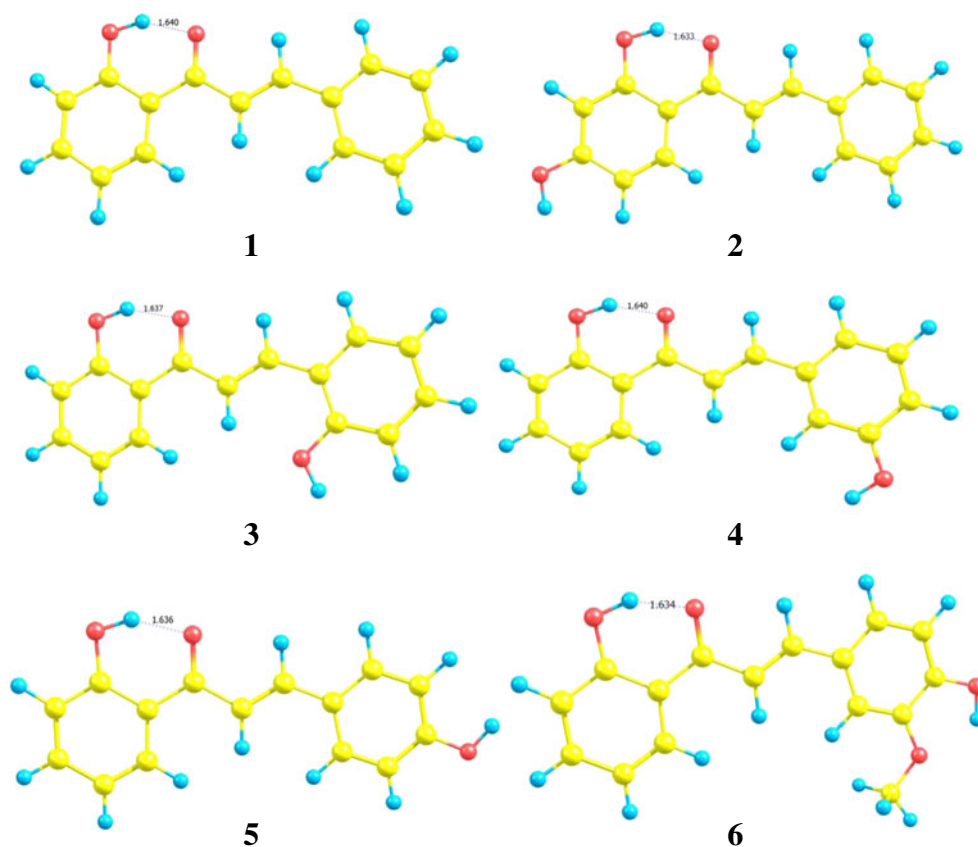


Table 1 The O-H BDE, IP, PA and ETE (in kcal mol⁻¹) values of hydroxychalcones **1–6** and the related compounds. The experimental antioxidant ratios are also included for comparison

Compound	BDE				IP		PA ^b		ETE		Antioxidant ratio(%) ^a
	Radical	Gas	Benzene	Water	Gas	Water	Gas	Water	Gas	Water	
1	2'-OH	105.3	104.2	102.7	175.9	126.7	349.7	52.0	69.0	117.6	0
2	2'-OH	105.3	104.4	103.3	177.7	142.9	349.9	53.3	69.3	117.4	0
	4'-OH	88.8	88.6	88.6							
3	2'-OH	105	103.9	102.5	174.9	140.9	352.3	52.5	66.2	116.9	69
	2-OH	82.3	83.4	85.9							
4	2'-OH	105.1	104.2	102.7	178.5	141.1	348.7	52.1	69.9	117.5	32
	3-OH	86.6	86.9	88.5							
5	2'-OH	104.9	103.9	102.6	170.8	132.4	351.3	53.1	67.7	117.0	68
	4-OH	83	83.6	85.1							
6	2'-OH	98.6	96.1	91.9	173.6	135.8	351.0	52.9	61.3	106.1	61
	4-OH	83	83.6	85.2							
CA ^c		82.6	82.3	82.1	178.8	139.0	329.8	42.5	66.4	106.7	61
Phenol		85.0 ^d			191.6 ^d						
PMHC ^c		73.1 ^d			154.2 ^d						

^a Ref. [42]

^b The enthalpy values of 1.48 and -245.05 kcal mol⁻¹ are used for H⁺ in gas phase and water, respectively [62, 67]

^c CA, ethyl *p*-coumarate; 2,2,5,7,8-pentamethyl-6-chromanol (PMHC), a model for the physiological antioxidant α -tocopherol

^d ref. [43]

reaction. From Table 1, we can also see that the BDE of 4'-OH in **2** is 88.8, 88.6 and 88.6 kcal mol⁻¹ in gas, benzene and water respectively. These values are larger than the corresponding data of OH on the B ring for **3–6**. This clearly confirms that HAT from the B ring is easier than that of the A ring, and the B ring is considered as the primary target for radical attack. This result is in line with previous studies on chalcones [40, 43, 44] and structure-activity relationship of antioxidant flavonoids [61].

It can be noted that the BDE values of 2'-OH are all significantly greater than that of phenol (85.0 kcal mol⁻¹ at this level of theory), which is generally chosen as the zero compound. Abstraction of the H atom from the hydroxyl in positions 2' implies the breaking of the hydrogen bonds formed with the adjacent carbonyl group. Hence, the hydrogen atom transfers for these 2'-OH are difficult to occur compared with phenol. In addition, the BDEs of 4'-OH (88.8 kcal mol⁻¹) is also larger than that of phenol (85.0 kcal mol⁻¹), indicating a chalcone with hydroxyl group(s) on the A ring cannot act as radical trapping compound. This is consistent with the experimental results [42], *i.e.*, compounds **1** and **2** did not show any peroxy radical trapping activity.

For compounds **2–6** possessing two phenolic hydroxyls, the one with the lowest BDE determines their radical-scavenging activities. Table 1 clearly shows that the BDEs declined in the order **1**>**2**>**4**>**5**=**6**>**3** in both gas and benzene phases, so the sequence of hydrogen donating ability of the hydroxychalcones is: **3**>**5**=**6**>**4**>**2**>**1**. In water phase, the sequence is slightly different from those in gas and benzene phases, with the order of **5**≈**6**>**3**>**4**>**2**>**1**. In addition, the BDE values of **3**, **5** and **6** are similar to those of ethyl *p*-coumarate (**CA**), indicating the activity of these three compounds might be comparable to the activity of **CA**. In general, our results are consistent with the conclusions reported by Ohkatsu and Satoh [42].

As presented in Table 1, a chalcone with a hydroxyl group on the 2- or 4-position of the B ring has higher H-atom donation ability compared with the 3-hydroxyl group. The lower activity of the latter probably due to the lower stability of the phenoxy radical given by the expansion of the conjugated system as shown in Fig. 4. As generally accepted, a molecule that can be written as several resonance structures of approximately equal energies has greater stability than does a molecule without or with less multiple resonance structures. In the case of the 2- or 4-OH-substituted chalcone, the phenoxy radical formed by hydrogen abstraction is well stabilized by the aromatic resonance (Fig. 4a), whereas the 3-OH-substituted chalcone is less stabilized (Fig. 4b).

As revealed by the BDE difference between **5** and **6** (Table 1), the introduction of the electron-donating methoxy group in the *o*-position of 4-OH (guaiacol moiety) has almost no effect on the O-H BDE of 4-OH. The BDEs of 4-OH for **4** in different media are almost equal to those of **5**. This

indicates that the presence of -OCH₃ in the ortho-position has a slightly stabilizing effect on the radicalized molecule due to the compromise between the electron-donor and electron-withdrawing capabilities of this group. This phenomenon is consistent with that of guaiacol [6].

Comparing the BDEs (Table 1) in the environments studied, we observe that in solvent medium these values are higher than in gas phase for OH in the B ring. An increase of 1.9–3.6 kcal mol⁻¹ was obtained for BDE values when computation was carried out in polar water. Smaller difference (0.3–1.1 kcal mol⁻¹) was found in the nonpolar benzene. Such a finding agrees with that reported for structurally related auronos [54] and *trans*-resveratrols [5], *i.e.*, an increasing of the BDE values in going from gas phase to solvent. Hence, it can be concluded that these media decrease the ability of the hydroxyl groups to homolytic dissociation. Contrary to the OH in the B ring, a reverse solvent effect on BDE was observed for the OH in the A ring. A decrease of 0.2–2.6 and 0.2–1.1 kcal mol⁻¹ was obtained for BDE values when computation was carried out in polar water and nonpolar benzene, respectively. These observations can be justified by the presence of strong intramolecular hydrogen bonds O7H4···O10, which are weakened more and more when the solvent polarity increases. Indeed, polar solvents ease the charge separation. Moreover, the higher the solvent polarity, the easier the separation of charges [62].

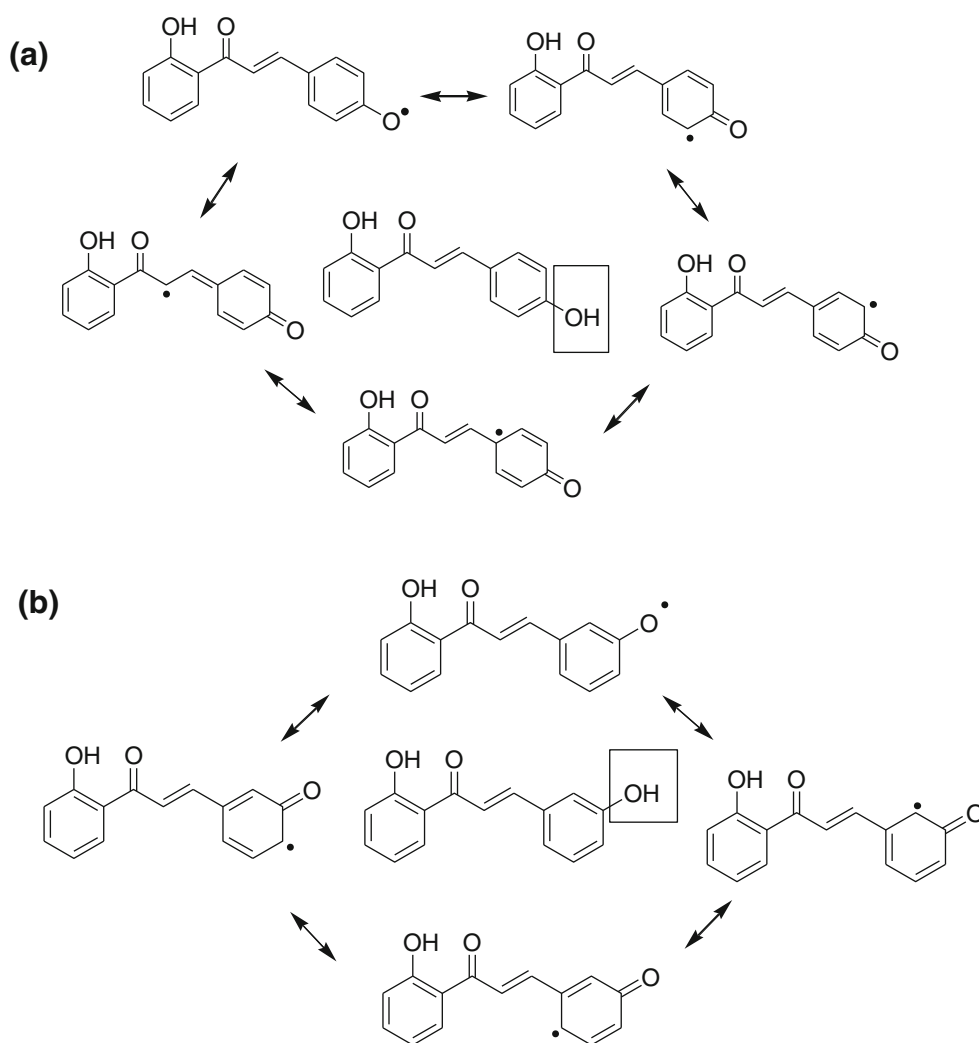
It can be seen from Table 1, the in-gas BDE values of O-H in the B ring (82.3–86.6 kcal mol⁻¹) are smaller or slightly larger than that of phenol (85.0 kcal mol⁻¹) but larger than that of α -tocopherol analogue, **PMHC** (73.1 kcal mol⁻¹) calculated at the same level of theory. This means that the H-atom donation ability of these compounds is stronger than that of phenol but weaker than that of **PMHC**. Moreover, the BDEs of O-H in the B ring for **3**, **5** and **6** are similar to or smaller than that of **CA** antioxidant (82.6 kcal mol⁻¹), indicating that these three compounds are of comparable or better hydrogen donating ability than the **CA** antioxidant.

IP and solvent effect

According to ET-PT mechanism, IP is another important physical factor indicating the range of electron donation. Low IP values are favorable to raise the electron-transfer reactivity while high IP values decrease the electron-transfer rate between antioxidant and free radicals. The IPs of hydroxychalcones **1–6** and related compounds in the gas phase as well as in water are presented in Table 1.

As can be seen from Table 1, the sequence of IP values in gas-phase is **5**<**6**<**3**<**1**<**2**<**4**, whereas the tendency of IP values in water solution is quite different: **1**<**5**<**6**<**3**<**4**<**2**. By comparison, we found that the trend for IP values is different from that of BDE values. This discrepancy can be attributed to the fact that BDE is affected by the local

Fig. 4 The resonance forms of (a) 4- and (b) 3-phenoxy radical formed by hydrogen abstraction



phenomena induced by the substituents, whereas the IP value is affected by the structure of the whole molecule [10]. In other words, within the mechanism of the electron transfer, the main factors affecting the value of IP are the extended delocalization and conjugation of the π -electrons, rather than the presence of particular functional groups.

The presence of the water medium involves a significant decrease of the absolute values of IP. For instance, the IP value of **5** that is the most active, changes from 170.8–132.4 kcal mol⁻¹, in going from the gas-phase to the water medium. This confirms that polar solvents largely ease the electron transfer. Comparatively to solvent effect on BDEs, the solvent influences the IPs drastically, since their effect is within 34.0–49.2 kcal mol⁻¹. This is not unexpected because it is well known that cation radicals are charged and they are quite sensitive to the polarity of various solvents. This result agrees with those pointed out in the literature [63, 64].

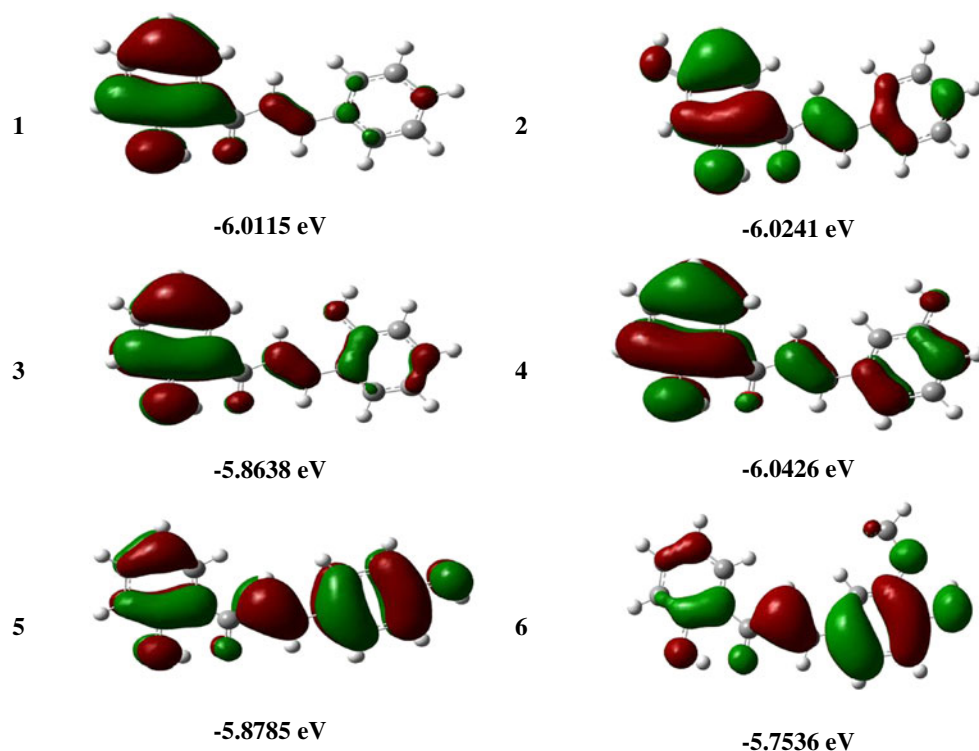
As shown in Table 1, the in-gas IPs of hydroxychalcones **1–6** are about 13~21 kcal mol⁻¹ lower than that of phenol (191.6 kcal mol⁻¹), while other flavonoids like catechin, rutin, scutellarin and baicalin are 30~40 kcal mol⁻¹ lower

than that of phenol [65]. Thus, when the electron transfer mechanism predominates for the scavenging of free radicals, hydroxychalcones **1–6** are not expected to be as efficient as other flavonoids. In addition, the IPs of **1–6** are similar to or smaller than that of **CA**, but, they are higher than that of **PMHC**. In previous literature [66], the antioxidant mechanism of α -tocopherol was certified to be H-atom transfer because it is difficult to donate electrons, thus the hydroxychalcones act as the α -tocopherol, and they are also difficult to donate electrons.

Frontier orbitals and spin density

The energy and distribution of the highest occupied molecular orbital (HOMO) are important parameters in describing the free-radical scavenging activities of phenolic antioxidants. The molecules with a higher energy of the HOMO have stronger electron donating ability. Besides, more active redox sites of antioxidant molecule are characterized by high density of the HOMO. According to the results presented in Fig. 5, compound **6** provided the highest HOMO energy, followed by **3**, **5**, **1**, **2**, **4**. This clearly indicates that **6** has the

Fig. 5 The orbital distribution and energy of HOMO for hydroxychalcones **1–6** computed at B3LYP/6-31G(d) level of theory in gas phase. *Green* and *red* regions denote positive or negative phase of orbital (isovalue=0.02)



strongest electron-donating capability among the studied compounds. Furthermore, the predicted electron-donating capability sequence according to the HOMO energies was found to be almost the same as that for the IP. As depicted in Fig. 5, HOMOs of the compounds **1–4** present similar distribution. These HOMO orbitals are mainly localized on the ring A and 3-phenylpropenal. On the contrary, the greatest contribution to the HOMO of compounds **5** and **6** mainly comes from the ring B and 3-phenylpropenal. Clearly, the HOMO distribution of phenolic oxygen atom on ring B is larger for **5** and **6** than for the others. Thus, we can anticipate that the OH groups on the B ring would be easily attacked by free radicals and other reactive agents in the real biological systems.

As local descriptor of reactivity, the spin density is often considered to be a realistic parameter and provides a better representation of the reactivity. The more delocalized spin density in the radical, the easier the radical formed and thus the lower the BDE. The importance of the spin density for the description of flavonoids has been pointed out by recent papers [5, 7–9, 43, 44, 54, 64]. Therefore, we have decided to analyze the spin density on the various radicals, in order to rationalize the differences in reactivity of the OH sites in hydrochalcones and consequently the differences in BDE.

As depicted in Fig. 6 and Fig. S1, the spin population appears to be slightly more delocalized for the radicals on the B ring than those located on the A ring. As an example, the spin density is 0.629 on the O-atom in the 2'-OH radical for **3**, whereas it is 0.577 for the 2-OH radical. Therefore, the O-H BDE is lower in the B ring than in the A ring. On the other

hand, the spin densities of **1r**, **2r-a**, **3r-a**, **4r-a** and **5r-a** radicals mainly distribute on the 2'-phenolic oxygen atom and the carbonyl group, whereas in **6r-a** radical the spin densities come mainly from the phenolic oxygen atom and the A ring. Meanwhile, the spin density on the phenolic oxygen atom is 0.434 for **6r-a**, significantly smaller than that of other 2'-phenolic radicals. This can explain why **6r-a** has lower BDE value than others.

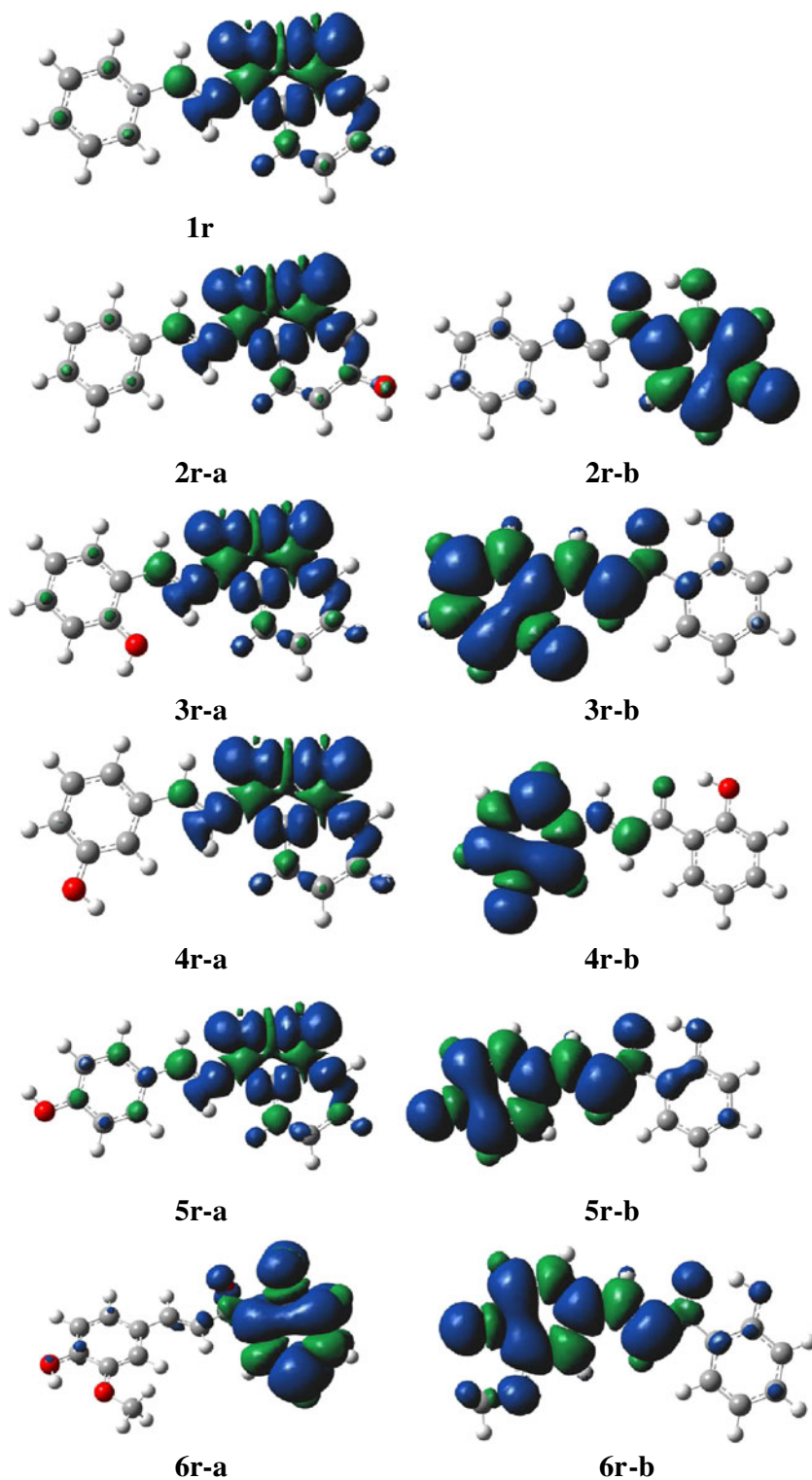
The spin population can also explain the difference between 2- or 4-OH and 3-OH in antioxidant activity. As can be seen from Fig. 6, the spin densities of **4r-b** radical mainly distribute on the phenolic oxygen atom and B ring, whereas in **3r-b**, **5r-b** and **6r-b** radicals the spin densities delocalize not only on the phenolic oxygen and the B ring, but also on the enone system. This indicates that the spin densities in **3r-b**, **5r-b** and **6r-b** radicals are more delocalized and thus these three radicals are more stable than **4r-b**. Thus, the BDE of O-H on ring B is lower in **3**, **5** and **6** than in the other molecules.

Comparing the radicals **5r-b** with **6r-b**, we find that these radicals have similar spin density distribution on the phenolic O-atom. The spin density is 0.306 and 0.344 in **5r-b** and **6r-b**, respectively (Fig. S1). This can explain why these two radicals have very similar BDE values and experimental antioxidant activities (Table 1).

Antioxidant mechanisms

According to a previous paper by Wright et al. [10], the mechanism dominating the antioxidant activity of a certain

Fig. 6 Plots of spin densities in the radicals formed by H-removal from the B and A rings for hydroxychalcones **1–6**. *Green* and *blue* regions denote positive or negative density (isovalue=0.0004)



phenolic compound can be inferred from the relative values of its IP and BDE with respect to phenol (ΔIP and ΔBDE , respectively). They believed that HAT dominates when $\Delta\text{IP} \geq -36 \text{ kcal mol}^{-1}$ and ΔBDE is around $-10 \text{ kcal mol}^{-1}$, whereas electron transfer is predominant when $\Delta\text{IP} \leq -45 \text{ kcal mol}^{-1}$. According to the results in Table 1, the ΔIP values in

gas phase are in the range of $13\text{--}21 \text{ kcal mol}^{-1}$, whereas the ΔBDE values range from -2.7 to $3.8 \text{ kcal mol}^{-1}$. Therefore, HAT mechanism may be more likely for the dominant antioxidant mechanism in the gas phase. From Table 1, it can also be seen that the calculated gas-phase BDE are significantly lower than the gas-phase IP and PA values. These

results further suggest that HAT is thermodynamically more favored than ET-PT and SPLET in the gas phase.

As can be seen from Table 1, the calculated gas-phase PA of the title molecules are significantly higher than the O-H BDE and the IP values. The PA values obtained in water are far away lower than those in the gas phase due to the large solvation enthalpies of proton [62, 67]. The differences between gas phase and water exceed 279 kcal mol⁻¹. This means solvent favors the deprotonation process. Moreover, the values of PA in water are significantly lower than the corresponding values of BDE and IP. Therefore, SPLET represents the most probable reaction pathway from the thermodynamic point of view in water. Computed ETEs of anion species are significantly lower than those of neutral species (IPs) in the two media. Accordingly, single electron transfer process from the anionic form is more preferable than that from the neutral form, which agrees with the results obtained by other studies [68–70]. Furthermore, all ETEs in water are higher than those in gas phase. In the case of water phase all ETEs are higher than PA while in the case of gas phase all ETEs are lower than PA.

In summary, among the three mechanisms, HAT is thermodynamically preferred in gas phase and SPLET is more favored in water. However, it must be stressed that the relative importance of HAT, ET-PT, or SPLET is not only determined by microenvironmental features (lipid phase, aqueous phase) but also governed by the characteristics of the scavenged radical [71].

Conclusions

In this work, B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d) theoretical method and polarizable continuum model (PCM) method have been applied to study the radical scavenging activity of a series of 2'-hydroxychalcones. The O-H BDE, adiabatic IP, PA, and ETE have been computed both in the gas phase and in solutions. In addition, other descriptors such as HOMO orbital distribution and spin density have also been discussed.

The theoretical results confirm the important role of the B ring in the antioxidant properties of hydroxychalcones. HAT from the B ring is easier than that of A ring, and the B ring is considered as the primary target for radical attack. On the basis of the obtained results, the BDE declined in the order 1>2>4>5=6>3 in both gas and benzene phases, whereas the sequence in water phase is slightly different from those in gas and benzene phases. In general, the sequence of hydrogen donating ability of the hydroxychalcones is: 3>5=6>4>2>1, which is consistent with the experimental result [42]. Our calculations also show that a chalcone with a hydroxyl group on the 2- or 4-position of the B ring has higher H-atom donation ability compared with 3-OH. The introduction of the methoxy group (OCH₃) in the o-position of 4-OH has

almost no effect on the O-H BDE. In solvent medium BDE values are higher than in gas phase for OH in the B ring, while a reverse solvent effect was observed for OH in A ring.

The sequence of IP values in gas-phase is 5<6<3<1<2<4, whereas the tendency in water is quite different: 1<5<6<3<4<2. Solvent influences the IPs drastically, with a significant decrease of the absolute values of IP. In addition, the results reveal that the trend for calculated IP values is different from that of BDE values. As expected, the IP and ETE values depend significantly on the phase because of the stabilization of charged species by polar solvents. The calculated gas-phase PA of the title molecules are significantly higher than the O-H BDE and IP values, and solvent favors the deprotonation process.

Based on the analysis above, it can be concluded that the HAT is the most favored mechanism for explaining the radical-scavenging activity of 2'-hydroxychalcone in the gas phase, whereas SPLET mechanism is the thermodynamically preferred pathway in aqueous solutions. Among the compounds studied 2',2-dihydroxychalcone (3) and 2',4-dihydroxychalcone (5) are predicted to be potential free radical scavengers and deserve further exploitation as candidates. Summarizing, our study contributes to the ongoing interest on the understanding of the antioxidant activity of chalcones for better exploitation in the field of food chemistry and pharmacy.

Acknowledgments This work was supported by the Science and Technology Plan Projects of Xuzhou (XZZD1224, XF11C037, XF11C062), Zhen Xing Project of XZMC, the Natural Science Foundation of Education Ministry of Jiangsu Province (No.11KJB350005), the Priority Academic Program Development of Jiangsu Higher Education Institutions and the Innovative Practice Training Program for Students of Jiangsu Higher Education Institutions (2011–1003). The authors are grateful to Prof. X.D. Gong (Department of Chemistry, Nanjing University of Science and Technology) for providing calculation convenience.

References

- Pieta PG (2000) Flavonoids as antioxidants. *J Nat Prod* 63:1035–1042. doi:10.1021/np9904509
- Farbstein D, Kozak-Blickstein A, Levy AP (2010) Antioxidant vitamins and their use in preventing cardiovascular disease. *Molecules* 15:8098–8110. doi:10.3390/molecules15118098
- Ndhala AR, Moyo M, Van Staden J (2010) Natural antioxidants: fascinating or mythical biomolecules? *Molecules* 15:6905–6930. doi:10.3390/molecules15106905
- Queiroz AN, Gomes BAQ, Moraes WM, Borges RS (2009) A theoretical antioxidant pharmacophore for resveratrol. *Eur J Med Chem* 44:1644–1649. doi:10.1016/j.ejmech.2008.09.023
- Mikulski D, Gomiak R, Molski M (2010) A theoretical study of the structure–radical scavenging activity of trans-resveratrol analogues and cis-resveratrol in gas phase and water environment. *Eur J Med Chem* 45:1015–1027. doi:10.1016/j.ejmech.2009.11.044

6. Leopoldini M, Russo N, Toscano M (2011) The molecular basis of working mechanisms of natural polyphenolic antioxidants. *Food Chem* 125:288–306. doi:10.1016/j.foodchem.2010.08.012
7. Anouar E, Calliste CA, Košinova P, DiMeo F, Duroux JL, Champavier Y, Marakchi K, Trouillas P (2009) Free radical scavenging properties of guaiacol oligomers: a combined experimental and quantum study of guaiacyl-moiety role. *J Phys Chem A* 113:13881–13891. doi:10.1021/jp906285b
8. Trouillas P, Marsal B, Siri D, Lazzaroni R, Duroux JL (2006) A DFT study of the reactivity of OH groups in quercetin and taxifolin antioxidants: the specificity of the 3-OH site. *Food Chem* 97:679–688. doi:10.1016/j.foodchem.2005.05
9. Leopoldini M, Pitarch IP, Russo N, Toscano M (2004) Structure, conformation, and electronic properties of apigenin, luteolin, and taxifolin antioxidants. A first principle theoretical study. *J Phys Chem A* 108:92–96. doi:10.1021/jp035901j
10. Wright JS, Johnson ER, DiLabio GA (2001) Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. *J Am Chem Soc* 123:1173–1183. doi:10.1021/ja002455u
11. Reis M, Lobato B, Lameira J, Santos AS, Alves CN (2007) A theoretical study of phenolic compounds with antioxidant properties. *Eur J Med Chem* 42:440–446. doi:10.1016/j.ejmech.2006.11.008
12. Sadasivama K, Kumaresan R (2011) A comparative DFT study on the antioxidant activity of apigenin and scutellarein flavonoid compounds. *Mol Phys* 109:839–852. doi:10.1080/00268976.2011.556576
13. Amić D, Stepanić V, Lučić B, Marković Z, Dimitrić Marković JM (2013) PM6 study of free radical scavenging mechanisms of flavonoids: why does O-H bond dissociation enthalpy effectively represent free radical scavenging activity? *J Mol Model*. doi:10.1007/s00894-013-1800-5
14. Go ML, Wu X, Liu XL (2005) Chalcones: an update on cytotoxic and chemoprotective properties. *Curr Med Chem* 12:481–499. doi:10.2174/0929867053363153
15. Awasthi SK, Mishra N, Kumar B, Sharma M, Bhattacharya A, Mishra LC, Bhasin VK (2009) Potent antimalarial activity of newly synthesized substituted chalcone analogs in vitro. *Med Chem Res* 18:407–420. doi:10.1007/s00044-008-9137-9
16. Lim SS, Kim HS, Lee DU (2007) In vitro antimalarial activity of flavonoids and chalcones. *Bull Korean Chem Soc* 28:2495–2497. doi:10.5012/bkcs.2007.28.12.2495
17. Anto RJ, Sukumarana K, Kuttana G, Raob MNA, Subbarajuv V, Kuttana R (1995) Anticancer and antioxidant activity of synthetic chalcones and related compounds. *Cancer Lett* 97:33–37
18. Kachadourian R, Day BJ, Pugazhenti S, Franklin CC, Genoux-Bastide E, Mahaffey G, Gauthier C, Di Pietro A, Boumendjel A (2012) A synthetic chalcone as a potent inducer of glutathione biosynthesis. *J Med Chem* 55:1382–1388. doi:10.1021/jm2016073
19. Yadav HL, Gupta P, Pawar RS, Singour PK, Patil UK (2011) Synthesis and biological evaluation of anti-inflammatory activity of 1,3 diphenyl propenone derivatives. *Med Chem Res* 20:461–465. doi:10.1007/s00044-010-9339-9
20. Hamdi N, Fischmeister C, Puerta MC, Valerga P (2011) A rapid access to new coumarinyl chalcone and substituted chromeno[4,3-c]pyrazol-4(1*H*)-ones and their antibacterial and DPPH radical scavenging activities. *Med Chem Res* 20:522–530. doi:10.1007/s00044-010-9326-1
21. Awasthi SK, Mishra N, Dixit SK, Singh A, Yadav M, Yadav SS, Rathaur S (2009) Antifilarial activity of 1,3-diarylpropen-1-one: Effect on glutathione-S-transferase, a phase-II detoxification enzyme. *AmJTrop Med Hyg* 80:764–768
22. López SN, Castelli MV, Zacchino SA, Domínguez JN, Lobo G, Charris-Charris J, Cortés JC, Ribas J, Devia C, Rodríguez AM, Enriz RD (2001) In vitro antifungal evaluation and structure-activity relationships of a new series of chalcone derivatives and synthetic analogues, with inhibitory properties against polymers of the fungal cell wall. *Bioorg Med Chem* 9:1999–2013. doi:10.1016/S0968-0896(01)00116-X
23. Yayli N, Ucuncu O, Yasar A, Kucuk M, Akyuz E, Karaoglu SA (2006) Synthesis and biological activities of N-alkyl derivatives of o-, m-, and p-nitro (E)-4- azachalcones and stereoselective photochemistry in solution with theoretical calculations. *Turk J Chem* 30:505–514
24. Begum NA, Roy N, Laskar RA, Roy K (2011) Mosquito larvicidal studies of some chalcone analogues and their derived products: structure–activity relationship analysis. *Med Chem Res* 20:184–191. doi:10.1007/s00044-010-9305-6
25. Kaushik S, Kumar N, Drabu S (2010) Synthesis and anticonvulsant activities of phenoxychalcones. *The Pharma Research* 3:257–262
26. Sivakumar PM, Prabhakar PK, Doble M (2011) Synthesis, antioxidant evaluation, and quantitative structure–activity relationship studies of chalcones. *Med Chem Res* 20:482–492. doi:10.1007/s00044-010-9342-1
27. Vogel S, Ohmayer S, Brunner G, Heilmann J (2008) Natural and non-natural prenylated chalcones: synthesis, cytotoxicity and anti-oxidative activity. *Bioorg Med Chem* 16:4286–4293. doi:10.1016/j.bmc.2008.02.079
28. Najafian M, Ebrahim-Habibi A, Hezareh N, Yaghmaei P, Parivar K, Larijani B (2011) Trans-chalcone: a novel small molecule inhibitor of mammalian alpha-amylase. *Mol Biol Rep* 38:1617–1620. doi:10.1007/s11033-010-0271-3
29. Zarghi A, Zebardast T, Hakimion F, Shirazi FH, Rao PN, Knaus EE (2006) Synthesis and biological evaluation of 1,3-diphenylprop-2-en-1-ones possessing a methanesulfonamido or an azido pharmacophore as cyclooxygenase-1/2 inhibitors. *Bioorg Med Chem* 14:7044–7050. doi:10.1016/j.bmc.2006.06.022
30. Chimenti F, Fioravanti R, Bolasco A, Chimenti P, Secci D, Rossi F, Yáñez M, Orallo F, Alcaro S (2009) Chalcones: a valid scaffold for monoamine oxidases inhibitors. *J Med Chem* 52:2818–2824. doi:10.1021/jm801590u
31. Calliste CA, Le Bail JC, Trouillas P, Pouget C, Habrioux G, Chulia AJ, Duroux JL (2001) Chalcones: structural requirements for antioxidant, estrogenic and antiproliferative activities. *Anticancer Res* 21:3949–3956
32. Rezk BM, Haenen GR, van der Vijgh WJ, Bast A (2002) The antioxidant activity of phloretin: the disclosure of a new antioxidant pharmacophore in flavonoids. *Biochem Bioph Res Comm* 295:9–13. doi:10.1016/S0006-291X(02)00618-6
33. Nishida J, Kawabata J (2006) DPPH radical scavenging reaction of hydroxy- and methoxychalcones. *Biosci Biotechnol Biochem* 70:193–202. doi:10.1271/bbb.70.193
34. Kim BT, O KJ, Chun JC, Hwang KJ (2008) Synthesis of dihydroxylated chalcone derivatives with diverse substitution patterns and their radical scavenging ability toward DPPH free radicals. *Bull Korean Chem Soc* 29:1125–1130. doi:10.5012/bkcs.2008.29.6.1125
35. Mohamad H, Abas F, Permana D, Lajis NH, Ali AM, Sukari MA, Hin TYY, Kikuzaki H, Nakatani N (2004) DPPH free radical scavenger components from the fruits of *Alpinia rafflesiana* Wall Ex. Bak. (Zingiberaceae). *Z Naturforsch* 59c:811–815
36. Qian YP, Shang YJ, Teng QF, Chang J, Fan GJ, Wei X, Li RR, Li HP, Yao XJ, Dai F, Zhou B (2011) Hydroxychalcones as potent antioxidants: structure–activity relationship analysis and mechanism considerations. *Food Chem* 126:241–248. doi:10.1016/j.foodchem.2010.11.011
37. Todorova IT, Batovska DI, Stamboliyska BA, Parushev SP (2011) Evaluation of the radical scavenging activity of a series of synthetic hydroxychalcones towards the DPPH radical. *J Serb Chem Soc* 76:491–497. doi:10.2298/JSC100517043T
38. Moa MJG, Mandado M, Cordeiro MNDS, Mosquera RA (2007) QTAIM electron density study of natural chalcones. *Chem Phys Lett* 446:1–7. doi:10.1016/j.cplett.2007.08.021

39. Cotellet N, Hapiot P, Pinson J, Rolando C, Vézin H (2005) Polyphenols deriving from chalcones: investigations of redox activities. *J Phys Chem B* 109:23720–23729
40. Kozłowski D, Trouillas P, Calliste C, Marsal P, Lazzaroni R, Duroux JL (2007) Density functional theory study of the conformational, electronic, and antioxidant properties of natural chalcones. *J Phys Chem A* 111:1138–1145. doi:10.1021/jp066496
41. Chen WJ, Song JR, Guo P, Wen ZY (2006) Butein, a more effective antioxidant than α -tocopherol. *J Mol Struct (THEOCHEM)* 763:161–164. doi:10.1016/j.theochem.2005.12.035
42. Ohkatsu Y, Satoh T (2008) Antioxidant and photo-antioxidant activities of chalcone derivatives. *J Jpn Petrol Inst* 51:298–308. doi:10.1627/jpi.51.298
43. Xue YS, Zhang L, Li YL, Yu D, Zheng YG, An L, Gong XD, Liu Y (2013) A DFT study on the structure and radical scavenging activity of newly synthesized hydroxychalcones. *J Phys Org Chem* 26:240–248. doi:10.1002/poc.3074
44. Xue YS, Zheng YG, An L, Qian Y, Yu D, Gong XD, Liu Y (2012) A theoretical study of the structure–radical scavenging activity of hydroxychalcones. *Comput Theoret Chem* 982:74–83. doi:10.1016/j.comptc.2011.12.020
45. Senthilkumar K, Kumaresan R (2012) A DFT study on the structural, electronic properties and radical scavenging mechanisms of calycosin, glycitein, pratensein and prunetin. *Comput Theoret Chem* 985:14–22. doi:10.1016/j.comptc.2012.01.028
46. Nenadis N, Tsimidou MZ (2012) Contribution of DFT computed molecular descriptors in the study of radical scavenging activity trend of natural hydroxybenzaldehydes and corresponding acids. *Food Res Int* 48:538–543. doi:10.1016/j.foodres.2012.05.014
47. Rezabal E, Mercero JM, Lopez X, Ugalde JM (2007) A theoretical study of the principles regulating the specificity for Al(III) against Mg(II) in protein cavities. *J Inorg Biochem* 101:1192–1200. doi:10.1016/j.jinorgbio.2007.06.010
48. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE et al. (2004) Gaussian 03, revision D.01. Gaussian, Inc Wallingford
49. Becke AD (1993) Density-functional thermochemistry. III. The role of exact exchange. *J Chem Phys* 98:5648–5652. doi:10.1063/1.464913
50. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys Rev B* 37:785–789
51. Galano A, Francisco-Marquez M (2009) Peroxyl-radical-scavenging activity of garlic: 2-propenesulfenic acid versus allicin. *J Phys Chem B* 113:16077–16081. doi:10.1021/jp907906h
52. Matxain JM, Padro D, Ristila M, Strid A, Eriksson LA (2009) Evidence of high OH radical quenching efficiency by Vitamin B6. *J Phys Chem B* 113:9629–9632. doi:10.1021/jp903023c
53. Najafi M, Mood KH, Zahedi M, Klein E (2011) DFT/B3LYP study of the substituent effect on the reaction enthalpies of the individual steps of single electron transfer-proton transfer and sequential proton loss electron transfer mechanisms of chroman derivatives antioxidant action. *Comput Theor Chem* 969:1–12. doi:10.1016/j.comptc.2011.05.006
54. Nenadis N, Sigalas MP (2008) A DFT study on the radical scavenging activity of maritimetin and related aurones. *J Phys Chem A* 112:12196–12202. doi:10.1021/jp8058905
55. Zhang HY, Sun YM, Wang XL (2003) Substituent effects on O-H bond dissociation enthalpies and ionization potentials of catechols: a DFT study and its implications in the rational design of phenolic antioxidants and elucidation of structure-activity relationships for flavonoid antioxidants. *Chem Eur J* 9:502–508. doi:10.1002/chem.200390052
56. Hernandez-Marin E, Galano A, Martínez A (2013) Cis carotenoids: colorful molecules and free radical quenchers. *J Phys Chem B*. doi:10.1021/jp401647n
57. Tomasi J, Mennucci B, Cammi R (2005) Quantum mechanical continuum solvation models. *Chem Rev* 105:2999–3094. doi:10.1021/cr9904009
58. Larsen M, Kromann H, Kharazmi A, Nielsen SF (2005) Conformationally restricted anti-plasmodial chalcones. *Bioorg Med Chem Lett* 15:4858–4861. doi:10.1016/j.bmcl.2005.07.012
59. Xue YS, Gong XD (2009) The conformational, electronic and spectral properties of chalcones: a density functional theory study. *J Mol Struct (THEOCHEM)* 901:226–231. doi:10.1016/j.theochem.2009.01.034
60. Xue YS, Mou J, Liu Y, Gong XD, Yang YH, An L (2010) An ab initio simulation of the UV/Visible spectra of substituted chalcones. *Cent Eur J Chem* 8:928–936. doi:10.2478/s11532-010-0058-3
61. Tsimidou M, Nenadis N, Zhang HY (2006) Structure radical scavenging activity relationships of flavonoids and phenolic acids. In: Boskou D, Gerothanassis I, Kefalas P (eds) *Natural antioxidant phenols. Sources, structure-activity relationship, current trends in analysis and characterization*. Research Signpost, Kerala
62. Fifen JJ, Nsangou M, Dhaouadi N, Motapon O, Jaidane N (2011) Solvent effects on the antioxidant activity of 3,4-dihydroxyphenylpyruvic acid: DFT and TD-DFT studies. *Comput Theoret Chem* 66:232–243. doi:10.1016/j.comptc.2011.03.006
63. Rimarčik J, Lukeš V, Klein E, Ilčín M (2010) Study of the solvent effect on the enthalpies of homolytic and heterolytic N–H bond cleavage in p-phenylenediamine and tetracyano-p-phenylenediamine. *J Mol Struct (THEOCHEM)* 952:25–30. doi:10.1016/j.theochem.2010.04.002
64. Leopoldini M, Rondinelli F, Russo N, Toscano M (2010) Pyranoanthocyanins: a theoretical investigation on their antioxidant activity. *J Agric Food Chem* 58:8862–8871. doi:10.1021/jf101693k
65. Ji HF, Tang GY, Zhang HY (2005) Theoretical elucidation of DPPH radical-scavenging activity difference of antioxidant xanthenes. *QSAR Comb Sci* 24:826–830. doi:10.1002/qsar.200430917
66. Leopoldini M, Marino T, Russo N, Toscano M (2004) Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. *J Phys Chem A* 108:4916–4922. doi:10.1021/jp037247d
67. Bartmess JE (1994) The thermodynamics of the electron and the proton. *J Phys Chem* 98:6420–6424. doi:10.1021/j100076a029
68. Li MJ, Liu WX, Peng CR, Ren QH, Lu WC, Deng W (2013) A DFT study on reaction of eupatilin with hydroxyl radical in solution. *Int J Quantum Chem* 113:966–974. doi:10.1002/qua.24060
69. Francisco-Marquez M, Galano A (2012) On the OH and OOH scavenging activity of 3-Methyl-1-pyridin-2-yl-5-pyrazolone: comparisons with its parent compound, Edaravone. *Int J Quantum Chem* 112:3441–3448. doi:10.1002/qua.24046
70. Perez-Gonzalez A, Galano A (2011) OH radical scavenging activity of Edaravone: mechanism and kinetics. *J Phys Chem B* 115:1306–1314. doi:10.1021/jp110400t
71. Zhang HY, Ji HF (2006) How vitamin E scavenges DPPH radicals in polar protic media. *New J Chem* 30:503–504. doi:10.1039/B600025H