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ARTICLE INFO

ABSTRACT

The free radical scavenging activity of a series of 2,4,5-trimethoxy chalcones has been computationally explored using the density functional theory (DFT) method. Three potential working mechanisms, hydrogen atom transfer (HAT), stepwise electron transfer proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET) have been investigated. The physiochemical parameters including O–H bond dissociation enthalpy (BDE), ionisation potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA) and electron transfer enthalpy (ETE) have been calculated in gas phase and solvents. The order of antioxidant efficiencies predicted theoretically in this work is in good agreement with that reported by experimental results. The results obtained demonstrate that HAT would be the most favourable mechanism in the gas and benzene phases, whereas the SPLET mechanism is the thermodynamically preferred pathway in polar media. In addition, the importance of the A-ring on the radical scavenging capabilities of chalcones was also confirmed.

1. Introduction

Cellular oxidative stress, produced by an increase in free radicals, plays a major role in the pathogenesis of ageing and other diseases, such as cancer, atherosclerosis, diabetes and Alzheimer's disease (Maritim, Sanders, & Watkins, 2003; Pieta, 2000). Therefore, the research and development of antioxidants have attracted a great deal of attention in recent years. Phenolic compounds, an important class of antioxidants, have been widely applied in the fields of commerce and biology to inhibit oxidation. Previous studies have suggested that the antioxidant capacity of phenolic compounds is mainly owing to their ability to scavenge radicals (Ndhlala, Moyo, & Van Staden, 2010). It is widely accepted that the radical scavenging abilities of these phenolic compounds depend greatly on the number and arrangement of phenolic hydroxyl groups. The nature of the scavenged radicals and solvents play an important role in the antioxidant mechanism (Alberto, Russo, Grand, & Galano, 2013; Iuga, Alvarez-Idaboy, & Russo, 2012; Leopoldini, Chiodo, Russo, & Toscano, 2011).

Chalcones (1,3-diaryl-2-propen-1-ones, Fig. 1) represent an important subgroup of the polyphenolic family. Structurally, chalcones are open-chain flavonoids bearing two aromatic rings linked by a three-carbon enone moiety. In addition, chalcones are biosynthetic precursors of flavonoids (Go, Wu, & Liu, 2005). In the last decade, chalcones have attracted great interest owing to their broad range of biological activities, such as antimalarial, anticancer, anti-inflammatory, antibacterial and antioxidant activities (Batovska & Todorova, 2010; Go et al., 2005).

To date, several reports have been published on the antioxidant activity of chalcones, especially on hydroxychalcones (Calliste et al., 2001; Chen, Song, Guo, & Wen, 2006; Kozlowski et al., 2007; Rezk, Haenen, van der Vijgh, & Bast, 2002; Todorova, Batovska, Stamboliyska, & Parushev, 2011). These experimental and theoretical studies have demonstrated that chalcones are efficient in the scavenging of various radicals. Moreover, some of them, such as 2,3,4,6-tetrahydroxychalcone and 2′,4′,3,4-tetrahydroxychalcone (butein), exhibit a more potent radical-scavenging activity than vitamin C and/or α-tocopherol (Chen et al., 2006). Available literature also suggests that the radical-scavenging activities of chalcones are mainly affected by the aryl groups and their substitution patterns. Hydroxyl groups are one of the key moieties to enhance the antioxidant activity due to its easy conversion to phenoxy radicals through the hydrogen transfer mechanism (Rezk et al., 2002).

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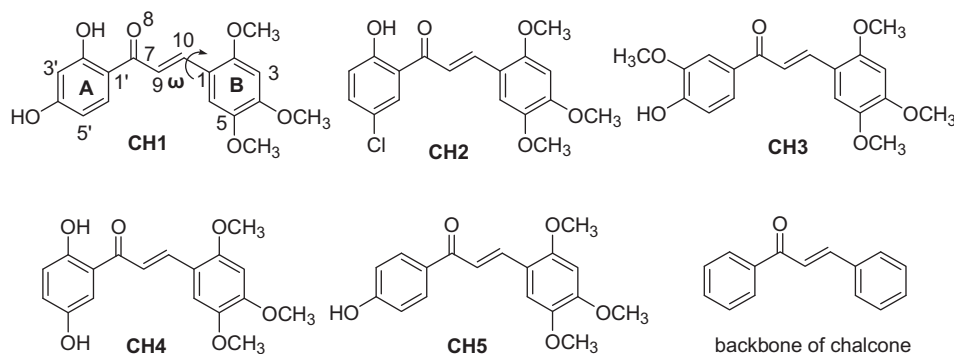*E-mail addresses:* xueyunsheng@xzmc.edu.cn (Y. Xue), njuliuyi2003@gmail.com (Y. Liu).

Fig. 1. The backbone structure of chalcone and molecular structures and atomic numbering of the studied 2,4,5-trimethoxy chalcones **CH1**–**CH5**.
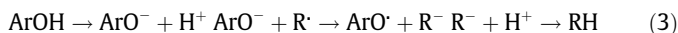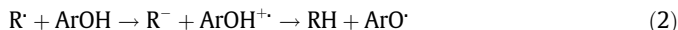
Very recently, a series of 26 derivatives of 2,4,5-trimethoxy chalcones and analogues were synthesised by Shenvi et al. (2013), and the antioxidant activities were evaluated by means of DPPH, NO and PhNHNH2 assays. The former two methods are based on radical scavenging, while the latter is based on lipid peroxidation. Among the synthesised chalcones, five of them bear OH group(s). Their experimental results displayed that 2,4,5-trimethoxy-2′,5′-dihydroxychalcone (IC$_{50}$ = 2.653 μg/ml) showed superior DPPH radical scavenging activity over other tested chalcones and the reference antioxidant ascorbic acid (IC$_{50}$ = 3.039 μg/ml) in DPPH assay.

In recent years, a lot of computational studies have been devoted to the elucidation of the antioxidant activity of phenolic compounds (Amic et al., 2014; Francisco-Marquez & Galano, 2012; Kozlowski et al., 2007; Leopoldini, Russo, & Toscano, 2011; Mazzone, Malaj, Russo, & Toscano, 2013; Nenadis & Tsimidou, 2012; Sadasivama & Kumaresan, 2011; Trouillas, Marsal, Siri, Lazzaroni, & Duroux, 2006; Wright, Johnson, & DiLabio, 2001). Antioxidant activity of the considered molecules can be evaluated thermodynamically through several physicochemical parameters such as bond dissociation enthalpy (BDE), proton dissociation enthalpy (PDE), proton affinity (PA), ionisation potential (IP) and electron transfer enthalpy (ETE). Moreover, density functional theory (DFT) has been found to give accurate evaluations about these parameters.

In light of these facts, and as a part of our ongoing work concerning the antioxidant activity of chalcones and other phenolics antioxidants (Xue, Zheng, An, Dou, & Liu, 2014; Xue et al., 2012, 2013a, 2013b), herein we wish to utilise the DFT method to investigate the radical scavenging properties of five recently synthesised 2,4,5-trimethoxy hydroxychalcones, **CH1**–**CH5** (Fig. 1 Shenvi et al., 2013). The aim of this study is to elucidate and compare their potentials as antiradical agents, in order to provide new clues for antioxidant development. The aforementioned thermodynamic descriptors including BDE, IP, PDE, PA, and ETE were calculated to examine the action mechanisms involved in the process of free radical scavenging for the title compounds. In addition, the influence of the polarity of solvent on the activity was also discussed.

2. Materials and methods

As reported in literatures, the phenolic antioxidants play a protective role in the oxidative process by three possible mechanisms (Leopoldini et al., 2011; Nenadis & Tsimidou, 2012; Wright et al., 2001), namely, hydrogen atom transfer (HAT, Eq. (1)), single electron transfer followed by proton transfer (SET-PT, Eq. (2)) and sequential proton loss electron transfer (SPLET, Eq. (3)).

$$R^{\cdot} + ArOH \rightarrow RH + ArO^{\cdot} \tag{1}$$

$$R^{\cdot} + ArOH \rightarrow R^{-} + ArOH^{+\cdot} \rightarrow RH + ArO^{\cdot} \tag{2}$$

$$ArOH \rightarrow ArO^{-} + H^{+}\ ArO^{-} + R^{\cdot} \rightarrow ArO^{\cdot} + R^{-}\ R^{-} + H^{+} \rightarrow RH \tag{3}$$

In the first mechanism (HAT), antioxidant (ArOH) reacts with the free radical (R$^{\cdot}$), by transferring a hydrogen atom to R$^{\cdot}$ through homolytic rupture of the O–H bond. The reactivity of an ArOH can be estimated by the O–H BDE, where the lower the BDE value, the higher the expected activity. The SET-PT mechanism involves two steps: electron abstraction from ArOH, followed by proton transfer from the radical cation, ArOH$^{+\cdot}$. In this case, the IP and PDE are the most significant energetic factor for the scavenging activity evaluation. Molecules with low IP and PDE values are expected to have high activity. The SPLET mechanism is governed by proton affinity (PA), as well as the electron transfer enthalpy (ETE) of the phenoxide anion, ArO^{-}. Thus, in the present study BDE, IP, PDE, PA and ETE values were used as the main molecular descriptors to elucidate the radical scavenging activity of the investigated compounds.

The BDE values were calculated according to the formula

$$BDE = H_r + H_h - H_p$$

where H_r, H_h and H_p are the enthalpies of the phenoxyl radical, hydrogen atom and the parent molecule at 298.15 K, respectively. Double bond dissociation enthalpy (BDE$_D$) corresponds to a second H abstraction from the ArO$^{\cdot}$ radical. BDE$_D$ are calculated by the following formula:

$$BDE_D = H_{rs} + H_h - H_r$$

where H_{rs} is the enthalpy of the phenoxyl radical generated from the second hydrogen atom transfer. The IP and PDE values were obtained via the equations of

$$IP = H_{cr} + H_e - H_p \text{ and } PDE = H_r + H_H^+ - H_{cr}$$

where H_{cr}, H_e and H_H^+ are the enthalpies of the cation radical, electron and proton, respectively. The PA and ETE values were calculated from the following equations

$$PA = H_{an} + H_H^+ - H_p \text{ and } ETE = H_r + H_e - H_{an}$$

where H_{an} is the enthalpy of anion. The molecular enthalpy (H) at 298.15 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). As in our previous studies (Xue et al., 2012, 2013a, 2013b) gas-phase corrections were employed to calculate the molecular enthalpy in the aqueous phase. In order to calculate the aforementioned parameters, the enthalpies of H-atom, proton, and electron in the gas phase and solvents are needed. The gas phase enthalpy of a hydrogen atom predicted using the B3LYP/6-311++G(2d,2p) method is −0.499897 hartree (Nenadis

& Tsimidou, 2012). The gas phase enthalpy of the proton and electron are 0.002363 and 0.001198 hartree, respectively (Bartmess, 1994). Proton and electron solvation enthalpies were taken from the data reported by Rimarcik, Lukes, Klein, and Ilcin (2010), and hydrogen atom solvation enthalpies from Parker (1992) and Bizarro, Costa Cabral, dos Santos, and Martinho Simões (1999).

All calculations were carried out using the Gaussian 09 software package (Frisch et al., 2009). Density functional theory (DFT) method combining with B3LYP hybrid functional (Becke, 1993; Lee, Yang, & Parr, 1988) has been chosen due to its successful application in the studies of radical scavenging activity on chalcones (Xue et al., 2012, 2013a, 2013b) and other phenolic compounds (Leopoldini et al., 2011; Nenadis & Tsimidou, 2012; Xue et al., 2014). In order to determine the starting geometry with minimum energy, a conformational analysis was performed. A fully relaxed potential energy scan was carried out at the B3LYP/6-31G(d) level. Subsequently, a further geometry optimisation was performed at the same level of theory. The analytical vibrational frequency analysis was performed using the same level of theory to determine stationary points on the potential energy surface. Single point energy (SPE) calculations were performed at the B3LYP/6-311++G(2d,2p) level using the B3LYP/6-31G(d) geometries and used to refine electronic energies.

The frontier orbital distribution and energy of HOMO and LUMO orbitals as well as the spin density of the radicals were determined at the (U)B3LYP/6-31G(d) level of theory. Solvation effects were taken into account on the single-point level by employing the self-consistent reaction field (SCRF) method with a polarised continuum model (PCM) (Tomasi, Mennucci, & Cammi, 2005). Since the physiological medium of human living cells is water and the possible site of action for antioxidants is also the lipid membranes, SPE computations were performed in both water ($\varepsilon = 78.39$) and benzene ($\varepsilon = 2.27$) media. In addition, a methanol solution ($\varepsilon = 32.61$) was also considered for comparing with the experimental results.

3. Results and discussion

3.1. Conformational analysis and geometrical structures

The conformational and geometrical features are significant to elucidate the relationship between the molecular structure and antioxidant activity of considered compounds. Chalcones exist as either the *trans*- or the *cis*-isomer. Due to the strong steric effects between the B-ring and the carbonyl group in the *cis*-isomer, the *trans*-isomer is thermodynamically the most stable form in most cases. For *trans*-isomer, there exits two conformations, *s-trans* and *s-cis*, with respect to the O=C–Cα=Cβ moiety. Our previous studies (Xue et al., 2012, 2013a, 2013b) have shown that *s-cis* conformer is more stable than *s-trans* one. Therefore, in this work, we decided to focus on the *trans-s-cis* conformer for compounds **CH1**–**CH5**.

Potential energy profiles for **CH1**–**CH5** as a function of torsion angle, ω, have been characterised by exploring in steps of 10° values from 0° to 180° at the B3LYP/6-31G(d) level of theory in gas phase. The plots of the potential energy profile are presented in Fig. S1 in Supporting Information. As can be seen from Fig. S1, the most stable structures of **CH1**–**CH5** are found at $\omega = 0°$ followed by a relative minimum at $\omega = 180°$ with average energy difference of only 0.06 kcal/mol. The potential energy is in the range of 7.29–8.62 kcal/mol, which suggests the probable coexistence of the two conformers that are practically isoenergetic.

After the minimum energy conformations were obtained, further geometry optimisation was performed with the B3LYP/6-31G(d) method. The optimised structures of **CH1**–**CH5** are presented in Fig. S2. An intramolecular hydrogen bond (IHB) between the carbonylic oxygen and the 2′-hydroxylic hydrogen was found in molecules **CH1**, **CH2** and **CH4**. These IHBs are expected to favour coplanarity among the A-ring, enone system and the B-ring.

The optimised geometrical parameters for neutral molecules and their radicals were collected in Tables S1–S4 in Supporting Information. By comparison, it can be seen that no significant geometrical change has been observed when going from the neutral molecule to the phenoxy (ArO·) and cation (ArOH·⁺) radicals as well as the anion forms (ArO⁻). From the data of dihedral angle of neutral molecules (Table S1), it can be observed that compounds **CH1**–**CH4**, with 2′-hydroxyl or guaiacol groups in the A-ring, are completely planar, while **CH5** has some degree of deviation from the planarity due to the torsion between the A-ring and the plane of the enone system. When a hydrogen atom was removed from the neutral form (Table S2), no significant changes were observed for the dihedral angles O8-C7-C9-C10, C1′-C7-C9-C10 and C7-C9-C10-C1. On the contrary, the dihedral angle C2′-C1′-C7-C8 changes largely, especially for 2′-O radicals, due to the disappearance of the IHB between the C=O and the 2′-OH. When an electron was removed from the neutral form (Table S3), all the cation radicals, with the exception of **CH5-ca**, retained planarity and consequently conjugation is similar to the parent molecule. As far as the anion forms are concerned, the anions generated from the 2′-OH of **CH1**, **CH2** and **CH4** have the nonplanar geometries, while other phenoxyl anions still retain planarity (Table S4).

3.2. Analysis of the HAT mechanism

The calculated BDE values in gas phase as well as in solvents (benzene, methanol and water) are presented in Table 1. In the same table, the experimental results (Shenvi et al., 2013) on the radical scavenging activity are also given in terms of inhibition concentration (IC₅₀). As discussed above, the 2′-hydroxyl groups in compounds **CH1**, **CH2** and **CH4** form IHB with the adjacent carbonylic oxygen. Abstraction of the H atom from 2′-OH implies the breaking of the IHB. This will cause a higher BDE for the 2′-hydroxyl group. Table 1 clearly shows that the BDE values in the gas phase of 2′-OH is in the 91.5–99.3 kcal/mol range, significantly larger than that of other OH groups (76.2–91.8 kcal/mol). Moreover, the BDE values of 2′-OH are all significantly greater than that of phenol (83.6 kcal/mol at the same level of theory), which is generally chosen as the reference compound. Hence, the hydrogen atom

Table 1
The bond dissociation enthalpy (BDE) values of hydroxychalcones **CH1**–**CH5** in the gas phase and solvents. The experimental IC₅₀ are also included for comparison.

Comp	BDE (kcal/mol)				IC₅₀[a] (μg/ml)
	Gas	Benzene	Methanol	Water	
CH1					4.368
2′-OH	99.3	98.6	95.0	92.6	
4′-OH	91.8	93.1	92.8	90.7	
CH2					5.073
2′-OH	96.0	95.6	92.4	90.0	
CH3					4.130
4′-OH	83.0	83.4	81.6	79.4	
CH4					2.653
2′-OH	91.5	90.5	86.9	84.5	
5′-OH	76.2	77.7	77.8	75.7	
(5′, 2′) –OH[b]	73.7	73.3	68.9	66.0	
CH5					4.293
4′-OH	83.5	85.0	85.1	82.9	
Phenol	83.6	84.6	83.7	81.5	
Ascorbic acid	74.0	75.6	75.3	73.1	3.039

[a] IC₅₀, the necessary concentration of an antioxidant needed for the inhibition of radical formation by 50% from DPPH assay, see Shenvi et al. (2013).
[b] The BDE of 2′-OH group obtained from the ArO radical formed after the H-loss from 5′-OH.

transfer from 2′-OH is difficult to occur compared to phenol, and this group can be neglected with respect to the H-atom donating reaction.

For compounds **CH1** and **CH4**, which possess two phenolic hydroxyls, their radical-scavenging activity is determined by the one with the lowest BDE. Analysis of the data in Table 1 shows that the BDEs decline in the order **CH2** > **CH1** > **CH5** > **CH3** > **CH4** in both gas and benzene phases, so the sequence of hydrogen donating ability is: **CH4** > **CH3** > **CH5** > **CH1** > **CH2**. The sequences found in methanol and water media are the same, **CH1** > **CH2** > **CH5** > **CH3** > **CH4** for BDEs and **CH4** > **CH3** > **CH5** > **CH2** > **CH1** for activity. Clearly, **CH4** is always the most active one among the studied compounds independent of the media. Generally, the predicted order of hydrogen donating ability from BDEs is in line with the experimental results from DPPH assay (Shenvi et al., 2013), i.e. the radical trapping activity declines in the order of **CH4** > **CH3** > **CH5** > **CH1** > **CH2**.

It can be seen from Table 1 that the BDE of 5′-OH in **CH4** is 76.2, 77.7, 77.8 and 75.7 kcal/mol in gas, benzene, methanol and water, respectively. These values are significantly lower than the corresponding data of 4′-OH in other molecules. This clearly confirms that HAT from 5′-OH is easier than from 4′-OH, and 5′-OH is considered as the primary target for radical attack. As revealed by the BDE difference between **CH3** and **CH5** (Table 1), the introduction of the OCH$_3$ group in the o-position of 4′-OH has a slight effect on the BDE of 4′-OH. This indicates that the presence of –OCH$_3$ has a stabilising effect on the radicalised molecule, due to the compromise between the electron-donating and electron-withdrawing capabilities of this group. This situation is similar with that of guaiacol (Leopoldini et al., 2011).

In order to explain the differences in BDE and consequently the differences in the reactivity of the OH sites, the spin density distribution of radicals were calculated and presented in Fig. 2. Spin density is a reasonably reliable parameter in rationalising the stability of radical species (Mazzone et al., 2013; Nenadis & Sigalas, 2011; Trouillas et al., 2006). As can be seen from Fig. 2, the spin densities of all radicals mainly distribute on the A-ring. The spin density on the O-atom of the 2′-OH radical in **CH1**, **CH2** and **CH4** is 0.43, 0.41 and 0.40, respectively. This indicates that the stabilisation of these radicals increases in the order of **CH1** < **CH2** < **CH4**, and consequently, the BDE values of 2′-OH decrease in the same order. The spin density on the O-atom of the 4′-OH radical in **CH3** is 0.35, lower than that of the 5′-OH radical in **CH4** (0.41), while the in-gas BDE value of the former is greater by 6.8 kcal/mol than that of the latter.

Analysis of the data in Table 1 shows that the solvent media affects the BDE values slightly. More specifically, the average deviation of BDEs for 2′-OH between gas phase and water is ~6.6 kcal/mol. For 2′-OH, the BDE values decrease stepwise from gas phase to water. This means these media can increase the ability of the hydroxyl groups to homolytic dissociation. This observation may be due to the presence of IHBs between the 2′-OH and the carbonyl group, which are weakened more and more when the solvent polarity increases. A similar phenomenon has also been observed in previous studies (Nenadis & Sigalas, 2011; Xue et al., 2013b, 2014).

Compared to 2′-OH, a different solvent effect on BDE was observed for 4′-OH and 5′-OH groups. For **CH1-R4**′ and **CH3-R4**′, the highest BDE values were found in benzene solution, which are on average 0.8 kcal/mol higher than the gas-phase ones. With
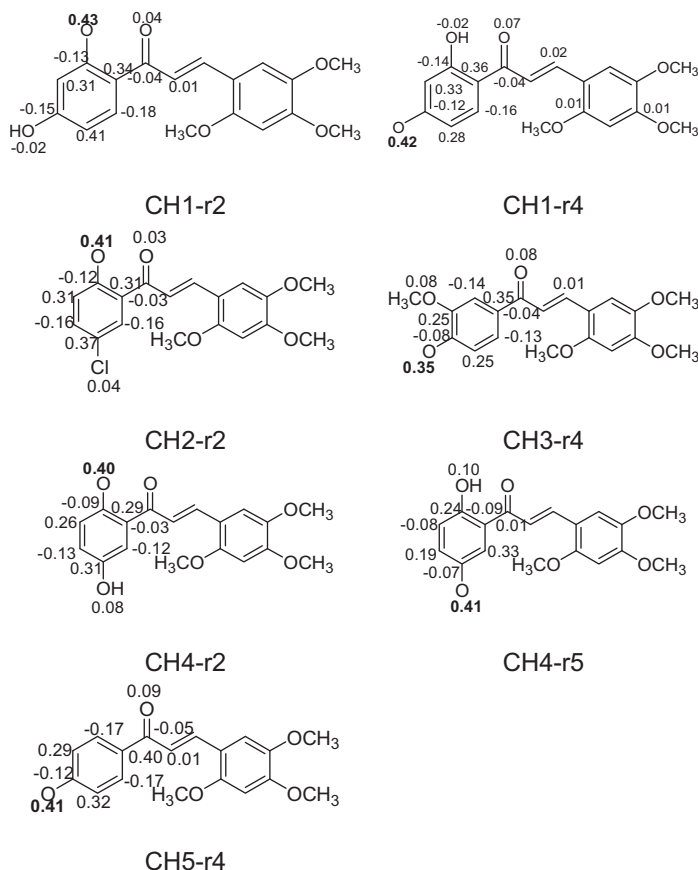

Fig. 2. Spin density distribution of phenoxy radicals of 2,4,5-trimethoxy chalcones **CH1**–**CH5** computed at the B3LYP/6-31G(d) level in gas phase.

the increasing of the solvent polarity, BDEs tend to decrease slightly from benzene to water. The decrease of BDE in a polar media may be owing to the better stabilisation of the corresponding radical in the presence of the polar media, as shown from the spin density distribution of the 4′-OH radical of **CH3** in the gas phase and in water (Fig. S3). As presented in Fig. S3, the spin density on the phenolic oxygen atom is 0.35 in the gas phase whereas it is 0.33 in water. This means the stabilisation of the radical is higher in water than in the gas phase. Considering **CH4-R5′** and **CH5-R4′**, a small increase in BDEs was observed in benzene and methanol media in comparison to those in gas phase. The highest BDE value was reached in methanol, and then decreased from methanol to water. From Table 1, we can see that all the BDEs in water are lower than those in gas phase. A close inspection on the BDE values in isoflavones (Lengyel, Rimarcik, Vaganekc, & Klein, 2013) and p-phenylenediamine (Rimarcik et al., 2010) revealed a similar trend.

It can be seen from Table 1, the lowest in-gas BDE values of **CH3** (83.0 kcal/mol) and **CH4** (76.2 kcal/mol) are smaller than that of phenol (83.6 kcal/mol), while others are similar to or larger than that of phenol. This means that the H-atom donation ability of **CH4** and **CH3** are stronger than that of phenol, while **CH1**, **CH2** and **CH5** are not sufficient enough to provide a good activity. Moreover, the lowest BDE value of **CH4** is slighter larger than that of known food antioxidant ascorbic acid (74.0 kcal/mol), indicating that **CH4** is of comparable hydrogen donating ability with the ascorbic acid antioxidant. This predicted trend is somewhat different from the experimental results obtained from the DPPH test, i.e., **CH4** presented higher activity than ascorbic acid. To improve such a correlation, we investigated the capacity of a second HAT mechanism and calculated the BDE_D parameter for **CH4**. Recently published reports have indicated that the BDE_D parameter showed a good correlation to the antioxidant activity of guaiacol oligomers (Anouar et al., 2009) as well as other flavonoids such as quercetin (Amić et al., 2014).

It can be seen from Table 1, the BDE_Ds of **CH4** ranges from 73.7 kcal/mol in gas phase to 66.0 kcal/mol in water. Clearly, these BDE_Ds are lower than the corresponding BDEs of **CH4** as well as the reference antioxidant ascorbic acid. This demonstrated the high antioxidant capacity of the ArO· radical formed after a first HAT action. The BDE_Ds are in line with the experimental results, i.e., **CH4** presented higher activity than ascorbic acid.

According to above discussion, **CH4** is expected to be a potent radical scavenger and deserves to be further studied. Meanwhile, these results also suggest the important role of the A-ring on the antioxidant activity of chalcones. Indeed, the role of the B-ring in flavonoids, including chalcones, has been extensively studied in the literature; however, fewer studies have been reported on the role of the A-ring. To our knowledge, only one paper has theoretically explored the importance of the A-ring in chalcones (Kozlowski et al., 2007). Here, the importance of the A-ring on the radical scavenging capabilities was confirmed again as illustrated by compound **CH4**. Although OH substitution at C5′ is unusual for chalcones, this group could strongly participate in the antioxidant activity as shown in above results.

3.3. Analysis of SET-PT mechanism

Although the DPPH radicals usually react with phenols via hydrogen abstraction, we also performed calculations on the SET-PT mechanism, because some studies have reported the role of this mechanism with DPPH (Nakanishi et al., 2005).

The calculated IPs and PDEs in the gas phase and solvents for the title compounds are summarised in Table 2. From Table 2, it can be seen that the lowest value of IP is found for **CH3** in all studied media, indicating that the electron donating ability of **CH3** is always stronger than others irrespective of the media. Analysis of Table 2 shows that the sequence of IP values in gas-phase is **CH3 < CH5 < CH4 < CH1 < CH2**, whereas the tendency of IP values in water is quite different: **CH3 < CH1 < CH5 < CH2 < CH4**. The IP values in benzene and methanol increase in the order of **CH3 < CH5 < CH1 < CH4 < CH2** and **CH3 < CH5 < CH1 < CH2 < CH4**, respectively, which are also somewhat different from that in gas phase. In comparison with BDEs, the trend of IP values is significantly different from that of BDE values. Such a discrepancy can be assigned to the fact that BDE is affected by the local phenomena induced by the substituents, whereas the IP value is affected by the structure of the whole molecule (Wright et al., 2001).

When the solvent effect was taken into account, a significant decrease of the IP value was observed. As an example, the IP value of **CH3** that is the most active, changes from 154.3 to 97.7 kcal/mol in going from the gas phase to the water solution. This confirms that polar solvents largely ease the electron transfer and thus favour the free radical scavenging process. Compared to BDEs (Table 1), the solvent affects the IP values drastically. This is not surprising because it is well known that cation radicals are charged and they are quite sensitive to the polarity of solvents. This result is in agreement with those documented in previous literature (Fifen, Nsangou, Dhaouadi, Motapon, & Jaidane, 2011; Rimarcik et al., 2010).

As can be seen from Table 2, the in-gas IPs of **CH1–CH5** are about 31–38 kcal/mol and 33–40 kcal/mol lower than that of phenol (192.3 kcal/mol) and ascorbic acid (193.9 kcal/mol), respectively. This means that **CH1–CH5** are expected to be more efficient than phenol and ascorbic acid, and can serve as good candidates to work within the electron transfer mechanism. It is interesting to

Table 2
The ionisation potential (IP) and proton dissociation enthalpy (PDE) values of hydroxychalcones **CH1–CH5** in the gas phase and solvents.

Comp	IP (kcal/mol)				PDE (kcal/mol)			
	Gas	Benzene	Methanol	Water	Gas	Benzene	Methanol	Water
CH1	157.9	137.7	105.1	98.7				
2′-OH					257.3	60.8	37.0	42.4
4′-OH					249.8	55.4	34.8	40.5
CH2	160.7	139.1	105.2	101.2				
2′-OH					251.2	56.4	34.3	37.3
CH3	154.3	135.4	104.1	97.7				
4′-OH					244.7	47.8	24.6	30.2
CH4	156.8	138.2	107.0	101.8				
2′-OH					250.6	52.2	27.0	31.2
5′-OH					235.4	39.3	18.0	22.4
CH5	156.5	136.5	104.4	99.2				
4′-OH					242.9	48.4	27.8	32.2
Phenol	192.3	163.2	125.0	119.6	207.2	21.2	5.8	10.5
Ascorbic acid	193.9	166.9	129.4	123.9	196.0	8.5	−7.0	−2.3

note that the IPs of other flavonoids like catechin, rutin, scutellarin and baicalin are 30–40 kcal/mol lower than that of phenol (Leopoldini, Rondinelli, Russo, & Toscano, 2010). Accordingly, when the ET mechanism predominates for the scavenging of free radicals, **CH1**–**CH5** are expected to be as efficient as these flavonoids.

It can be seen from Table 2 that the lowest PDEs of **CH1**–**CH5** in gas phase can be arranged in the following order: **CH4** < **CH5** < **CH3** < **CH1** < **CH2**. This trend is analogous with that of BDEs, because the second step of the SET-PT mechanism results in the phenoxy radical formation. The orders in solvents are somewhat different from that in gas phase. Clearly, **CH4** has the lowest PDE value in all four studied media.

The high solvation enthalpies of proton decrease the PDE value significantly. As observed in Table 2, PDEs decrease in the order: gas phase > benzene > water > methanol. The average deviation of PDE between methanol and gas-phase reaches about 218 kcal/mol. Such a finding is similar with that reported for *p*-phenylenediamine (Rimarcik et al., 2010) and eupatilin (Li et al., 2013).

When comparing with the experimental results, the trend of IPs is significantly different from that of DPPH radical scavenging activity. For example, compound **CH4**, being the most efficient in the experiment, was predicted to be less active according to the computed IP values. Moreover, from the thermodynamic analysis of ET-PT mechanism (Table S5), it can be seen that the ET process in the gas phase is endothermic, with ΔG^{ET} values ranging from 76.2 to 86.5 kcal/mol. Even in water, the ET process was still endothermic, with ΔG^{ET} values in the range of 6.3–19.7 kcal/mol. Therefore, in the DPPH· assay, SET is not likely to be favoured.

3.4. Analysis of SPLET mechanism

In SPLET, the electron transfer occurs from a deprotonated form of hydroxychalcones. Previous studies for phenolic antioxidants have confirmed the significant role of SPLET in polar solvents (Foti, Daquino, & Geraci, 2004; Musialik & Litwinienko, 2005). Thus, the possible contribution of phenolates was investigated to explore the DPPH· scavenging activity.

Table 3 presents the Pα and ETE values in gas phase and solvents. By comparison, we can see that 2′-OH PAs are always larger than that of 4′-OH and 5′-OH in all the studied environments. This demonstrates that formation of 2′-phenoxy anion is more difficult than other anions. This can be ascribe to the formation of IHB between 2′-OH and adjacent carbonyl group. It clearly shows that the lowest PAs increase in the order of **CH1** < **CH5** < **CH3** < **CH4** < **CH2** in all the four media, and 4′-OH tends to has smaller PA than other OH groups. The only structural difference in **CH3** and **CH5** is the OMe group at C3′ in the former. Its effect on 4′-OH PA is small.

Similar to the case of PDE, the PA values decrease drastically from gas phase to solution owing to the high solvation enthalpies of proton. The average deviations between PA in the gas phase and different solvents are 238.5 (benzene), 294.8 (methanol) and 292.2 (water) kcal/mol, respectively. This indicates that solvents, especially polar solvents, seem to favour the deprotonation process. Analysis of the PA values in Table 3 shows that the lowest gas-phase PAs for the title compounds are in the range of 332.2–349.4 kcal/mol, which is similar with the values ranging from 312.5 to 327.5 kcal/mol reported in the literature for polyphenols (Leopoldini, Russo, & Toscano, 2006). Additionally, with the exception of **CH2**, the lowest gas-phase PAs of the other four compounds are lower than that of phenol, but larger than that of ascorbic acid. This is the case for PAs in solvents. Thus, deprotonation of phenolic-OH for the four hydroxychalcones is easier than phenol but more difficult than ascorbic acid.

As far as the ETEs are concerned, the lowest gas-phase ETEs follow the sequence: **CH4** < **CH1** < **CH3** < **CH2** < **CH5**, while a somewhat different order is found in the solvents. Clearly, **CH4** has the lowest ETE value in all the studied media. This means once the phenoxide anion is generated in a polar media, **CH4** is the most active among the five studied compounds. In comparison to the IP values of the neutral form (Table 2), the corresponding ETE values were significantly lower. This implies that single electron transfer processes from the anionic form are more favourable than that of the neutral form. This matches well with the results obtained from previous studies (Francisco-Marquez & Galano, 2012; Lengyel et al., 2013).

From the above discussion, the PAs are significantly lower than the corresponding values of BDE and IP, indicating that deprotonation is favoured in polar media. To explore the possibility of the reaction between the generated chalcone anions and DPPH·⁻, we calculated the electron affinity (EA) of DPPH·. The values of EA are −88.2 and −84.2 kcal/mol in methanol and water, respectively. In comparison with the ETE values of the anions, it can be inferred that the electron transfer between the chalcone anion and DPPH· is thermodynamically permitted (ETE + EA is negative), suggesting SPLET represents the most probable mechanism in polar media. In this sense, the SPLET mechanism could possibly account for the decreased activity of **CH1**, **CH2** and **CH5**, which was not successfully predicted by the HAT mechanism (inactive predicted by BDEs).

3.5. Thermodynamically preferred mechanism

As mentioned in Section 2, the HAT, SET-PT and SPLET mechanisms are mainly governed by BDE, IP and PA descriptors,

Table 3
The proton affinity (PA) and electron transfer enthalpy (ETE) values of hydroxychalcones **CH1**–**CH5** in the gas phase and solvents.

Comp	PA (kcal/mol)				ETE (kcal/mol)			
	Gas	Benzene	Methanol	Water	Gas	Benzene	Methanol	Water
CH1								
2′-OH	354.6	114.6	55.9	58.5	60.7	83.9	86.2	82.6
4′-OH	332.2	95.3	41.6	44.6	75.5	97.8	98.3	94.6
CH2								
2′-OH	349.4	110.7	53.3	56.0	62.6	84.8	86.1	82.5
CH3								
4′-OH	338.1	100.3	44.5	47.2	60.9	83.0	84.3	80.6
CH4								
2′-OH	353.3	113.1	53.6	57.4	54.1	77.3	80.3	75.5
5′-OH	344.4	105.6	50.2	50.9	47.7	71.9	74.8	73.3
CH5								
4′-OH	333.7	96.7	42.8	45.7	65.6	88.2	89.4	85.7
Phenol	347.9	106.0	49.1	51.9	51.7	78.4	81.8	78.2
Ascorbic acid	326.0	87.9	34.3	37.2	63.9	87.5	88.2	84.4

respectively. Thus, these descriptors can be used to determine the thermodynamically preferred reaction pathway involved in the free radical scavenging process. By comparison, we found that the calculated gas-phase IPs and PAs of the title compounds are significantly higher than the BDEs, and the latter presents the strong correlation with the antioxidant activity on DPPH·. Therefore, HAT represents the most favourable process in the gas phase from a thermodynamic point of view. In the benzene phase, PAs are lower than IPs, but still larger than BDEs. This indicates that HAT remains the favoured process in benzene and SET-PT is least preferred. In polar methanol and water solutions, the PAs are significantly lower than the corresponding values of BDE and IP. Therefore, SPLET represents the most likely mechanism in polar media.

In summary, among the three mechanisms, HAT is the thermodynamically dominant process in gas phase and non-polar solvents, while SPLET is more favoured in polar media. It should be mentioned, however, that the preponderant mechanism of an antioxidant is not only determined by the chemical nature of the antioxidant, but also governed by the micro-environmental features (lipid phase, aqueous phase, pH) as well as the chemical nature of the scavenged radical (Litwinienko & Ingold, 2007; Nenadis & Tsimidou, 2012; Rimarcik et al., 2010). Detailed kinetic data including rate constants and branching ratios are needed to further identify the preponderant mechanism.

3.6. Frontier molecular orbitals

The energy and distribution of the frontier orbitals are important parameters in describing the free-radical scavenging activities of phenolic antioxidants. Fig. 3 gives the calculated frontier orbital distributions and energies in the gas phase for CH1–CH5. From Fig. 3, it can be seen that the HOMOs and LUMOs of compounds CH1–CH5 present similar distributions with each other. More specifically, the HOMO orbitals are mainly localised on the ring B and enone moiety, whereas the LUMOs are delocalised over the whole molecule. The only exception was found for CH4, where the HOMO is delocalised on the whole molecule. Clearly, the HOMO distribution of the phenolic oxygen atom on CH4 is larger than that of the others. This means that the OH groups on the A-ring would be more easily attacked by free radicals for CH4 than for others.

As for the HOMO energies (Fig. 3), compound CH3 seems to have the highest HOMO energy (−5.1283 eV), followed by CH5 (−5.1693 eV), CH4 (−5.2502 eV), CH1 (−5.2804 eV), and CH2 (−5.4219 eV). Because of the higher the HOMO energy, the easier the electron is being excited; thus CH3 has the strongest

electron-donating capability among the studied compounds. By comparison, the predicted electron-donating capability sequence, according to the HOMO energies, was found to be the same as that for the IP.

4. Conclusion

In the present work, DFT calculations under the level of B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d) have been utilised to explore the structure, molecular properties and radical scavenging activity of five 2,4,5-trimethoxy hydroxychalcones. The thermodynamic parameters, such as O–H BDE, IP, PDE, PA and ETE, have been computed both in the gas phase and in solvents to evaluate the possible mechanism involved in the process of radical scavenging.

As for the HAT mechanism, the sequence of hydrogen donating ability of the title compounds deduced from O–H BDEs is: CH4 > CH3 > CH5 > CH1 > CH2, which is in line with the experimental results (Shenvi et al., 2013). Hydroxyl group on the 5′-position in the A ring has higher H-atom donation ability than 2′- and 4′-OH, and could strongly participate in the radical scavenging activity. The introduction of a methoxy group (OCH$_3$) in the o-position of 4′-OH has a slight effect on the O–H BDE. As illustrated by compound CH4, the importance of the A-ring on the radical scavenging capabilities was confirmed. Parameter BDE$_D$ related to the second HAT mechanism better accounts for DPPH radical scavenging efficiency than the BDEs related to the first HAT mechanism. The results obtained also show that solvents have a slight effect on BDE values.

Our results reveal that the sequence of IP values is different from that of BDE values, and solvent influences the IPs drastically. By comparison, CH1–CH5 are expected to be more efficient in donating electrons than phenols and ascorbic acid. The order of the lowest in-gas PDEs of CH1–CH5 is analogous with that of BDEs. Among the studied compounds, CH4 had the lowest PDE value in all four studied media. The calculated gas-phase PAs are significantly higher than the O–H BDEs and IPs. 4′-OH seems to have a smaller PA than other OH groups. Similar to the case of PDE, the PA values decrease drastically from the gas phase to solution. Obtained results also show that the solvent is able to cause a significant change in the reaction enthalpies of the SET-PT and SPLET mechanisms, that may result in a change in the thermodynamically preferred mechanism.

From the above discussion, it can be concluded that HAT is the most favoured mechanism for explaining the radical-scavenging activity of the title compounds in the gas phase and non-polar benzene solutions, whereas the SPLET mechanism is the
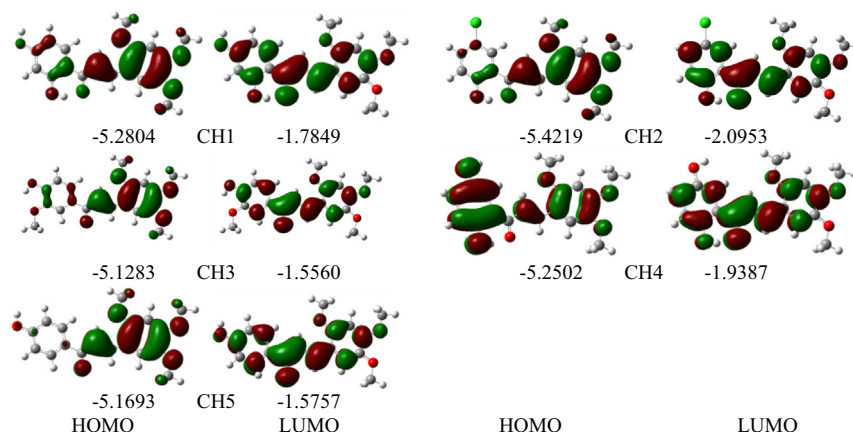

−5.2804	CH1	−1.7849
−5.1283	CH3	−1.5560
−5.1693	CH5	−1.5757
HOMO	LUMO	

−5.4219	CH2	−2.0953
−5.2502	CH4	−1.9387
HOMO	LUMO	

Fig. 3. The orbital distribution and energy (in eV) of HOMO and LUMO for 2,4,5-trimethoxy chalcones CH1–CH5 computed at the B3LYP/6-31G(d) level in gas phase.

thermodynamically preferred pathway in polar media. Among the studied compounds, 2,4,5-trimethoxy-2′,5′-dihydroxychalcone (**CH4**) is predicted to be a potential free radical scavenger and deserves further exploitation. To summarise, these results will contribute to the ongoing interest on the understanding of the activity of polyphenolic antioxidants and open new perspectives towards a rational exploitation of chalcones in the field of food chemistry and pharmacy.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.08.106.

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