



# Antioxidant activity of thiourea derivatives: An experimental and theoretical study



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## ABSTRACT

Antioxidant ability of thiourea derivatives was characterized based on the measurement of the color change of 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS<sup>•+</sup>) when reacting with the studied compounds. The values of IC<sub>50</sub><sub>DPPH</sub> [IC<sub>50</sub><sub>ABTS</sub>] of DPTU (1,3-diphenyl-2-thiourea) and BPTU (1-benzyl-3-phenyl-2-thiourea) are of 0.710 ± 0.001 mM/[0.044 ± 0.001 mM] and 11.000 ± 0.015 mM/[2.400 ± 0.021 mM], respectively. As a result, it could be concluded that DPTU could capture free radicals better than BPTU and quantum chemical calculations also confirmed this evaluation. Although bond dissociation energy values of bonds in BPTU molecule are less than those in DPTU molecule, but the reaction rates of DPTU are greater than those of BPTU when reacting with HOO<sup>•</sup> (selected as a representative free radical), so the IC<sub>50</sub> values of DPTU were smaller than IC<sub>50</sub> of BPTU. On the basis of kinetic calculations, hydrogen atom transfer (HAT) is supposed to be a preferred mechanism over single electron transfer (SET) when thiourea derivatives reacted with free radicals. The amount of products obtained according to HAT mechanism accounts for 99.99% compared with the product amount of HAT and SET mechanisms.

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## 1. Introduction

Thiourea and its derivatives are known to be a powerful scavenger of O<sub>2</sub><sup>-</sup> and OH<sup>•</sup> [1,2]. In 2011, Sudzhaev and coworkers [3] synthesized several thiourea derivatives including 1-(2-aminoethyl)thiourea, N,N'-(iminodiethane-2,1-diyl)bis(thiourea), 1-[1-methyl-2-(phenylamino)ethyl] thiourea, then examined their antioxidant properties through reactions of cumene oxidation. These thiourea derivatives could inhibit the autooxidation of cumene by scavenging of cumylperoxy radicals and simultaneously affecting the catalytic decomposition of cumyl hydroperoxide. In 2015, thiazolidine and pyrrolidine compounds containing a thiourea moiety were prepared and evaluated the free radicals scavenging ability [4]. Several compounds were selected and experimentally evaluated their antioxidant. The resulting data showed that all of them are useful for preventing oxidative stress in biological systems. In 2017, free radicals scavenging of urea, thiourea and selenourea derivatives in both gas phase and aqueous medium were computationally investigated using density functional theory [5]. The results showed thiourea and selenourea

could scavenge superoxide radical anions efficiently through single hydrogen abstraction. While urea, thiourea, selenourea and their derivatives would also scavenge hydroxyl radical efficiently by the mechanism of addition to carbon sites of the molecules. In 2020, Naz *et al.* synthesized and studied the properties of 1,3-bis(3,4-dichlorophenyl)thiourea, 1,3-bis(2,6-dimethylphenyl)thiourea, diethyl 4,4'-(thiocarbonylbis(azanediyl))dibenzoate, 1,3-bis(4-nitrophenyl)thiourea and 1,3-di-p-tolylthiourea [6]. These compounds showed their promising antibacterial and antioxidant potential, being very active against glucose-6-phosphatase. Among them 1,3-bis(3,4-dichlorophenyl)thiourea was more active against DPPH<sup>•</sup> and ABTS<sup>•+</sup> free radicals than the others.

The above studies showed that thiourea derivatives have an excellent potential for free radical capturing and antioxidant ability. However, up to date, the antioxidant potential of derivatives such as DPTU and BPTU, etc. has not been studied in detail. These are inexpensive compounds and their molecules have N–H bonds that are easily severed. Therefore, they are promised as potential candidates to be studied for an evaluation of their antioxidants.

In this work, the antioxidant ability of DPTU and BPTU were evaluated using tandem experimental and computational methods at the M05-2X/6-311++G(d,p) level of theory for calculating thermochemical parameters and reaction kinetics [7,8].

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## 2. Experimental

### 2.1. Materials

DPTU (1,3-diphenyl-2-thiourea) and BPTU (1-benzyl-3-phenyl-2-thiourea) were from AK Scientific, USA. Absolute ethanol of HPLC grade was from Fisher. 2,2-diphenyl-1-picrylhydrazyl; 2,2'-azino bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and  $K_2S_2O_8$  were obtained from Sigma-Aldrich.

### 2.2. Methods

#### 2.2.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH $\cdot$ ) assay

DPPH $\cdot$  was diluted in ethanol at a concentration of  $6.7 \times 10^{-5}$  M, and then the solution was coated with aluminum foil to protect from the effects of light. Different concentrations of antioxidants were added to DPPH $\cdot$  with a volume ratio of 3: 1. The reaction solution was shaken vigorously and kept in the dark for 30 min. TCC-240A SHIMADZU UV/Vis spectrophotometer was used to measure the absorbance of the solutions at 515 nm. DPPH $\cdot$  free radical scavenging ability was determined by the below equation:

$$SA_{DPPH\cdot} \% = \frac{A_c - A_s}{A_c} \cdot 100 \quad (1)$$

$SA_{DPPH\cdot} \%$ : DPPH $\cdot$  scavenging ability;

$A_c$ : absorbance of the control containing DPPH $\cdot$  (Control = 1 mL of DPPH $\cdot$  + 3 mL of ethanol);

$A_s$ : absorbance of the sample containing DPPH $\cdot$  (Sample = 1 mL of DPPH $\cdot$  + 3 mL of antioxidant at the different concentrations).

#### 2.2.2. 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS $^{+\cdot}$ ) assay

ABTS $^{+\cdot}$  free radical cation scavenging ability was conducted according to the modified procedure of Re *et al.* [9]. First, the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was dissolved in water to obtain a stock solution with a concentration of 7 mM. The ABTS $^{+\cdot}$  free radical cation was produced by the reaction of 7 mM 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt solution with 140 mM  $K_2S_2O_8$ . The concentration of  $K_2S_2O_8$  after mixing was 2.45 mM. The solution mixture was incubated in the dark at room temperature. After 16 h, this mixture was diluted with ethanol to adjust the absorbance of the solution at 734 nm at  $0.7 \pm 0.05$  on the spectrophotometer at room temperature. Tests were conducted by adding 1 mL of antioxidants (at the different concentrations) to 3 mL of a solution containing ABTS $^{+\cdot}$  free radical cation and incubating at room temperature for 6 min. The absorbance was measured at 734 nm. The experiments were repeated three times and results were expressed as  $IC_{50_{ABTS}}$  (mM) values. ABTS $^{+\cdot}$  free radical cation scavenging ability was calculated as follows:

$$SA_{ABTS^{+\cdot}} \% = \frac{A'_c - A'_s}{A'_c} \cdot 100 \quad (2)$$

$SA_{ABTS^{+\cdot}} \%$ : ABTS $^{+\cdot}$  free radical cation scavenging ability;

$A'_c$ : absorbance of the control containing ABTS $^{+\cdot}$  (Control = 3 mL of ABTS $^{+\cdot}$  + 1 mL of ethanol);

$A'_s$ : absorbance of the sample containing ABTS $^{+\cdot}$  (Sample = 3 mL of ABTS $^{+\cdot}$  + 1 mL of antioxidant at the different concentrations).

## 3. Computational methods

In this study, all computations were performed by the Gaussian 16 software [10]. The M05-2X method was proposed by the developers [11], and many reports have indicated the reliability of this method for evaluating the reaction kinetics [12–14]. In this work, a combination of M05-2X functional at the basis set of 6-311++G (d,p) was applied to calculate the essential thermochemical parameters and reaction paths in the reaction between the thiourea derivatives and HOO $\cdot$  free radical then predicting their mechanisms.

The antioxidant ability of a particular compound could be assessed by the following mechanisms, namely hydrogen atom transfer (HAT) mechanism, single electron transfer (SET) mechanism, single electron transfer followed by proton transfer (SETPT) and sequential proton loss electron transfer (SPLET)... [15–22]. The details of reaction mechanisms and the calculations of thermochemical parameters are described in Table S6. Enthalpies of electron (in the gas phase and ethanol) were referenced from the previous report of Rimarčík *et al.* [23].

The reaction rate constant ( $k$ ) and activated Gibbs free energy of HAT mechanism were calculated by Eyringpy software [24]. According to the theory of Marcus [25,26], the SET reaction relied on the transition state to determine the electron transfer activation barrier ( $\Delta G_{ET}^\ddagger$ ) based on two thermodynamic parameters: *i*) the free energy of reaction ( $\Delta G_{ET}^0$ ) and *ii*) the nuclear reorganization energy ( $\lambda$ ):

$$\Delta G_{ET}^\ddagger = \frac{\lambda}{4} \left( 1 + \frac{\Delta G_{ET}^0}{\lambda} \right)^2 \quad (3)$$

Appropriately,  $\lambda$  is determined by the formula (4):

$$\lambda \approx \Delta E_{ET} - \Delta G_{ET}^0 \quad (4)$$

In which  $\Delta E_{ET}$  is the non-adiabatic energy difference between reactants and vertical products. This approach is similar to that of Nelsen *et al.* which has been used for a large number of spontaneous reactions [27].

The rate constant ( $k$ ) is calculated in the gas phase using the conventional transition state theory [14,20].

$$k = \sigma \cdot \kappa \cdot \frac{k_B \cdot T}{h} e^{-\frac{\Delta G^\ddagger}{RT}} \quad (5)$$

where  $\sigma$  is total symmetry number of reaction;  $\kappa$  is the coefficient related to the tunneling correction which was calculated according to the method of Wigner [28] and Eckart [29];  $k_B$  is Boltzmann constant;  $T$  is the temperature (K);  $h$  is Planck constant;  $R$  is gas constant;  $\Delta G^\ddagger$  is Gibbs free energy of activation.

If the reaction occurred with different mechanisms such as HAT, SET, the total rate constant would be calculated by the formula (6) [14]:

$$k_{tot} = k_{HAT} + k_{SET} \quad (6)$$

in which  $k_{HAT}$ ,  $k_{SET}$  are the HAT, SET rate constants, respectively.

$$k_{HAT} = \sum k_{HAT(i)} \quad (7)$$

$$k_{SET} = \sum k_{SET(i)} \quad (8)$$

*i* represents each reaction path which might be happened in each mechanism.

The relative amount of products ( $\Gamma_i$  %) yielded by the different reaction mechanisms is estimated according to formula (9):

$$\Gamma_i = \frac{k_i}{k_{\text{tot}}} \cdot 100 \quad (9)$$

In addition, AIM2000 software was used to analyze and display atoms in molecules designed by Biegler – König *et al.* [30].

## 4. Results and discussion

### 4.1. DPPH<sup>•</sup> radical scavenging assay

In this free radical scavenging assay, the 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) radical, which forms a purple solution becomes reduced when it reacts with any antioxidant that can donate a hydrogen atom, forming the yellow-colored diphenylpicrylhydrazine. The absorbance of the mixed solution containing DPPH<sup>•</sup> is detected at 515 nm.

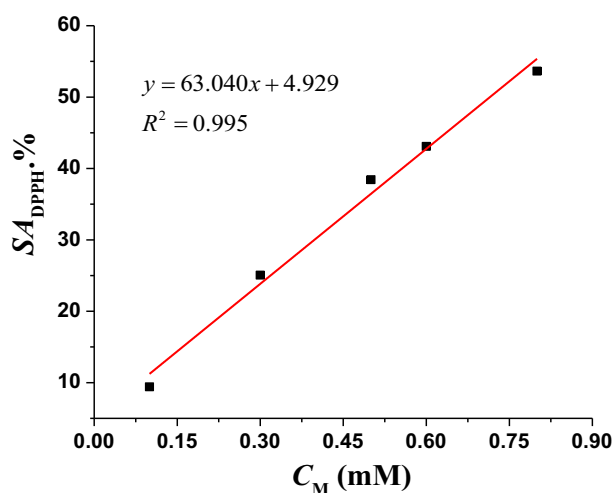
As can be seen in Fig. S1, the change in optical density of DPPH<sup>•</sup> solution shows that the DPPH<sup>•</sup> free radical scavenging capacity of antioxidants is varied by concentration of DPTU and BPTU. The higher the concentration of antioxidants, the less absorbance of

the solution. DPPH<sup>•</sup> free radical scavenging ability is assessed by IC<sub>50</sub><sub>DPPH</sub> value. The IC<sub>50</sub><sub>DPPH</sub> value is defined as the sample concentration that can inhibit 50% of the original DPPH<sup>•</sup> amount. The smaller the IC<sub>50</sub><sub>DPPH</sub> value, the more active the sample is. From the relationship between DPPH<sup>•</sup> scavenging efficiency and concentration of DPTU and BPTU (Fig. 1), the IC<sub>50</sub><sub>DPPH</sub> values of DPTU and BPTU are calculated. DPTU and BPTU exhibit DPPH<sup>•</sup> scavenging activity with IC<sub>50</sub><sub>DPPH</sub> values of  $0.710 \pm 0.001$  mM,  $11.000 \pm 0.01$  5 mM, respectively. It means that DPPH<sup>•</sup> capturing capacity of BPTU is not as strong as that of DPTU.

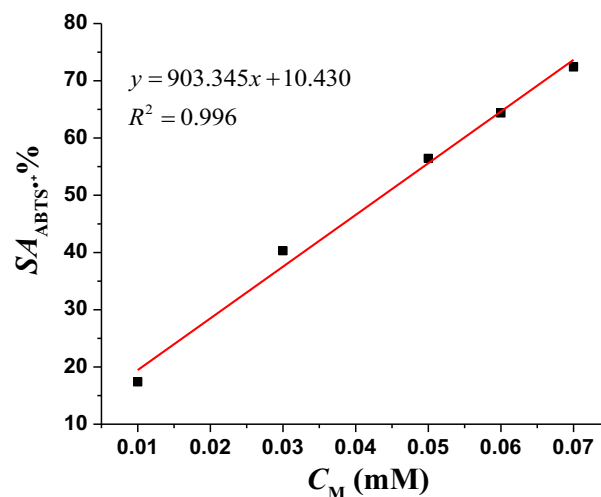
### 4.2. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS<sup>•+</sup>) assay

Antioxidant ability of thiourea derivatives is assessed through ABTS<sup>•+</sup> assay. In this assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) is oxidized to its radical cation ABTS<sup>•+</sup>, produced a blue colored solution ( $\lambda_{\text{max}} = 734$  nm), with potassium persulfate. When the reaction of ABTS<sup>•+</sup> with antioxidant is finished, the blue color of the solution turns colorless.

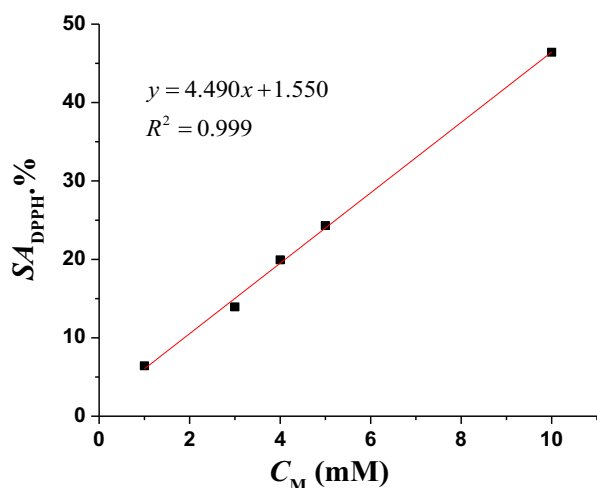
Fig. S2 shows ultraviolet–visible spectra of ABTS<sup>•+</sup> with different concentrations of DPTU and BPTU. Similarly to DPPH<sup>•</sup> assay, as the



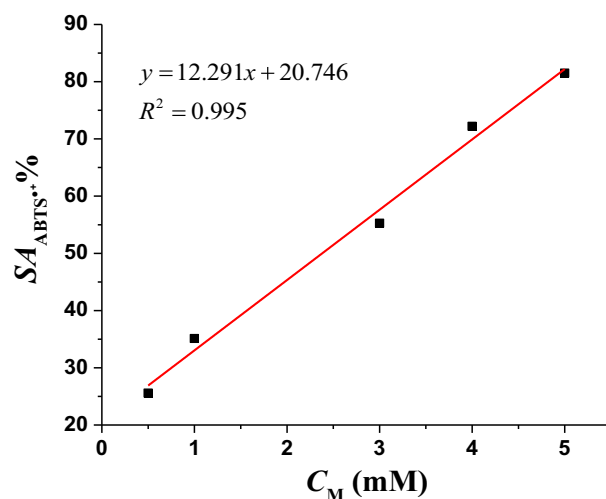
(a) DPTU



(a) DPTU



(b) BPTU



(b) BPTU

Fig. 1. Relationship between DPPH<sup>•</sup> scavenging efficiency and concentration of (a) DPTU and (b) BPTU.

Fig. 2. Relationship between ABTS<sup>•+</sup> scavenging efficiency and concentration of (a) DPTU and (b) BPTU.

concentration of thiourea derivatives increases, the visible ultraviolet spectral lines of the solution are gradually lower. This proves that the absorbance of the solution is decreasing, or in other words, the amount of  $\text{ABTS}^+$  in the solution is decreased.

$\text{IC}_{50_{\text{ABTS}}}$  of DPTU and BPTU are extrapolated from diagrams showing the relationship between  $\text{ABTS}^+$  free radical cation scavenging ability ( $\text{SA}_{\text{ABTS}^+}$ ) and antioxidant concentrations (Fig. 2). DPTU has  $\text{IC}_{50_{\text{ABTS}}}$  value of  $0.044 \pm 0.001$  mM while BPTU has this value of  $2.400 \pm 0.021$  mM. This demonstrates that DPTU is able to neutralize  $\text{ABTS}^+$  free radical cations better than BPTU.

Thus, two experimental methods (DPPH $\cdot$  and  $\text{ABTS}^+$ ) show DPTU can quench free radicals better than BPTU.

#### 4.3. Computational results

The structures of 1,3-diphenyl-2-thiourea (DPTU) and 1-benzyl-3-phenyl-2-thiourea (BPTU) were optimized at M05-2X/6-311++g(d,p) and shown in Fig. 3.

The thermochemical parameters of DPTU and BPTU were calculated both in the gas phase and ethanol at M05-2X/6-311++g(d,p) and given in Table 1.

BDE is an important parameter in HAT mechanism to evaluate the hydrogen atom transfer ability [31]. The lowest BDE values of DPTU are found at the N2–H17 and N3–H18 bonds (91.2 and 89.8 kcal·mol $^{-1}$  in the gas phase, 92.4 and 91.1 kcal·mol $^{-1}$  in ethanol, respectively). Meanwhile, BPTU has these lowest BDE values for the N2–H17, N3–H20 and C4–H18 bonds (97.5, 87.7 and 79.2 kcal·mol $^{-1}$  in the gas phase and 101.4, 90.3 and 81.0 kcal·mol $^{-1}$  in ethanol). And these BDE values tend to increase slightly by 1.2–3.9 kcal·mol $^{-1}$  in the ethanol.

There are many steps in SETPT and SPLET mechanisms. However, the first step plays the most crucial role from the thermochemical viewpoint [32]. In those first steps, the lowest quantities IE and PA will decide which mechanism is more dominant.

For SETPT mechanism, IE is a key factor showing the ability of electron donation [33]. From Table 1, IE values of DPTU and BPTU are not significantly different. However, IE values display a significant variation when comparing the calculated values in the gas phase and ethanol. Specifically, IE decreases from 49.6 kcal·mol $^{-1}$  to 50.2 kcal·mol $^{-1}$ . This means that the electron exchange process takes precedence over the ethanol solvent.

In SPLET mechanism, the calculated results of PA values in gas are higher than in ethanol for DPTU and BPTU. This is explained by the relative high enthalpies of proton and anion solvation.

In both media, the BDE values of the bonds are significantly lower than the sum of IE and PDE or PA and ETE. Therefore, it

can be concluded that HAT is the most predominant reaction pathway in the gas phase and SPLET is the most preferred mechanism in ethanol from the thermochemical viewpoint.

For a more comprehensive evaluation of thiourea derivatives to extinguish free radicals, their interactions with  $\text{HOO}\cdot$  have been studied in detail with two reaction mechanisms, HAT and SET. These reactions depend on many factors such as the nature of the free radicals, the molecular structure characteristics of antioxidants and the research environment [34].

According to Ingold and coworkers, a substance which can donate hydrogen atom perfectly must have low bond dissociation energy and the reaction speed must be fast [35]. Therefore, we proceed to construct the potential surface and calculate the reaction rate constant ( $k$ ) for the reactions of DPTU and BPTU with the  $\text{HOO}\cdot$  (selected as a representative radical).

Fig. 4 shows potential surface of reaction at the N2–H17, N3–H18 for DPTU and the N2–H17, N3–H20, C4–H18 for BPTU. All reactions start with the formation of intermediate 1 (Inter 1), through transition state (TS), intermediate 2 (Inter 2) and finally produce the products. In DPTU +  $\text{HOO}\cdot$  reaction, Inter 1, TS and Inter 2 are formed with the relative energies of  $-14.0$ ,  $2.6$  and  $1.6$  kcal·mol $^{-1}$  corresponding to reaction at the N2–H17. These values are  $-8.2$ ,  $11.1$  and  $-5.0$  kcal·mol $^{-1}$  for reaction of DPTU at the N3–H18. BPTU may have three HAT reaction sites: N2–H17, N3–H20 and C4–H18. The relative energy values of Inter 1, TS and Inter 2 are  $-14.1$ ,  $127.4$  and  $3.7$  kcal·mol $^{-1}$  for reaction of BPTU at the N2–H17;  $-4.1$ ,  $9.0$  and  $-5.2$  kcal·mol $^{-1}$  for reaction of BPTU at the N3–H20;  $-10.7$ ,  $5.5$  and  $-12.7$  kcal·mol $^{-1}$  for reaction of BPTU at the C4–H18. Clearly, the N2–H17 site of DPTU has the lowest transition energy barrier, so this may be the most preferred position in the hydrogen atom transfer reaction with  $\text{HOO}\cdot$  free radicals.

To evaluate the  $\text{HOO}\cdot$  free radical capturing reaction rates of the studied compounds, Gibbs free energy of activation ( $\Delta G^\ddagger$ ) and rate constant ( $k$ ) are calculated at M05-2X/6-311++G(d,p) at 298 K in the gas phase (Table 2).

In the HAT mechanism, the rate constants of DPTU are calculated (based on Eyringpy software) with the values of  $5.12 \times 10^4$  M $^{-1}$ s $^{-1}$  at the N2–H17 bond and  $5.00 \times 10^1$  M $^{-1}$ s $^{-1}$  at the N3–H18 bond. Thus the total rate constant of DPTU is estimated at  $5.12 \times 10^4$  M $^{-1}$ s $^{-1}$ . Meanwhile, this corresponding value of BPTU in the hydrogen atom transfer reaction with  $\text{HOO}\cdot$  is  $9.81 \times 10^2$  M $^{-1}$ s $^{-1}$ .

The activated Gibbs free energy of DPTU also has the lowest value of 11.6 kcal·mol $^{-1}$  at the N2–H17 position. This proves that the reaction of DPTU according to HAT mechanism at this bond is the most favorable. This is completely consistent with the

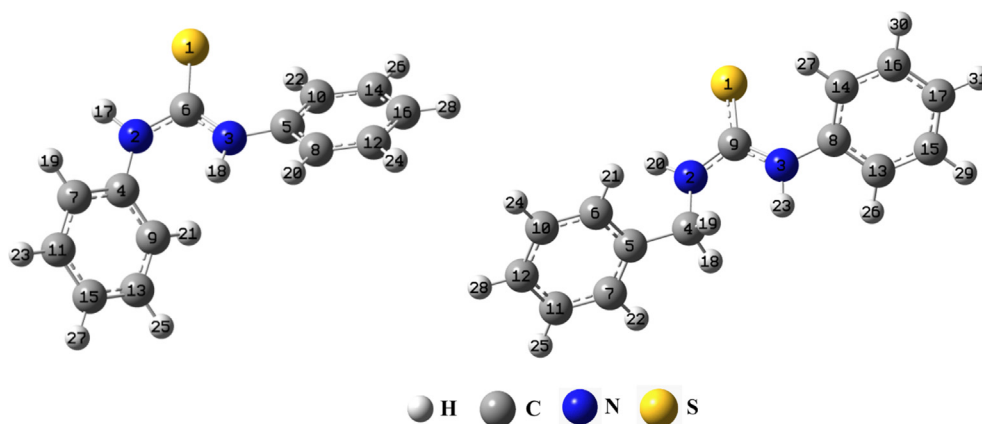
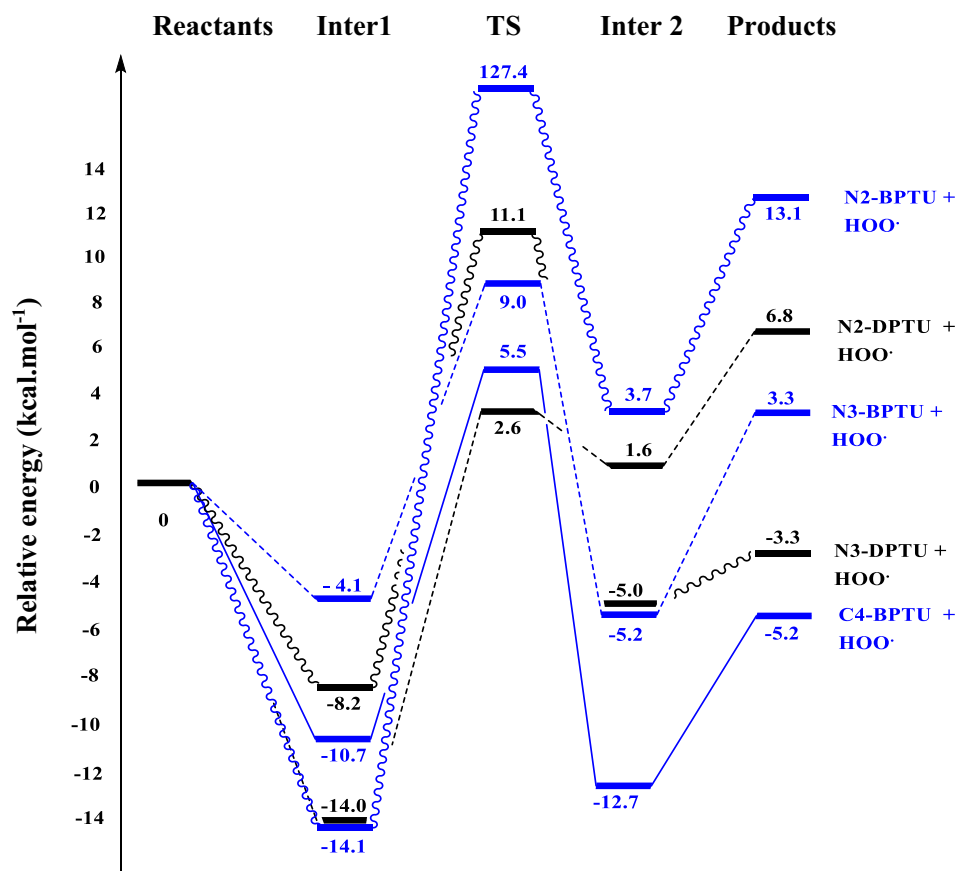


Fig. 3. Optimized structures of DPTU and BPTU at M05-2X/6-311++g(d,p).

**Table 1**  
Thermochemical parameters of DPTU and BPTU in the gas phase and ethanol at M05-2X/6-311++g (d, p).

Thermochemical parameters (kcal.mol <sup>-1</sup> )	DPTU		BPTU		
	N2-H17	N3-H18	N2-H17	N3-H20	C4-H18
BDE	91.2 (92.4)	89.8 (91.1)	97.5 (101.4)	87.7 (90.3)	79.2 (81.0)
IE	173.5 (123.3)		171.6 (122.0)		
PDE	231.7 (12.9)	230.3 (11.7)	239.9 (23.3)	230.1 (12.2)	221.6 (2.9)
PA	338.9 (43.0)	329.8 (37.4)	344.3 (48.1)	332.0 (38.7)	350.4 (65.7)
ETE	66.4 (93.2)	74.0 (97.6)	67.3 (97.3)	69.8 (95.5)	42.8 (59.3)

Data in ethanol are enclosed in parentheses.



**Fig. 4.** Potential energy surface of DPTU and BPTU reaction with HOO<sup>•</sup> calculated in the gas phase at the M05-2X/6-311++g(d,p) level of theory.

**Table 2**  
Activated Gibbs free energy ( $\Delta G^\ddagger$ ) and rate constant ( $k$ ) calculated at M05-2X/6-311++g(d,p) theory level at 298.15 K in gas phase.

Reactions	$\Delta G^\ddagger$ (kcal.mol <sup>-1</sup> )	$k$ (M <sup>-1</sup> s <sup>-1</sup> )	$k_{\text{total}}$ (M <sup>-1</sup> s <sup>-1</sup> )
DPTU-N2-H17 + HOO <sup>•</sup>	11.6	$5.12 \times 10^4$	$5.12 \times 10^4$
DPTU-N3-H18 + HOO <sup>•</sup>	21.6	$5.00 \times 10^1$	
BPTU-N2-H17 + HOO <sup>•</sup>	62.6	$5.54 \times 10^{-105}$	$9.81 \times 10^2$
BPTU-N3-H20 + HOO <sup>•</sup>	18.3	$1.99 \times 10^2$	
BPTU-C4-H18 + HOO <sup>•</sup>	15.3	$7.83 \times 10^2$	

**Table 3**  
Enthalpies, Gibbs free energies and rate constants at 298.15 K for the reactions (10), (11), (12) and (13) in the gas phase at M05-2X/6-311++G(d,p).

	$\Delta H^0$ (kcal.mol <sup>-1</sup> )	$\Delta G^0$ (kcal.mol <sup>-1</sup> )	$k_{\text{SET}}$ (M <sup>-1</sup> s <sup>-1</sup> )	$k_{\text{total}}$ (M <sup>-1</sup> s <sup>-1</sup> )
Reaction (10)	150.2	149.8	$1.46 \times 10^{-237}$	$1.46 \times 10^{-237}$
Reaction (11)	270.8	271.0	0	
Reaction (12)	148.3	148.2	$1.92 \times 10^{-242}$	$1.92 \times 10^{-242}$
Reaction (13)	279.4	278.7	0	

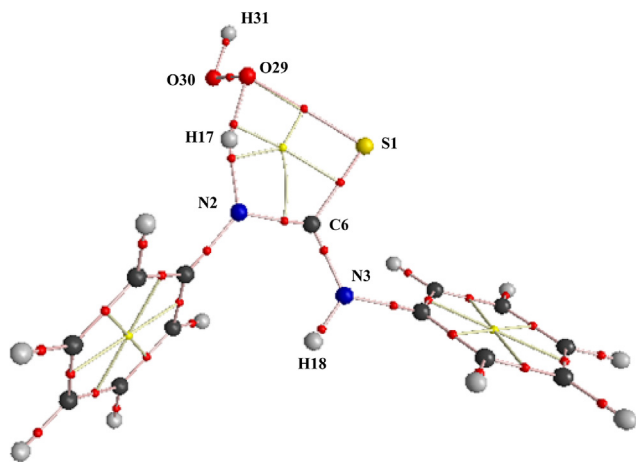
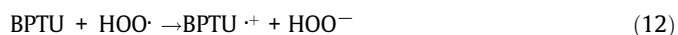
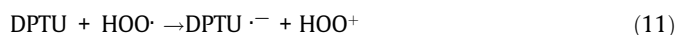
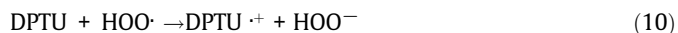


Fig. 5. Geometry of the formed transition state when DPTU approaches HOO· at the N2–H17 bond.

potential surface constructed in Fig. 4. Although BPTU has the lowest BDE value at the C4–H18 bond, the reaction speed at this position is not greater than that of DPTU's bonds. Therefore, this is also a reason why the IC50 values of DPTU are smaller than IC50 values of BPTU.

SET is the next mechanism which is applied to evaluate the antioxidant ability of thiourea derivatives in the gas phase. The processes of giving electron (reactions (10) and (12)), receiving electron (reactions (11) and (13)) between thiourea derivatives and HOO· can happen as follows:



Enthalpy ( $\Delta H^0$ ) and Gibbs free energy ( $\Delta G^0$ ) at 298.15 K for reaction (10) have values of 150.2 and 149.8 kcal·mol<sup>-1</sup>, respectively, while these values of reaction (11) are 270.8 and 271.0 kcal·mol<sup>-1</sup> (Table 3). This proves that reaction (10) is thermodynamically more favorable than reaction (11). Similarly, reaction (12) takes precedence over reaction (13). DPTU and BPTU tend to give electron more easily than they accept electron from HOO· free radicals.

The rate constants of electron donating reaction and electron accepting reaction are calculated according to the theory of Marcus [26].  $k_{\text{SET}}$  values are  $1.46 \times 10^{-237} \text{ M}^{-1}\text{s}^{-1}$ ,  $1.92 \times 10^{-242} \text{ M}^{-1}\text{s}^{-1}$  for reactions (10) and (12) and zero for both reactions (11) and (13). Compared with the value of  $k_{\text{HAT}}$ , the rate constant values of  $k_{\text{SET}}$  are minimal. This proves that the electron exchanging mechanism is negligible when thiourea derivatives react with HOO·.

Table 4

Parameters at the critical points in the intermolecular interaction of the transition state.

Contacts	$\rho(r)$ (e·au <sup>-3</sup> )	R(Å)	$\nabla^2\rho(r)$ (e·au <sup>-5</sup> )	$G(r)/ V(r) $	$H(r)$ (hartrees·au <sup>-3</sup> )	$E_{\text{HB}}$ (kJ·mol <sup>-1</sup> )
N2···H17	0.16	1.27	-0.21	0.36	-0.12	-253.87
H17···O29	0.18	1.20	-0.36	0.34	-0.18	-363.51
O29···O30	0.31	1.41	-0.06	0.48	-0.23	-592.26
O30···H31	0.37	0.96	-2.65	0.09	-0.73	-1046.28
O29···S1	0.05	2.43	0.11	1.06	0.00	-40.34

The relative amount of products ( $\Gamma_i$  %) yielded by HAT reaction mechanisms accounts for 99.99% of the total products created by the two mechanisms (HAT, SET) for both DPTU and BPTU. The N2–H17 bond of DPTU is evaluated as the position that easily transfers hydrogen as well as reacts with the highest speed in studied thiourea derivatives. Therefore, the reaction at this bond is chosen for AIM analysis to comprehend the nature of new bonds in detail when DPTU approaches HOO· free radical [36,37].

As can be seen in Fig. 5, the structure of TS is stabilized by H···O contacts. The distances of these contacts are in the range of 0.96–1.20 Å (Table 4), which are shorter than the total van der Waals radii of the considered atoms (2.72 Å). This confirms the existence of intermolecular interactions in the TS structure. The appearance of small red spheres represents bonding critical points (BCP), while the yellow ball represents a ring critical point (RCP). DPTU molecule approaches HOO· free radical leading to the formation of a pentagonal ring.

From Table 4, electron density ( $\rho(r)$ ) and Laplacian value ( $\nabla^2\rho(r)$ ) at the BCP related to O29···S1 contact are 0.05 e·au<sup>-3</sup> and 0.11 e·au<sup>-5</sup>. The lowest electron density value and the positive Laplacian value indicate that the O29···S1 contact has no covalent nature [38]. Conversely, the N2···H17, H17···O29, O29···O30, O30···H31 contacts have the negative values of  $\nabla^2\rho(r)$  and  $H(r)$ , and the ratios of  $G(r)/|V(r)|$  are smaller than 1. Therefore, they are considered to be covalent. The electron density and binding energy value at the critical point of the O30···H31 contact are much greater than these of the N2···H17, H17···O29, and O29···O30 contacts, which prove that the O30···H31 contact plays a significant role in a stabilization of the TS in a reaction of DPTU and HOO·.

## 5. Conclusions

The antioxidant ability of DPTU and BPTU is assessed by their capacity to capture DPPH· and ABTS· free radicals. In addition, quantum chemistry calculations are applied to calculate thermochemical parameters and construct the potential surface of the reaction with HOO· as well as to calculate the reaction rate constants. The results are multiple:

1. DPTU is better than BPTU in capturing DPPH· and ABTS· free radicals. The IC50<sub>DPPH</sub> and IC50<sub>ABTS</sub> values of DPTU are  $0.710 \pm 0.001 \text{ mM}$  and  $0.044 \pm 0.001 \text{ mM}$  respectively while these values of BPTU are  $11.000 \pm 0.015 \text{ mM}$  and  $2.400 \pm 0.021 \text{ mM}$ .
2. HAT mechanism is preferred over SET when thiourea derivatives react with HOO· free radicals. The amount of products obtained according to HAT mechanism accounts for 99.99% compared with the product amount of HAT and SET mechanisms.
3. The most preferred reaction position of DPTU is the N2–H17 with the reaction rate of  $5.12 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$  according to the HAT mechanism.
4. BDE is not a parameter that completely determines a substance's antioxidant ability. The reaction rate is a very important factor when assessing the antioxidant resistance of a compound.

- Quantum chemistry calculations are consistent with the experimental results based on DPPH<sup>•</sup> assay and ABTS<sup>•+</sup> assay.
- AIM analysis proves that O30...H31 interaction in transition state of DPTU when reacting with HOO<sup>•</sup> at the N2–H17 bond plays an essential role in stabilizing this transition.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molliq.2021.117149>.

## References

- C.D. Georgiou, N. Tairis, A. Sotiropoulou, Hydroxyl radical scavengers inhibit lateral-type sclerotial differentiation and growth in phytopathogenic fungi, *Mycologia* 92 (5) (2019) 825–834, <https://doi.org/10.1080/00275514.2000.12061226>.
- W. Wang, M.N. Schuchmann, H.P. Schuchmann, W. Knolle, J. von Sonntag, Radical cations in the OH<sup>•</sup>-radical-induced oxidation of thiourea and tetramethylthiourea in aqueous solution, *J. Am. Chem. Soc.* 121 (1) (1999) 238–245, <https://doi.org/10.1021/ja983275b>.
- A.R. Sudzhaev, I.A. Rzaeva, R.A. Nadzhafova, Y.S. Safarov, M.A. Allakhverdiev, Antioxidant properties of some thiourea derivatives, *Russ. J. Appl. Chem.* 84 (8) (2011) 1394–1397, <https://doi.org/10.1134/s1070427211080167>.
- T.L. Da Silva, L.M.F. Miolo, F.S.S. Sousa, L.M.P. Brod, L. Savegnago, P.H. Schneider, New thioureas based on thiazolidines with antioxidant potential, *Tetrahedron Lett.* 56 (48) (2015) 6674–6680, <https://doi.org/10.1016/j.tetlet.2015.10.037>.
- A.K. Prasad, P.C. Mishra, Scavenging of superoxide radical anion and hydroxyl radical by urea, thiourea, selenourea and their derivatives without any catalyst: a theoretical study, *Chem. Phys. Lett.* 684 (2017) 197–204, <https://doi.org/10.1016/j.cplett.2017.06.040>.
- S. Naz, M. Zahoor, M.N. Umar, S. Alghamdi, M.U.K. Sahibzada, W. UlBari, Synthesis, characterization, and pharmacological evaluation of thiourea derivatives, *Open Chem.* 18 (1) (2020) 764–777, <https://doi.org/10.1515/chem-2020-0139>.
- Q.V. Vo, P.C. Nam, M.V. Bay, N.M. Thong, L.T. Hieu, A. Mechler, A theoretical study of the radical scavenging activity of natural stilbenes, *RSC Adv.* 9 (72) (2019) 42020–42028, <https://doi.org/10.1039/c9ra08381b>.
- M. Leopoldini, S.G. Chiodo, N. Russo, M. Toscano, Detailed investigation of the OH radical quenching by natural antioxidant caffeic acid studied by quantum mechanical models, *J. Chem. Theory Comput.* 7 (12) (2011) 4218–4233, <https://doi.org/10.1021/ct200572p>.
- R. Re, N. Pellegrini, A. Prolegente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 26 (9–10) (1999) 1231–1237, [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3).
- M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, et al. *Gaussian 16 Rev. B.01*. Wallingford, CT2016.
- Y. Zhao, N.E. Schultz, D.G. Truhlar, Design of density functionals by combining the method of constraint satisfaction with parametrization for thermochemistry, thermochemical kinetics, and noncovalent interactions, *J. Chem. Theory Comput.* 2 (2) (2006) 364–382, <https://doi.org/10.1021/ct050276310.1021/ct0502763.s00110.1021/ct0502763.s002>.
- M.E. Alberto, N. Russo, A. Grand, A. Galano, A physicochemical examination of the free radical scavenging activity of Trolox: mechanism, kinetics and influence of the environment, *PCCP* 15 (13) (2013) 4642–4650, <https://doi.org/10.1039/c3cp43319f>.
- E. Vélez, J. Quijano, R. Notario, E. Pabón, J. Murillo, J. Leal, E. Zapata, G. Alarcón, A computational study of stereospecificity in the thermal elimination reaction of menthyl benzoate in the gas phase, *J. Phys. Chem.* 22 (10) (2009) 971–977, <https://doi.org/10.1002/poc.v22:1010.1002/poc.1547>.
- A. Galano, J.R. Alvarez-Idaboy, A computational methodology for accurate predictions of rate constants in solution: application to the assessment of primary antioxidant activity, *J. Comput. Chem.* 34 (28) (2013) 2430–2445, <https://doi.org/10.1002/jcc.23409>.
- J.M. Mayer, D.A. Hrovat, J.L. Thomas, W.T. Borden, Proton-coupled electron transfer versus hydrogen atom transfer in benzyl/toluene, methoxy/methanol, and phenoxy/phenol self-exchange reactions, *J. Am. Chem. Soc.* 124 (37) (2002) 11142–11147, <https://doi.org/10.1021/ja012732c>.
- A. Urbaniak, M. Szela, M. Molski, Theoretical investigation of stereochemistry and solvent influence on antioxidant activity of ferulic acid, *Comput. Theor. Chem.* 1012 (2013) 33–40, <https://doi.org/10.1016/j.cmpctc.2013.02.018>.
- M. Musialik, G. Litwinienko, Scavenging of dpph<sup>•</sup> radicals by vitamin E is accelerated by its partial ionization: the role of sequential proton loss electron transfer, *Org. Lett.* 7 (22) (2005) 4951–4954, <https://doi.org/10.1021/ol051962j>.
- N.M. Thong, D.T. Quang, N.H.T. Bui, D.Q. Dao, P.C. Nam, Antioxidant properties of xanthenes extracted from the pericarp of *Garcinia mangostana* (Mangosteen): a theoretical study, *Chem. Phys. Lett.* 625 (2015) 30–35, <https://doi.org/10.1016/j.cplett.2015.02.033>.
- L. Tabrizi, D.Q. Dao, T.A. Vu, Experimental and theoretical evaluation on the antioxidant activity of a copper(ii) complex based on lidocaine and ibuprofen amide-phenanthroline agents, *RSC Adv.* 9 (6) (2019) 3320–3335, <https://doi.org/10.1039/c8ra09763a>.
- N.M. Thong, Q.V. Vo, T.L. Huyen, M.V. Bay, D. Tuan, P.C. Nam, Theoretical study for exploring the diglycoside substituent effect on the antioxidative capability of isorhamnetin extracted from *anoechtichilus roxburghii*, *ACS Omega* 4 (12) (2019) 14996–15003, <https://doi.org/10.1021/acsomega.9b0178010.1021/acsomega.9b01780.s001>.
- H. Zhou, X. Li, Y. Shang, K. Chen, Radical scavenging activity of puerarin: a theoretical study, *Antioxidants* (Basel) 8 (12) (2019) 590, <https://doi.org/10.3390/antiox8120590>.
- P.T. Thuy, N.V. Trang, N.T. Son, Antioxidation of 2-phenylbenzofuran derivatives: structural-electronic effects and mechanisms, *RSC Adv.* 10 (11) (2020) 6315–6332, <https://doi.org/10.1039/c9ra10835a>.
- J. Rimarčík, V. Lukeš, E. Klein, M. Ilčin, 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 (1–3) (2010) 25–30, <https://doi.org/10.1016/j.theochem.2010.04.002>.
- E. Dzib, J.L. Cabellos, F. Ortíz-Chi, S. Pan, A. Galano, G. Merino, Eyringpy: a program for computing rate constants in the gas phase and in solution, *Int. J. Quantum Chem.* 119 (2) (2019) e25686, <https://doi.org/10.1002/qua.v119.210.1002/qua.25686>.
- R.A. Marcus, Chemical and electrochemical electron-transfer theory, *Annu. Rev. Phys. Chem.* 15 (1) (1964) 155–196, <https://doi.org/10.1146/annurev.pc.15.100164.001103>.
- R.A. Marcus, Electron transfer reactions in chemistry, *Theory Exp. Rev. Mod. Phys.* 65 (3) (1993) 599–610, <https://doi.org/10.1103/RevModPhys.65.599>.
- S.F. Nelsen, M.N. Weaver, Y. Luo, J.R. Pladziewicz, L.K. Ausman, T.L. Jentsch, J.J. O'Konek, Estimation of electronic coupling for intermolecular electron transfer from cross-reaction data, *J. Phys. Chem. A* 110 (41) (2006) 11665–11676, <https://doi.org/10.1021/jp064406v10.1021/jp064406v.s001>.
- E. Wigner, On the quantum correction for thermodynamic equilibrium, *Phys. Rev.* 40 (5) (1932) 749–759, <https://doi.org/10.1103/PhysRev.40.749>.
- C. Eckart, The penetration of a potential barrier by electrons, *Phys. Rev.* 35 (11) (1930) 1303–1309, <https://doi.org/10.1103/PhysRev.35.1303>.
- F. Biegler-König, J. Schönbohm, Update of the AIM2000-program for atoms in molecules, *J. Comput. Chem.* 23 (15) (2002) 1489–1494, <https://doi.org/10.1002/jcc.v23:1510.1002/jcc.10085>.
- Y.-Z. Zheng, G. Deng, R. Guo, D.-F. Chen, Z.-M. Fu, Substituent effects on the radical scavenging activity of isoflavonoid, *Int. J. Mol. Sci.* 20 (2) (2019) 397, <https://doi.org/10.3390/ijms20020397>.
- M.S. Blois, Antioxidant determinations by the use of a stable free radical, *Nature* 181 (4617) (1958) 1199–1200, <https://doi.org/10.1038/1811199a0>.
- D.Q. Dao, T.C. Ngo, N.M. Thong, P.C. Nam, Is vitamin A an antioxidant or a pro-oxidant?, *J. Phys. Chem. B* 121 (40) (2017) 9348–9357, <https://doi.org/10.1021/acs.jpcc.7b0706510.1021/acs.jpcc.7b07065.s001>.
- A. Galano, Relative antioxidant efficiency of a large series of carotenoids in terms of one electron transfer reactions, *J. Phys. Chem. B* 111 (44) (2007) 12898–12908, <https://doi.org/10.1021/jp074358u>.
- K.U. Ingold, D.A. Pratt, Advances in radical-trapping antioxidant chemistry in the 21st century: a kinetics and mechanisms perspective, *Chem. Rev.* 114 (18) (2014) 9022–9046, <https://doi.org/10.1021/ct500226n>.
- A. Filarowski, I. Majerz, AIM analysis of intramolecular hydrogen bonding in O-hydroxy aryl Schiff bases, *J. Phys. Chem. A* 112 (14) (2008) 3119–3126, <https://doi.org/10.1021/jp076253x>.
- I. Rozas, I. Alkorta, J. Elguero, Behavior of ylides containing N, O, and C atoms as hydrogen bond acceptors, *J. Am. Chem. Soc.* 122 (45) (2000) 11154–11161, <https://doi.org/10.1021/ja0017864>.
- L. Zhang, F. Ying, W. Wu, P. Hiberty, S. Shaik, Topology of electron charge density for chemical bonds from valence bond theory: a probe of bonding types, *Chemistry* 15 (12) (2009) 2979–2989, <https://doi.org/10.1002/chem.v15:1210.1002/chem.200802134>.