



# Understanding the Chemical Reactivity of Dihydroxyfumaric Acid and its Derivatives through Conceptual DFT

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**Abstract:** *The chemical reactivity descriptors have been calculated through Molecular Electron Density Theory encompassing Conceptual DFT. The validity of “Koopmans’ theorem in DFT” (KID) has been assessed by a comparison between the global descriptors (electronegativity, total hardness, and global electrophilicity) calculated through vertical energy values and those arising from the HOMO and LUMO values. These results suggest that the KID procedure is valid and may be used, in conjunction with the B3LYP/3-611G(d, p) level of theory in further studies of related compounds in the aqueous medium. The active sites for nucleophilic and electrophilic attacks have been identified and verified using the local reactivity descriptors: the dual descriptor, the electrophilic and nucleophilic Parr functions, the local reactivity difference index  $R_k$  and MEP maps. Obtained results suggest that the antioxidant/antiradical power of investigated compounds may be explained by the highest ambiphilic activation of the oxygen atoms of the hydroxyl groups in the ene-diol moiety.*

**Keywords:** *dihydroxyfumaric acid, conceptual DFT, Koopmans’ theorem in DFT, ambiphilic reactivity*

## 1. Introduction

Dihydroxyfumaric acid (DHF<sub>4</sub>) has recently attracted attention in the context of the “glyoxylate scenario”, which suggested that it might have been one of the central building blocks in the synthesis of complex organic macromolecular structures, such as sugars and nucleosides [1].

The chemical reactivity of dihydroxyfumaric acid is mainly due to its ene-diol moiety, which is readily oxidized or reduced, depending on the environment. The ene-diol fragment has the ability to efficiently capture electrons from free radicals, making DHF<sub>4</sub> a good radical scavenger, similar to ascorbic acid. Previous research showed that DHF and some of its salts and derivatives may be successfully used as antioxidants in wines [2, 3], inhibitors of nitrosoamines formation in vitro [4,5] and in vivo [6,7], as well as efficient free-radical scavengers [8, 9].

The ene-diol moiety is of interest from the synthetic point of view, as well. DHF<sub>4</sub> is prone to auto-oxidation and decarboxylation, but its dimethyl ester is stable in solution for days, making it a good starting point for further synthesis. The chemical structure of the DHF<sub>4</sub> and its dimethyl ester give two possible types of bond forming reactivity: through the C atom in the carboxylic acid fragment, which favors interactions with nucleophilic reagents, or through the ene-diol moiety, susceptible to electrophiles.

A number of new derivatives have been previously synthesized by our group [8, 9] by modifying the natural dihydroxyfumaric acid and its dimethyl ester, in the search for new synthons for targeted synthesis and to study the “structure-property” relationship. The antioxidant/antiradical activity of the compounds has been investigated using the DPPH radical and ABTS radical cation assays, and it was found that some of the new derivatives exhibited good antioxidant/antiradical activity, and others didn’t, despite the fact that the ene-diol was structurally preserved [8].

An understanding of the chemical interactions is essential for the synthesis of new derivatives with antioxidant/antiradical properties, therefore, this research seeks to obtain chemical reactivity information, including global and local reactivity descriptors, by means of the Density Functional Theory (DFT) derived concepts. This will allow to explain previously obtained results, and predict the active reaction sites for new derivatives.

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To our knowledge, this is the first DFT study of the investigated compounds to evaluate global and local reactivity descriptors. Therefore, we consider that this kind of study will contribute to get a better understanding of the chemical behavior, in the gas and aqueous phases, of these molecules, which can be of potential utility in the process of designing new antioxidant/antiradical compounds for food additives or pharmaceutical drugs.

## 2. Materials and methods

### 2.1. Theoretical background and methods

Density functional theory (DFT) offers extraordinary tools to qualitatively understand and quantitatively predict the global and local chemical reactivity of molecules.

Global descriptors measure the overall susceptibility of a system to different types of reactions, e.g., electrophilic or nucleophilic attacks, and assign a single value to the entire molecule.

The simplest global reactivity descriptors are the first ionization potential  $I$ , defined as the energy needed to remove an electron from a molecule, and electron affinity,  $A$ , which measures the ability of a molecule to accept electrons and form anions. The chemical potential ( $\mu$ ) represents the intrinsic Lewis acidity/basicity of a system and is always negative; electronegativity ( $\chi$ ) represents the tendency of atoms or molecules to attract electrons; the global hardness ( $\eta$ ) can be seen as the resistance to charge transfer and is always positive, electrophilicity ( $\omega$ ) represents the stabilization energy when the system acquires electrons from the environment up to saturation. Although the validity of the “Koopmans’ theorem within the DFT” (KID) approximation is controversial [10], the use of the energies of frontier molecular orbitals as an approximation to obtain  $I$  and  $A$  is supported by the Janak’s theorem [11]. In particular, it was found that the negative of Hartree-Fock and Kohn-Sham HOMO orbital defined upper and lower limits, respectively, for the experimental values of the first ionization potential [12]. Using a finite difference approximation and Koopmans’ theorem [13], these descriptors may be expressed by the following equations:

$$I = E_{N-1} - E_N \approx -\epsilon_H \quad (1)$$

$$A = E_N - E_{N+1} \approx -\epsilon_L \quad (2)$$

$$\mu = -\chi \approx -\frac{1}{2}(I + A) \approx \frac{1}{2}(\epsilon_L + \epsilon_H) \quad (3)$$

$$\eta \approx \frac{1}{2}(I - A) \approx \frac{1}{2}(\epsilon_L - \epsilon_H) \quad (4)$$

$$\omega = \frac{\mu^2}{2\eta} \approx \frac{(I+A)^2}{2(I-A)} \approx \frac{(\epsilon_L + \epsilon_H)^2}{2(\epsilon_H + \epsilon_L)} \quad (5)$$

where  $\epsilon_H$  and  $\epsilon_L$  are the energies of the highest occupied and the lowest unoccupied molecular orbitals, HOMO and LUMO, respectively and  $E_N$ ,  $E_{N-1}$ , and  $E_{N+1}$  represent the energy of the systems with  $N$ ,  $N - 1$ , and  $N + 1$  electrons, respectively. This suggests that the KID procedure can efficiently be used to calculate the global descriptors directly from the energies of the frontier orbitals, without resorting to the time-consuming calculations of the single point energies of the anion and cation.

Besides global reactivity descriptors, DFT allows the computation of local descriptors, which assign a value to every point in space, providing insight into the reactivity of the molecule at point  $r$  in space; they are regioselectivity indicators.

In the frames of DFT, the first local reactivity descriptor that is used to characterize electrostatic interactions between molecules is the electronic density  $\rho(\vec{r})$ . However, when the chemical reactions are due to mainly covalent interactions, a second order local reactivity descriptor is used, the Fukui



function, which allows the identification of the most reactive sites of a molecule, those prone to electrophilic, nucleophilic or radical attacks.

The Fukui function is defined in terms of the derivative of  $\rho(\vec{r})$  with respect to  $N$ , given by eq. (6), as follows:

$$f(\vec{r}) = \left( \frac{\partial \rho(\vec{r})}{\partial N} \right)_{v(\vec{r})} \quad (6)$$

where  $\rho(\vec{r})$ ,  $N$  and  $v(\vec{r})$  are the electronic density, number of electrons and external potential exerted by the nucleus, respectively. The function  $f(\vec{r})$  reflects the change of electron density induced by the change of absolute electron number with a fixed geometry and potential.

Depending on total electronic densities, the Fukui functions can be defined in two ways, based on the right and left derivatives of  $\rho(\vec{r})$  with respect to  $N$  (eq. 7 and 8), and the third definition gives the radical attack descriptor (eq. 9), in line with Perdew et al.'s [14] conclusions:

$$f^+(\vec{r}) = \left( \frac{\partial \rho(\vec{r})}{\partial N} \right)_{v(\vec{r})}^+ = \rho_{N+1}(\vec{r}) - \rho_N(\vec{r}) \quad (7)$$

$$f^-(\vec{r}) = \left( \frac{\partial \rho(\vec{r})}{\partial N} \right)_{v(\vec{r})}^- = \rho_N(\vec{r}) - \rho_{N-1}(\vec{r}) \quad (8)$$

$$f^0(\vec{r}) = \frac{1}{2} [\rho_{N+1}(\vec{r}) - \rho_{N-1}(\vec{r})] \quad (9)$$

where  $\rho(\vec{r})$  is the atomic charge at the  $r^{\text{th}}$  atomic site, for the neutral ( $N$ ), anionic ( $N+1$ ) and cationic ( $N-1$ ) chemical species.

The electrophilic descriptor  $f^+(\vec{r})$  measures the intramolecular reactivity at the site  $r$  toward a nucleophilic reagent, and the highest values of  $f^+(\vec{r})$  will imply the preferred site for a nucleophilic attack (by a nucleophile molecule). The nucleophilic Fukui function  $f^-(\vec{r})$  measures the intramolecular reactivity at the site  $r$  toward an electrophilic reagent, and the highest value indicates the preferred site for an electrophilic attack [15].

Although the Fukui function is very useful in revealing nucleophilic and electrophilic regions on a molecule, a more accurate local reactivity descriptor has been proposed by Morell et al. [16], i.e. the dual descriptor, expressed as the derivative of the Fukui function with respect to the electron number:

$$f^{(2)}(\vec{r}) = \left( \frac{\partial f(\vec{r})}{\partial N} \right)_{v(\vec{r})} \cong f^+(\vec{r}) - f^-(\vec{r}) \quad (10)$$

Thus, the dual descriptor may be approximated to the difference between the Fukui functions for nucleophilic and electrophilic attacks, and therefore it aids in revealing the true preferred sites for electrophilic and nucleophilic attacks simultaneously. The dual descriptor is especially useful in ambiguous situations, when no exact information can be derived from  $f^+(\vec{r})$  and  $f^-(\vec{r})$ , because it is a combination of the two. The dual descriptor is positive for locations susceptible to nucleophilic attacks, and negative where an electrophilic attack is more probable.

In terms of densities of frontier molecular orbitals (frontier molecular orbital approximation, FMOA), eq. (10) turns into eq. (11) as follows:

$$f^{(2)}(\vec{r}) = \rho_{LUMO}(\vec{r}) - \rho_{HOMO}(\vec{r}) \quad (11)$$

In this form, this expression implies that the local reactivity of the molecule depends only on the electronic densities of frontier molecular orbitals. Thus, the electronic densities of the remaining



occupied molecular densities are not involved, and the relaxation effects are excluded, making the dual descriptor a more accurate local reactivity descriptor than the  $f^+(\vec{r})$  and  $f^-(\vec{r})$  [16].

Recently, Luis R. Domingo introduced other local reactivity descriptors, within his concept of Molecular Electron Density Theory (MEDT), i.e. the Parr functions [17, 18] that can be considered as an alternative to the Fukui functions for describing the areas within the molecules where nucleophilic or electrophilic attacks will be favored. The Parr functions can be expressed by the following equations:

$$P^-(\vec{r}) = \rho_s^{rc}(\vec{r}) \quad (12)$$

$$P^+(\vec{r}) = \rho_s^{ra}(\vec{r}) \quad (13)$$

where  $P^-(\vec{r})$  and  $P^+(\vec{r})$  are the nucleophilic and electrophilic Parr functions, respectively, and  $\rho_s^{rc}(\vec{r})$  and  $\rho_s^{ra}(\vec{r})$  are related to the atomic spin density of the radical cation and radical anion, respectively [19].

It should be noted, however, that Fukui and Parr functions may give poor and even contradictory results for systems that present hyperconjugation, and this effect is stronger in the charged species than in the neutral species [19].

In order to overcome this possible shortcoming, the reactivity difference descriptor  $R_k$ , proposed by Chattaraj et al. [20] was also employed in this research, allowing to identify the centers with electrophilic ( $R_k = +$  n.nn) or nucleophilic ( $R_k = -$  n.nn) behavior as well as the ambiphilic ( $R_k = \pm$  n.nn) behavior, and to eliminate the centers with marginal reactivity [20]:

(a) if  $(1 < w_k/N_k < 2)$  or  $(1 < N_k/w_k < 2)$   
then  $R_k \approx (w_k + N_k)/2 \rightarrow$  ambiphilic ( $R_k = \pm$  n.nn)

(b) else  $R_k \approx (w_k - N_k)$   
for  $R_k > 0 \rightarrow$  electrophilic ( $R_k = +$  n.nn)  
for  $R_k < 0 \rightarrow$  nucleophilic ( $R_k = -$  n.nn)

(c) if  $|R_k| < 0.1$ , then  $R_k = 0$ .

The sign (+, -,  $\pm$ ) in the  $R_k$  index indicates the electrophilic or/and nucleophilic character of the center  $k$ , while the magnitude n.nn provides a measure of the local activation. The local electrophilicity ( $\omega_k$ ) and local nucleophilicity ( $N_k$ ), at the atomic site  $k$  are defined in terms of the related condensed Fukui functions,  $f_k^+$  and  $f_k^-$ , according to the following:

$$\omega_k = \omega \cdot f_k^+ \quad (9)$$

and

$$N_k = N \cdot f_k^- \quad (10)$$

where  $N$  is the nucleophilicity defined relative to tetracyanoethylene (TCE) [20]. TCE is used as a reference because it presents the lowest HOMO energy in a large series of molecules already investigated, allowing the creation of a normalized scale of positive values:

$$N = E_{HOMO(Nu)} (eV) - E_{HOMO(TCE)} (eV) \quad (11)$$

The Fukui functions condensed at the atomic site  $k$ , for the nucleophilic attack ( $f_k^+$ ) and for the electrophilic attack ( $f_k^-$ ) are given in terms of the respective population  $q_k(N)$  of the  $N$ -electronic system of  $k$  atomic site, as 13,14 the following:

$$f_k^+ = q_k(N + 1) - q_k(N) \quad (12)$$

and

$$f_k^- = q_k(N) - q_k(N - 1) \quad (13)$$

Another useful predictor of chemical reactivity, employed in this research for verification purposes, is the electrostatic potential, which shows negative potential regions, expected to be sites of protonation and nucleophilic attack, and positive potential regions, which may indicate electrophilic sites. This is an important predictive tool, since electrostatic attraction/repulsion is long-ranged compared to the electron-transfer effects characterized by the dual descriptor [21]. A classic molecular electrostatic potential (MEP) map indicates the red regions of negative electrostatic potential, the blue sites represent the regions of positive electrostatic potential and the parts of green color represent the regions of zero potential.

## 2.2. Computational methods

The following molecules have been investigated in this research: dihydroxyfumaric acid, dimethyl 2,3-dihydroxyfumarate, 2,3-dihydroxy-N1,N4-bis(2-hydroxyethyl)fumaramide and (*E*)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate. The molecular structures of investigated molecules were optimized at B3LYP/6-311G(d,p) in gas phase and the absence of negative values in the vibrational profile confirmed true minima. In order to analyze the effect of water, these structures were further used as the starting point for an optimization at B3LYP/6-311G(d, p) in water, employing the SMD solvation model. All calculations were performed with the ORCA computational software [22].

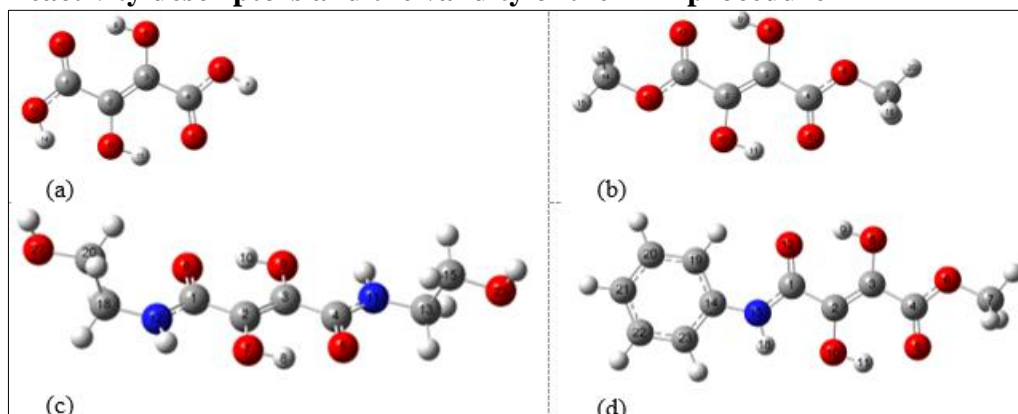
In order to compare the information derived from local reactivity descriptors, Fukui functions and Parr functions were computed. Due to the sensitivity of the Fukui function to the type of population analysis used, in the present work we calculated the values of the Fukui function by employing the Hirshfeld and NBO populations charges of the respective anions and cations. The Parr functions were calculated using the Hirshfeld and Mulliken atomic spin densities of the respective radical anions and radical cations.

In order to validate the use of energies of Kohn-Sham frontier molecular orbital to calculate reactivity descriptors coming from Conceptual DFT, the global descriptors were calculated using both techniques, the ionization potentials *I* and electron affinities *A* (in eV), and the HOMO and LUMO energies, in gas phase and in aqueous media using the SMD solvation model.

Cartesian coordinates of optimized molecules, in gas phase and in water, as well as Hirshfeld and NBO populations charges and Hirshfeld and Mulliken atomic spin densities are presented in the Supplementary material.

## 3. Results and discussions

### 3.1. Global reactivity descriptors and the validity of the KID procedure



**Figure 1.** The optimized structures of investigated compounds in aqueous phase: (a) dihydroxyfumaric acid, (b) dimethyl 2,3-dihydroxyfumarate, (3) 2,3-dihydroxy-N1,N4-bis(2-hydroxyethyl)fumaramide and (4) (*E*)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate



Figure 1 shows the optimized structures of investigated compounds in aqueous phase. Compounds (a), (b) and (d) are planar, while the fumaramide is twisted, with the dihedral angles  $\angle(\text{C}20\text{C}18\text{N}12\text{C}1) = 88^\circ$ ,  $\angle(\text{C}15\text{C}13\text{N}11\text{C}4) = 86^\circ$ ,  $\angle(\text{O}27\text{C}20\text{C}18\text{N}12) = 179.9^\circ$  and  $\angle(\text{O}23\text{C}15\text{C}13\text{N}11) = 179.8^\circ$ . Apart of this difference, the ene-diol moiety preserves its bonds and angles in the structures of investigated molecules at almost equal values.

Table 1 presents the values of the electronic energies calculated for the investigated compounds with the charges +1, 0 and -1, which corresponds to the cationic, neutral and anionic species, respectively. The energy difference indicates that water stabilizes the compounds by 25-80  $\text{kJ}\cdot\text{mol}^{-1}$ .

**Table 1.** Electronic energies in hartrees of the studied compounds, in gas phase and in water

Compound	Phase	Charge +1 (multiplicity = 2)	Charge 0 (multiplicity = 1)	Charge -1 (multiplicity = 2)
Dihydroxyfumaric acid	Gas	-605.7484223	-606.088655	-606.1035926
	Water	-605.8721992	-606.1047084	-606.1947288
Dimethyl ester of DHF4	Gas	-684.346181	-684.6495683	-684.6518663
	Water	-684.4335329	-684.6597513	-684.7452721
2,3-dihydroxy-N1,N4-bis(2-hydroxyethyl)fumaramide	Gas	-873.64297	-873.9349746	-873.9249985
	Water	-873.7486554	-873.9658111	-874.0341436
(E)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate	Gas	-856.1784784	-856.4595639	-856.4722537
	Water	-856.2537816	-856.470400	-856.5552357

The values presented in Table 1 were used to calculate the vertical ionization potentials  $I$  and electron affinities  $A$  (in eV), and global reactivity descriptors (electronegativity  $\chi$ , total hardness  $\eta$ , and global electrophilicity  $\omega$ ) of the studied molecules. These descriptors, along with the HOMO and LUMO orbital energies (in eV), are presented in Table 2.

**Table 2.** HOMO and LUMO orbital energies (in eV), the HOMO-LUMO gap  $\Delta E_{\text{HOMO-LUMO}}$  (eV), ionization potentials  $I$  and electron affinities  $A$  (in eV), and global electronegativity  $\chi$  (in eV), total hardness  $\eta$  (in eV), and global electrophilicity  $\omega$  (in eV) of the studied compounds

Property	Dihydroxyfumaric acid		Dimethyl 2,3-dihydroxy-fumarate		2,3-dihydroxy-N1,N4-bis(2-hydroxyethyl)fumaramide		(E)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate	
	Vacuum	Water	Vacuum	Water	Vacuum	Water	Vacuum	Water
$E_{\text{HOMO}}$	-6.91	-6.70	-6.05	-6.28	-5.90	-6.04	-6.00	-6.09
$E_{\text{LUMO}}$	-2.56	-2.56	-2.05	-2.15	-1.56	-1.76	-2.04	-2.09
$\Delta E_{\text{HOMO-LUMO}}$	4.35	4.14	4.00	4.13	4.34	4.28	3.96	4.00
$\chi$	4.73	4.63	4.05	4.22	3.73	3.90	4.02	4.09
$\eta$	2.17	2.07	2.00	2.07	2.17	2.14	1.98	2.00
$\omega$	10.31	10.36	8.20	8.60	6.41	7.11	8.16	8.36
$I$	9.26	6.33	8.26	6.16	7.95	5.91	7.65	5.89
$A$	0.41	2.45	0.06	2.33	-0.27	1.86	0.35	2.31
$\chi$	4.83	4.39	4.16	4.24	3.84	3.88	4.00	4.10
$\eta$	4.43	1.94	4.10	1.91	4.11	2.03	3.65	1.79
$\omega$	5.28	9.93	4.22	9.40	3.58	7.45	4.37	9.38

The upper part of the table shows the results derived assuming the validity of Koopmans' theorem, and the lower part shows the results derived from the calculated vertical  $I$  and  $A$ . As can be seen from Table 2, the Koopman's theorem holds well for the aqueous medium calculations. For the gas phase, the electronegativity is predicted surprisingly well, while the total hardness is 2-fold lower and the



electrophilicity is 2-fold higher than the data obtained from the data obtained employing the calculated vertical  $I$  and  $A$ . These results suggest that the KID procedure is valid and may be used in further studies of related compounds in aqueous media.

### 3.2. Comparative analysis of the local reactivity indices

The dual descriptor  $f^{(2)}(\vec{r})$ , and the electrophilic and nucleophilic Parr functions over the atoms of the studied compounds are shown in Tables 3-6. The actual values have been multiplied by 100 for an easier comparison. One of the main criticisms regarding the calculation of Fukui functions is the arbitrariness in the way of choosing the charges [23]. In this research, the Fukui functions were calculated using Hirshfeld [24] and NBO charges [25], and results were similar. Further, only results based on Hirshfeld charges are presented. Results for hydrogen atoms are omitted here, and are available in supplementary material.

**Table 3.** The condensed dual descriptor  $f_k^{(2)}$ , the electrophilic  $P_k^+$  and nucleophilic  $P_k^-$  Parr functions, and the local reactivity difference index  $R_k$  over the atoms of the dihydroxyfumaric acid molecule, calculated *in vacuo* and in water. The actual values of  $f^{(2)}(\vec{r})$ ,  $P_k^+$  and  $P_k^-$  have been multiplied by 100 for an easier comparison

Atom	$f_k^{(2)}$		$P_k^+$		$P_k^-$		$R_k$	
	Gas	Water	Gas	Water	Gas	Water	Gas	Water
<b>1 C</b>	<b>-7.1</b>	<b>-7.1</b>	<b>16.8</b>	<b>17.6</b>	-3.5	-2.7	-0.47	-1.02
<b>2 C</b>	3.8	4.2	<b>15.9</b>	<b>15.0</b>	<b>24.3</b>	<b>24.7</b>	<b>±0.37</b>	-0.66
<b>3 C</b>	3.8	3.9	<b>15.9</b>	<b>15.5</b>	<b>24.3</b>	<b>22.3</b>	<b>±0.37</b>	-0.68
<b>4 C</b>	<b>-7.1</b>	<b>-7.4</b>	<b>16.8</b>	<b>18.2</b>	-3.5	-2.5	-0.47	-1.04
5 O	-3.4	-4.6	12.4	11.4	7.3	5.0	-0.41	-0.94
6 O	-2.0	-3.2	2.7	3.1	-0.8	-0.1	-0.28	-0.57
<b>8 O</b>	<b>8.6</b>	<b>10.5</b>	2.6	2.8	<b>23.3</b>	<b>25.5</b>	<b>±0.34</b>	<b>±0.51</b>
<b>10 O</b>	<b>8.6</b>	<b>9.7</b>	2.6	2.7	<b>23.3</b>	<b>23.8</b>	<b>±0.34</b>	<b>±0.48</b>
12 O	-3.4	-4.3	12.4	11.4	7.3	5.5	-0.41	-0.94
13 O	-2.0	-2.9	2.7	3.0	-0.8	-0.2	-0.28	-0.58

From Tables 1S-8S (in supplementary materials) note that many values for  $f_k^+$ ,  $f_k^-$  and  $f_k^0$  of the investigated compounds are negative, both in gas and water. Basically this means that in the cationic state, the electron density increases and in the anionic state, the electron density decreases on all atoms. Ayres et al., has previously suggested the relation of this behavior with the reduction and oxidation of atomic centers in the molecule [26].

Thus, in the case of the investigated compounds, no conclusions can be accurately obtained from the electrophilic and nucleophilic Fukui function alone. Instead, the sign of the dual descriptor can describe unambiguously nucleophilic and electrophilic sites within the molecule, as previously mentioned. Specifically, if  $f^{(2)}(\vec{r}) > 0$ , then the site is favored for a nucleophilic attack, whereas if  $f^{(2)}(\vec{r}) < 0$ , then the site may be favored for an electrophilic attack [16,27].

For DHF<sub>4</sub> acid, the data presented in Table 3 shows that in the gas phase, according to the value of the dual descriptor, the preferred sites for the nucleophilic attack are the O8 and O10 ( $f_k^{(2)} = 8.6$ ) followed by C2 and C3 ( $f_k^{(2)} = 3.8$ ), while the most susceptible sites for an electrophilic attack are C1 and C4 ( $f_k^{(2)} = -7.1$ ). The calculated Parr functions indicate that the C1 and C4 ( $P_k^+ = 16.8$ ), and C2 and C3 ( $P_k^+ = 15.9$ ) sites will be preferred for a nucleophilic attack, while C2 and C3 ( $P_k^- = 24.3$ ) and O8 and O10 ( $P_k^- = 23.3$ ) will be the most prone sites for an electrophilic attack.

These results may seem controversial at first sight, but this may be explained by the fact that dihydroxyfumaric acid is a hyperconjugated system, stabilized by the interactions between the C=C and C=O double bonds, and the lone pairs of oxygen electrons, and therefore some of the groups may behave ambiphilic.



The MEP map of DFH<sub>4</sub> (Figure 2a) confirms that the region over C1 and C4 is positively charged, so an electrophile would be repelled, favoring these sites for a nucleophilic attack, as suggested by the Parr function, and confirmed by organic chemistry experiments, while the electrostatic potential over C2, C3 and O8, O10 is very close to neutral, suggesting that these sites could, indeed, be approached by (neutral or positive) electrophiles and (neutral or negative) nucleophiles.

To verify this statement, the local reactivity difference  $R_k$  index was computed (Table 3). The results confirm that C2, C3 and O8, O10 are ambiphilic sites that can act as electrophiles and nucleophiles in the gas phase. In the aqueous media, the reactivity descriptors slightly increase and the trend remains the same, with the only difference that O8 and O10 are shown as the only ambiphilic reactive sites, with  $R_k$  of  $\pm 0.48$  and  $\pm 0.51$ .

Table 4 presents the condensed dual descriptor  $f_k^{(2)}$ , the electrophilic  $P_k^+$  and nucleophilic  $P_k^-$  Parr functions, and the local reactivity difference index  $R_k$  over the atoms of the dimethyl 2,3-dihydroxyfumarate molecule, calculated in the gas phase and in water. In general, the data shows a similar picture to the case of the DHF<sub>4</sub> acid, with the difference that there are a lot more ambiphilic sites in the gas phase. In water, however, the atomic sites O8 and O10 are the only ambiphilic sites, with  $R_k$  of  $\pm 0.49$ , equivalent to the value obtained in the case of DHF<sub>4</sub> in aqueous media.

These data offer a good explanation of the high antioxidant/antiradical activity of DHF<sub>4</sub> and dimethyl 2,3-dihydroxyfumarate, reported previously for the DPPH radical and ABTS radical cation. The ambiphilic nature of the ene-diol moiety, with the highest reactivity on the hydroxyl oxygen atoms ensures the accepting of electrons from the reactive radical species and, at the same time, an efficient transfer of hydrogen atoms during radical quenching.

**Table 4.** The condensed dual descriptor  $f_k^{(2)}$ , the electrophilic  $P_k^+$  and nucleophilic  $P_k^-$  Parr functions, and the local reactivity difference index  $R_k$  over the atoms of the dimethyl 2,3-dihydroxyfumarate molecule, calculated *in vacuo* and in water. The actual values of  $f_k^{(2)}$ ,  $P_k^+$  and  $P_k^-$  have been multiplied by 100 for an easier comparison

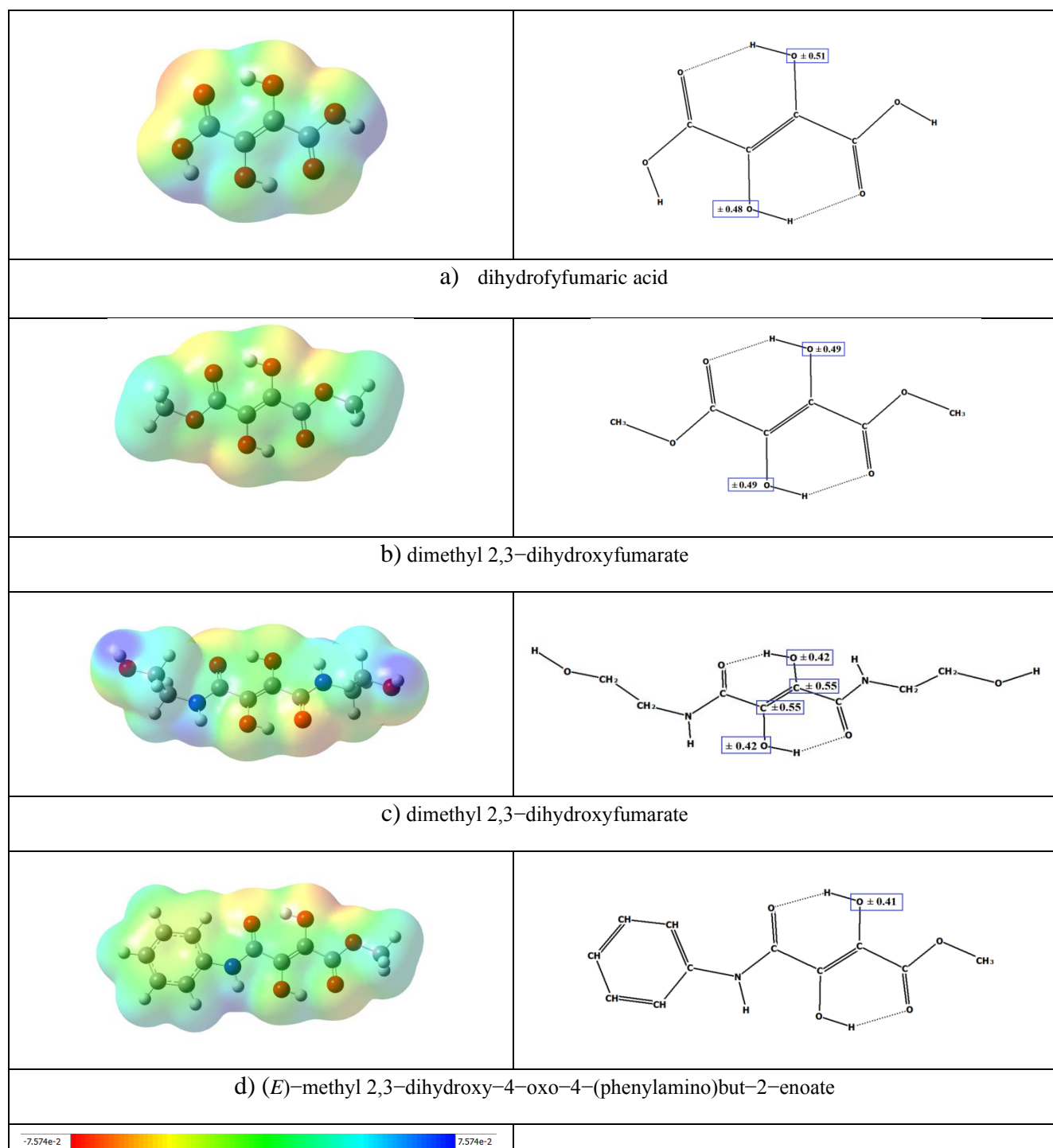
Atom	$f_k^{(2)}$		$P_k^+$		$P_k^-$		$R_k$	
	Gas	Water	Gas	Water	Gas	Water	Gas	Water
<b>1 C</b>	<b>-7.4</b>	<b>-7.1</b>	<b>19.4</b>	<b>18.3</b>	-2.8	-2.5	-0.34	-0.91
<b>2 C</b>	4.2	4.3	12.6	14.5	<b>23.1</b>	<b>23.7</b>	<b><math>\pm 0.35</math></b>	-0.57
<b>3 C</b>	4.2	4.2	12.6	14.5	<b>23.1</b>	<b>23.6</b>	<b><math>\pm 0.35</math></b>	-0.57
<b>4 C</b>	<b>-7.4</b>	<b>-7.1</b>	<b>19.4</b>	<b>18.2</b>	-2.8	-2.5	-0.34	-0.91
5 O	-4.1	-4.6	12.7	11.4	4.4	4.2	-0.23	-0.83
6 O	-1.6	-2.5	2.6	3.4	0.6	0.5	$\pm 0.15$	-0.43
7 C	-0.4	-0.7	-0.3	-0.4	0.0	0.0	$\pm 0.09$	-0.15
<b>8 O</b>	<b>9.2</b>	<b>10.3</b>	2.8	2.7	<b>25.2</b>	<b>24.7</b>	<b><math>\pm 0.38</math></b>	<b><math>\pm 0.49</math></b>
<b>10 O</b>	<b>9.2</b>	<b>10.3</b>	2.8	2.6	<b>25.2</b>	<b>24.7</b>	<b><math>\pm 0.38</math></b>	<b><math>\pm 0.49</math></b>
12 O	-4.1	-4.6	12.7	11.4	4.4	4.2	-0.23	-0.82
13 O	-1.6	-2.5	2.6	3.4	0.6	0.5	$\pm 0.15$	-0.43
14 C	-0.4	-0.7	-0.3	-0.4	0.0	0.0	$\pm 0.09$	-0.15

The following two new derivatives of DHF<sub>4</sub> were previously synthesized and characterized by our group: 2,3-dihydroxy-N<sup>1</sup>,N<sup>4</sup>-bis(2-hydroxyethyl)fumaramide and (*E*)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate [8,9]. The antioxidant/antiradical activity of these compounds was investigated [8, 9], and it was found that while the enoate exhibited a similar antiradical activity for the ABTS and a slightly lower antiradical activity towards DPPH, compared to the dimethyl ester, the fumaramide didn't exhibit any antiradical activity in these assays. This suggests that, although the ene-diol moiety was structurally preserved, its reactivity was altered by the incorporation of two monoethanolamine fragments.

Table 5 presents the condensed dual descriptor  $f_k^{(2)}$ , the electrophilic  $P_k^+$  and nucleophilic  $P_k^-$  Parr functions, and the local reactivity difference index  $R_k$  over the atoms of the 2,3-dihydroxy-N<sup>1</sup>,N<sup>4</sup>-bis(2-hydroxyethyl)fumaramide molecule, calculated *in vacuo* and in water. In general, the results show



the same reactive sites as for the previous investigated molecules. There is however, a very significant difference, i.e. the increased reactivity of the C2 and C3 sites, that is higher than that for the two oxygen atoms of the hydroxylic groups attached to the C2=C3 double bond. According to the reactivity difference index, C2 and C3 are the most preferred sites for nucleophilic and electrophilic attacks in gas phase. In contrast to the data obtained for DHF<sub>4</sub> and its dimethyl ester, in the aqueous phase, the reactivity difference index  $R_k$  of the fumaramide confirms the ambiphilicity of the C2, C3 ( $R_k = \pm 0.55$ ) and its value is higher than that of the O7 and O9 sites ( $R_k = \pm 0.42$ ). The MEP map of the fumaramide (Figure 2c) confirms that C2 and C3 sites are a strong region for nucleophilic and electrophilic attacks.



**Figure 2.** The MEP maps (on the left) and the ambiphilic  $R_k$  index (on the right) of the investigated molecules, in water



**Table 5.** The condensed dual descriptor  $f_k^{(2)}$ , the electrophilic  $P_k^+$  and nucleophilic  $P_k^-$  Parr functions, and the local reactivity difference index  $R_k$  over the atoms of the 2,3-dihydroxy-N1,N4-bis(2-hydroxyethyl)fumaramide molecule, calculated *in vacuo* and in water. The actual values of  $f_k^{(2)}$ ,  $P_k^+$  and  $P_k^-$  have been multiplied by 100 for an easier comparison

Atom	$f_k^{(2)}$		$P_k^+$		$P_k^-$		$R_k$	
	Gas	Water	Gas	Water	Gas	Water	Gas	Water
1 C	<b>-6.77</b>	<b>-6.26</b>	<b>18.19</b>	<b>16.82</b>	-2.99	-2.80	-0.25	-0.60
2 C	3.34	4.21	<b>14.27</b>	<b>16.03</b>	<b>23.04</b>	<b>24.98</b>	<b>±0.32</b>	<b>±0.55</b>
3 C	3.34	4.21	<b>14.27</b>	<b>15.98</b>	<b>23.04</b>	<b>24.96</b>	<b>±0.32</b>	<b>±0.55</b>
4 C	<b>-6.77</b>	<b>-6.27</b>	<b>18.18</b>	<b>16.87</b>	-2.99	-2.81	-0.25	-0.61
5 O	-4.32	-4.22	10.57	8.57	0.73	1.55	-0.16	-0.53
6 O	-4.32	-4.21	10.57	8.55	0.73	1.56	-0.16	-0.53
7 O	<b>7.33</b>	<b>9.71</b>	2.40	2.34	<b>20.34</b>	<b>22.84</b>	<b>0.24</b>	<b>±0.42</b>
9 O	<b>7.33</b>	<b>9.71</b>	2.40	2.34	<b>20.34</b>	<b>22.83</b>	<b>±0.31</b>	<b>±0.42</b>
11 N	-0.90	-2.01	3.86	6.11	5.35	3.80	±0.15	-0.31
12 N	-0.90	-2.01	3.86	6.10	5.34	3.78	±0.15	-0.31
13 C	-0.13	-0.45	-0.13	-0.39	-0.01	-0.19	±0.05	-0.08
15 C	0.18	-0.25	0.25	0.51	0.56	0.36	±0.04	-0.04
18 C	-0.13	-0.45	-0.13	-0.39	-0.01	-0.19	±0.05	-0.08
20 C	0.18	-0.24	0.25	0.52	0.56	0.36	±0.04	-0.04
23 O	1.17	-0.17	0.05	0.04	2.70	0.02	±0.08	-0.03
27 O	1.17	-0.17	0.05	0.04	2.70	0.02	±0.08	-0.03

Table 6 shows the condensed dual descriptor  $f_k^{(2)}$ , the electrophilic  $P_k^+$  and nucleophilic  $P_k^-$  Parr functions, and the local reactivity difference index  $R_k$  over the atoms of the (*E*)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate molecule, calculated in gas phase and in water. Although the dual descriptor suggests that C1 and C4 will be the preferred sites for an electrophilic attack, the  $P_k^+$  function indicates that C1 and C4 will be the preferred sites for a nucleophilic attack, while the  $P_k^-$  descriptor indicates that C3 is most prone to an electrophilic attack, followed by O8 and N13.

**Table 6.** The condensed dual descriptor  $f_k^{(2)}$ , the electrophilic  $P_k^+$  and nucleophilic  $P_k^-$  Parr functions, and the local reactivity difference index  $R_k$  over the atoms of the (*E*)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate molecule, calculated *in vacuo* and in water. The actual values of  $f_k^{(2)}$ ,  $P_k^+$  and  $P_k^-$  have been multiplied by 100 for an easier comparison

Atom	$f_k^{(2)}$		$P_k^+$		$P_k^-$		$R_k$	
	Gas	Water	Gas	Water	Gas	Water	Gas	Water
1 C	<b>-6.11</b>	<b>-6.40</b>	<b>18.35</b>	<b>17.87</b>	-3.80	-4.11	-0.28	-0.76
2 C	-0.20	-0.68	8.20	12.90	10.45	10.33	±0.23	-0.61
3 C	1.11	0.65	12.97	13.68	<b>16.54</b>	<b>20.43</b>	<b>±0.26</b>	-0.57
4 C	<b>-6.44</b>	<b>-7.28</b>	<b>16.25</b>	<b>17.17</b>	-1.76	-1.83	-0.30	-0.86
5 O	-4.25	-5.50	10.91	10.65	3.21	3.65	-0.23	-0.78
6 O	-2.10	-3.03	2.39	3.15	0.42	0.56	-0.11	-0.41
7 C	-0.70	-0.92	-0.30	-0.33	-0.02	-0.05	±0.07	-0.14
8 O	<b>4.83</b>	<b>5.66</b>	3.06	2.58	<b>16.88</b>	<b>17.68</b>	<b>±0.28</b>	<b>±0.41</b>
10 O	1.36	2.24	1.79	2.29	7.62	8.78	±0.20	-0.30
12 O	-4.83	-5.31	13.22	11.02	-3.77	-3.69	-0.24	-0.72
13 N	2.58	1.95	-1.90	1.49	<b>17.13</b>	<b>17.05</b>	<b>±0.15</b>	-0.22
14 C	3.11	3.31	3.46	-0.21	9.69	7.03	-0.09	±0.11
19 C	2.11	2.15	3.40	3.55	8.70	8.10	±0.11	±0.16
20 C	1.13	1.86	-1.21	-1.79	-4.53	-4.09	±0.12	±0.10
21 C	2.86	3.93	8.46	5.13	21.96	18.95	±0.24	±0.21
22 C	1.16	1.70	-1.64	-1.61	-4.07	-4.72	±0.13	±0.10
23 C	1.67	2.37	3.63	3.21	8.42	8.83	±0.13	±0.15



The MEP map (Figure 2d) shows a slightly positive region over C1 and C4, and a slightly negative region over C3, O8 and N13, thus supporting the conclusions based on the values of Parr functions. The local reactivity difference index  $R_k$  indicates that there are two almost equivalent ambiphilic sites in the gas phase, O8 ( $R_k = \pm 0.28$ ) and C3 ( $R_k = \pm 0.26$ ), and the most ambiphilic reactive site in the aqueous medium is O8 ( $R_k = \pm 0.41$ ).

#### 4. Conclusions

Conceptual DFT descriptors that predict and explain the chemical reactivity of molecular systems may be calculated with accuracy directly from the energies of the frontier orbitals only if the validity of the Koopman's theorem is ensured. The present research investigated the goodness of the B3LYP density functional together with the 3-611G(d,p) basis set in predicting and explaining the chemical reactivity of DHF<sub>4</sub> and its three derivatives, by checking how well it follows the KID procedure. It was found that the Koopman's theorem holds well for the aqueous medium calculations. For the gas phase, however, the total hardness is 2-fold lower and the electrophilicity is 2-fold higher than the data obtained from the data obtained employing the calculated vertical  $I$  and  $A$ . These results suggest that the KID procedure is valid and may be used, in conjunction with the B3LYP/3-611G(d,p) level of theory in further studies of related compounds in aqueous media.

Global chemical reactivity descriptors show that the investigated molecules have comparable HOMO-LUMO gaps, in the range of 4.00-4.28 eV. The electronegativity and electrophilicity are the highest for DHF<sub>4</sub> acid, and decrease in the order DFH<sub>4</sub> > dimethyl 2,3-dihydroxyfumarate > (E)-methyl 2,3-dihydroxy-4-oxo-4-(phenylamino)but-2-enoate > 2,3-dihydroxy-N1,N4-bis(2-hydroxyethyl)fumaramide. It should be noted that this is the exact order of the decrease of the antioxidant/antiradical activity previously studied for these compounds [8, 9].

Local reactivity descriptors and the MEP maps of investigated compounds have been computed in order to identify the atomic sites most prone to nucleophilic and electrophilic attacks. Obtained results suggest that the previously obtained antioxidant/antiradical power of investigated compounds towards DPPH radical and ABTS radical cation may be explained by the highest ambiphilic activation of the oxygen atoms of the hydroxyl groups in the ene-diol moiety. The fumaramide, with the highest ambiphilic activation index for the C2 and C3 sites, did not exhibit any antiradical activity in the mentioned assays, confirming the above conclusion.

The present research confirms the importance of understanding the chemical interactions for the synthesis of new derivatives with antioxidant/antiradical properties and shows the importance of Density Functional Theory derived concepts in predicting the active reaction sites for the design of useful new derivatives.

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#### References

1. ESCHENMOSER, A., On a hypothetical generational relationship between HCN and constituents of the reductive citric acid cycle, *Chem. Biodiversity*, **4**, 2007, 554 – 573. DOI: [10.1002/cbdv.200790050](https://doi.org/10.1002/cbdv.200790050)
2. SYCHEV, A., DUCA, GH., *Fruit growing, Viticulture and vinification of Moldova*, **12**, 1985, 39-41 (in Romanian)
3. DUCA, GH., *Homogeneous Catalysis with Metal Complexes: Fundamentals and Applications*; Berlin Heidelberg: Springer, Vol. XII, 2012, 478 p. DOI: [10.1007/978-3-642-24629-6](https://doi.org/10.1007/978-3-642-24629-6)
4. STEPANOV, I., CARMELLA, S. G., HECHT, S. S., DUCA, GH., Analysis of Tobacco-Specific Nitrosamines in Moldovan Cigarette Tobacco, *Journal of Agricultural and Food Chemistry*, **50**(10), 2002, 2793-2797. DOI: [10.1021/jf011552j](https://doi.org/10.1021/jf011552j)



5. DUCA, GH., Free radicals in natural water in *Free Radicals in Biology and Environment*; Dordrecht, NATO ASI Series 1997, 475-489. DOI: [10.1007/978-94-017-1607-9\\_34](https://doi.org/10.1007/978-94-017-1607-9_34)
6. PORUBIN, D., Inhibition of in vitro nitrosation of nornicotine, *Chemistry Journal of Moldova*, **2**, 2007, 3–7. DOI: [10.19261/cjm.2007.02\(1\).11](https://doi.org/10.19261/cjm.2007.02(1).11)
7. PORUBIN, D., HECHT, S., LI, Z.Z., GONTA M., STEPANOV, I., Endogenous Formation of N'-Nitrosornicotine in F344 Rats in the Presence of Some Antioxidants and Grape Seed Extract, *J. Agric. Food. Chem.*, **55**(17), 2007, 7199–7204. DOI: [10.1021/jf0712191](https://doi.org/10.1021/jf0712191)
8. SECARA, N., DUCA, GH., VLAD, L., MACAEV F., Observations on the antioxidant activity of novel dihydroxyfumaric acid derivatives, *Chem. J. Mold.*, **5**(2), 2010, 59–67. DOI: [10.19261/cjm.2010.05\(2\).08](https://doi.org/10.19261/cjm.2010.05(2).08)
9. SECARA, N., DUCA, GH., VLAD, L., MACAEV F., Occurrence and chemistry of dihydroxyfumaric acid, *Chem. J. Mold.*, **6**(1), 2011, 29-44. DOI: [10.19261/cjm.2011.06\(1\).14](https://doi.org/10.19261/cjm.2011.06(1).14)
10. MARTÍNEZ-ARAYA, J.I., SALGADO-MORÁN, G., GLOSSMAN-MITNIK, D., Computational Nutraceuticals: Chemical Reactivity Properties of the Flavonoid Naringin by Means of Conceptual DFT, *Journal of Chemistry*, 2013, Article ID 850297. DOI: [10.1155/2013/850297](https://doi.org/10.1155/2013/850297)
11. JANAK, J.F., Proof that  $\delta E_{\text{dft}}^{\text{ni}} = \epsilon$  in density-functional theory, *Physical Review B*, **18**(12), 1978, 7165–7168. DOI: [10.1103/PhysRevB.18.7165](https://doi.org/10.1103/PhysRevB.18.7165)
12. ZEVALLOS, J., TORO-LABBE A., A theoretical analysis of the Hohn-Sham and Hartree-Fock orbitals and their use in the determination of electronic properties, *Journal of the Chilean Chemical Society*, **48**(4), 2003, 39-47. DOI: [10.1155/2013/850297](https://doi.org/10.1155/2013/850297)
13. CRAMER, C. J., *Essentials of Computational Chemistry-Theories and Models*, John Wiley & Sons, New York, NY, USA, 2<sup>nd</sup> edition, 2004.
14. PERDEW, J. P., BURKE, K., ERNZERHOF, M. Generalized Gradient Approximation Made Simple. *Physical Review Letters*, **77**(18), 1996, 3865–3868. DOI: [10.1103/physrevlett.77.3865](https://doi.org/10.1103/physrevlett.77.3865).
15. PARR R. G., YANG, W., Density functional approach to the frontier-electron theory of chemical reactivity, *Journal of the American Chemical Society*, **106**(14), 1984, 4049–4050. DOI: [10.1021/ja00326a036](https://doi.org/10.1021/ja00326a036)
16. MORELL, C., GRAND, A., TORO-LABBÉ, A., New Dual Descriptor for Chemical Reactivity. *Phys. Chem. A*, **109**(1), 2005, 205–212, DOI: [10.1021/jp046577a](https://doi.org/10.1021/jp046577a)
17. CHATTARAJ, P. K., CHAKRABORTY, A., GIRI, S., Net electrophilicity, *The Journal of Physical Chemistry A*, **113**(37), 2009, 10068–10074. DOI: [10.1021/jp904674x](https://doi.org/10.1021/jp904674x)
18. GORELSKY, S., AOMix Program for Molecular Orbital Analysis-Version 6.5, Ottawa, Canada, University of Ottawa, 2011, <http://www.sg-chem.net/>
19. DOMINGO, L. R., PÉREZ, P., SÁEZ, J. A. Understanding the local reactivity in polar organic reactions through electrophilic and nucleophilic Parr functions. *RSC Adv.*, **3**(5), 2003, 1486–1494. doi:[10.1039/c2ra22886f](https://doi.org/10.1039/c2ra22886f)
20. CHATTARAJ, P. K., DULEY, S., DOMINGO, L. R. Understanding local electrophilicity/nucleophilicity activation through a single reactivity difference index. *Organic & Biomolecular Chemistry*, **10**(14), 2002, 2855. doi:[10.1039/c2ob06943a](https://doi.org/10.1039/c2ob06943a)
21. CÁRDENAS, C., RABI, N., AYERS, P. W., MORELL, C., JARAMILLO, P., FUENTEALBA, P. Chemical Reactivity Descriptors for Ambiphilic Reagents: Dual Descriptor, Local Hypersoftness, and Electrostatic Potential. *The Journal of Physical Chemistry A*, **113**(30), 2009, 8660–8667. doi:[10.1021/jp902792n](https://doi.org/10.1021/jp902792n)
22. NEESE, F., WENNMOHS, F., BECKER U., RIPLINGER C., *J. Chem. Phys.*, **152**, 2020, 224108, <https://doi.org/10.1063/5.0004608>
23. FUENTEALBA, P., FLOREZ, E., TIZNADO, W., *J. Chem. Theory Comput.*, **6**, 2010, 1470-1478. DOI:[10.1021/ct100022w](https://doi.org/10.1021/ct100022w)
24. LIU, S., RONG, C., LU, T., Information Conservation Principle Determines Electrophilicity, Nucleophilicity, and Regioselectivity, *J. Phys. Chem. A*, 2014, **118**, 3698–3704. doi.org/[10.1021/jp5032702](https://doi.org/10.1021/jp5032702)



26. NAZARI, F., ZALI, F. R., A Theoretical Study of Chemical Reactivity of Tartrazine Through DFT Reactivity Descriptors, *J. Mol. Struct. (Theochem)*, **817**, 2007, 11-18. DOI: [10.29356/jmcs.v58i4.50](https://doi.org/10.29356/jmcs.v58i4.50)
27. AYERS, P. W., MORRISON, R. C., ROY, R. K. *J. Chem. Phys.*, **116**, 2002, 8731-8734
28. Morell, C., Grand, A., Toro-Labbe, A., Theoretical support for using the  $\Delta f(r)$  descriptor, *Chemical Physics Letters*, 425(4-6), 2006, 342-346. DOI: [10.1016/j.cplett.2006.05.003](https://doi.org/10.1016/j.cplett.2006.05.003)

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