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Research Article

Theoretical Study about the Effect of Halogen Substitution on the Reactivity of Antitumor 3-Formylchromones and Their Free Radicals

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The mandatory presence of a chlorine atom on the aromatic ring of 6-hydroxy-3-formyl angular chromones, on the respiration inhibition of mammary carcinoma mouse, is explained through a computational study of these compounds. This study analyzes the reactivity of the neutral molecules and their free radicals, in gas phase and with water solvation, incorporated by the polarizable continuum medium (PCM) approach. Electrophilic reactivities were evaluated using Fukui (f^+) and Parr (P^+) functions. The stabilities of radical species formed by the abstraction of a hydrogen atom from the O-H bond were evaluated by bond dissociation enthalpy (BDE) and spin density (SD) calculations. This study has potential implications for the design of chromone analogues as anticancer compounds.

1. Introduction

Chromones are a group of natural oxygen-containing heterocyclic compounds, with a benzoannelated γ-pyrone ring; this motif is the core fragment of several flavonoids [1, 2]. The biological actions exhibited by this type of compounds include cytotoxic [2–4], neuroprotective [5, 6], HIV-inhibitory [7, 8], antimicrobial [9], antifungal [10], antioxidant [11, 12], and antidiabetics [13, 14] activities. Also they have been evaluated as cardiovascular agents calpain inhibitors [15] and calcium antagonists [16]. In cancer studies, activities such as cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation, and reversal of multidrug resistance have been identified [17]. Some flavonoids have already entered clinical trials; for example, flavopiridol, identified as the first cyclin dependent kinase inhibitor, entered phase II clinical trials [18]. Besides, stigmatellin A, a chromone isolated from the myxobacterium *Stigmatella aurantiaca*, is a powerful inhibitor of electron transport in mitochondria and chloroplasts [19]. Stigmatellin is one of the most potent inhibitors of the ubiquinol oxidation site (Qo site) of complex-III [20], although some chromones have also shown interesting activities on NADH: ubiquinone reductase (complex I) [21, 22].

The ethylenic double bond of 4-oxo-4H-benzopyran-3-carbaldehyde (3-formyl chromone) is a very reactive system towards nucleophiles, owing to the presence of aldehydic and pyrone carbonyl groups linked to it [23]. This feature makes these compounds susceptible to nucleophilic attack, inside the cell, resulting in covalent modifications of the nucleophilic sites present in biological molecules, such as thiols and amine groups in proteins. On the other hand, phenolic compounds may either stimulate or inhibit oxidative damage to biomolecules, acting as antioxidants or

Table 1: Bond dissociation energies (kcal/mol) for compounds **1–4** in gas phase and water solvated (PCM) phase.

Comp	R1	R2	BDE (O-H) gas phase	BDE (O-H) PCM water phase	IC_{50} (mM) TA3[a]
1	H	H	87.08	87.92	Inactive
2	H	Me	86.81	87.80	Inactive
3	Cl	H	87.64	86.94	0.12 ± 0.003
4	Cl	Me	86.84	86.77	0.12 ± 0.006

[a]Data taken from [27].

prooxidants [24]. Their ability to inhibit the growth and proliferation of certain malignant cells in vitro is strongly dependent on their structural characteristics [25]. These are two main mechanisms which may be closely related to the biological activities displayed by the compounds studied here.

Previously, we have described that the oxygen uptake inhibition by mouse mammary adenocarcinoma TA3/Ha cancer cells can be used as a quick test for preliminary screening of possible anticancer activity. Generally, IC_{50} for oxygen uptake inhibition is about one order of magnitude greater than IC_{50} for cytotoxicity [26] when a mitochondrial effect is involved. Based on these antecedents, we screened a set of simple and angular chromones for the inhibition of oxygen consumption by TA3/Ha tumor cells [27]. An interesting result arising from that study shows the mandatory presence of chlorine atom on angular structures to exert an effect on the respiration of tumor cells. IC50 values for oxygen uptake by chromones **3** and **4** (Table 1) are similar to that exhibited by a tricyclic hydroquinone, previously studied by us [28, 29], which exhibited cell cycle arrest and inhibits complex I-dependent respiration with selective antiproliferative effect on mouse mammary adenocarcinoma TA3/Ha cancer cells.

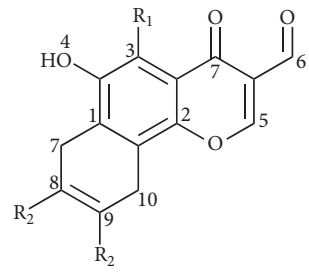
To explain the differences among the biological activities of these compounds, in this work we pursued to gain insight about the role of the chlorine atom on the chemical reactivity of the neutral molecules and also on the reactivity of free radicals generated from them. The main parameters to evaluate the free radical behavior of chromones **1–4** (Table 1), namely, bond dissociation energies (BDE) and spin density (SD), were assessed through density functional theory (DFT) calculations at M06-2x/6-311+G(d,p) level. Moreover, the reactivity of the chromones towards nucleophiles was studied through Fukui and Parr functions for nucleophilic attack.

2. Material and Methods

The calculations were carried out using the Gaussian09 program package [30]. All molecules and their radicals were optimized at density functional theory (DFT) M06-2x/6-311+G(d,p) level. The bond dissociation enthalpy (BDE) for the O-H homolytic bond breaking was calculated as follows:

$$\mathrm{BDE} = \mathrm{H}\left(\mathrm{RO}^{\bullet}\right) + \mathrm{H}\left(\mathrm{H}^{\bullet}\right) - \mathrm{H}\left(\mathrm{ROH}\right) \quad (1)$$

where H(ROH), H($RO^{\bullet}$), and H($H^{\bullet}$) are the enthalpies of the neutral molecules, their radicals, and the H-atom, respectively.



1–4

Figure 1: Structure of molecules **1–4** studied in this work.

Local reactivity indices, Fukui function (f^{+}) and Parr function (P^{+}), for electrophilic attack were calculated as previously described [31, 32].

3. Results

3.1. Free Radicals Properties. A computational study about some properties of the free radicals from the phenolic compounds **1–4** (Figure 1) was carried out. Bond dissociation enthalpy (BDE) associated with the bond breaking of labile atoms (e.g., hydrogen in the O-H bond) that lead to formation of radical species is the main parameter related to hydrogen atom transfer (HAT), the most important mechanism of antioxidant activity. Lower BDE values are associated with higher antioxidant activities of the molecules [33]. Table 1 shows the BDE values calculated in gas phase and in aqueous medium (polarizable continuum medium, PCM). Gas phase values show a higher BDE for compounds **1** and **3**, with R_2 = H, while compounds **2** and **4**, with R_2 = Me, present lower BDE. Nevertheless, when water solvation is included, the trend changes and compounds **1** and **2**, with R_1 = H, present higher BDE than **3** and **4**, with R_1 = Cl. The last result is according to the capability of oxygen uptake inhibition by mouse mammary adenocarcinoma TA3/Ha cancer cells (Table 1).

On the other hand, the stability of the free radicals is also a key factor related to their antioxidant activity. To obtain information about the stability of the free radicals generated from the studied compounds, spin densities (SD) were calculated for the four radical species formed by the abstraction of the hydrogen atom from the O-H bond. When SD is more delocalized, the formation of the radical is easier. SD and BDE are correlated; when SD is lower BDE also tend

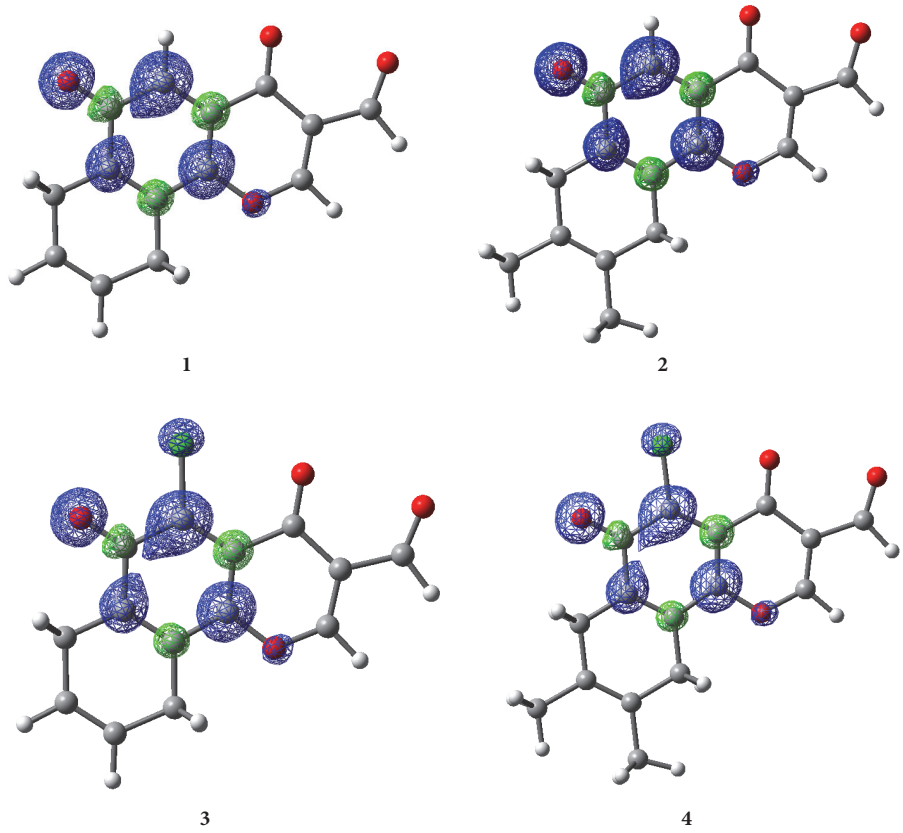


Figure 2: Spin density isosurface (isovalue = 0.004) for four radical molecules (water PCM solvated). Blue indicates positive and green indicates negative spin density values.

Table 2: Spin densities on carbons 1, 2, 3, and 4 both in gas phase and water (PCM) solvated.

Molecule	Spin density							
	Gas phase				Water phase			
	C1	C2	C3	O4	C1	C2	C3	O4
1	0.158	0.200	0.287	0.650	0.199	0.305	0.392	0.356
2	0.200	0.192	0.280	0.650	0.226	0.293	0.394	0.357
3	0.141	0.209	0.280	0.602	0.157	0.299	0.371	0.315
4	0.164	0.201	0.287	0.603	0.171	0.292	0.385	0.316

Table 3: Fukui (f^+) and Parr (P^+) functions for the nucleophilic attack at carbons 5, 6, and 7.

Molecule	f^+			P^+		
	C5	C6	C7	C5	C6	C7
1	0.217	0.156	−0.054	0.752	0.110	0.099
2	0.192	0.183	0.064	0.751	0.114	0.094
3	0.165	0.113	0.048	0.262	0.044	0.151
4	0.072	0.129	0.080	0.122	0.038	0.169

to be lower. Additionally, SD provides information about site reactivity in the radical molecule.

In Figure 2 the SD isosurfaces for the four radical molecules are displayed. The SD is mainly located on the aromatic ring in all cases. The presence of chlorine in molecules **3** and **4** polarizes some SD towards itself, leading to a more delocalized SD distribution.

Atoms with higher SD values are presented in Table 2. In gas phase, the atom with highest SD is O4 in all cases. Molecules **1** and **2** present similar values of SD on O4, and higher than SD on O4 of molecules **3** and **4**, which also present similar values between them. C3 presents the second higher SD, with **1** and **4** being higher than **2** and **3**. On the other hand, considering water solvation, the results are quite different. In this case C3 presents the highest SD in all cases. Molecules **1** and **2** (without chlorine) present higher values of SD on C3 compared with molecules **3** and **4**. The effect of chlorine atom is to decrease the SD on C3 for molecules **3** and **4**, which led to a more delocalized SD distribution and can explain the lower BDE observed in these molecules. These results also highlight the importance of the solvent to explain the antioxidant properties of these compounds and hence their biological activities.

3.2. Electrophilic Reactivity. In order to explore if the electrophilic reactivity of the four chromones towards biological nucleophiles, which could also explain the biological activity exerted by these compounds, Fukui (f^+) and Parr (P^+) functions were calculated (Table 3). We focus on the atoms

that can suffer a nucleophilic attack; carbon 5 can experience 1,4-addition, while carbons 6 and 7 can experience 1,2-addition. According to f^+, C5 is the most reactive site in molecules **1**, **2**, and **3** for a nucleophilic attack, while C6 is the most reactive site in molecule **4**. On the other hand, according to P^+ C5 is more susceptible for a nucleophilic attack in molecules **1**, **2**, and **3** while for molecule **4** C7 is the most reactive. Although f^+ and P^+ commonly give a similar trend; in some cases f^+ has generated some errors where P^+ have predicted correctly the experimental results [32]. These results also highlight the effect of the presence of the chlorine atom, which can modify the local electrophilic reactivity in electrophilic atoms and can account to explain the difference in their biological activities.

4. Conclusions

In this work, the free radicals behavior (BDE and SD) and electrophilic reactivity (f^+ and P^+) of a series of four chromones, which have previously shown antiproliferative activity towards mouse mammary adenocarcinoma TA3/Ha cancer cells, were explored. The presence of a chlorine atom on the aromatic carbon C3 is shown to be mandatory for the antiproliferative activity of these molecules.

The BDE for the O-H bond is shown to be lower for chromones possessing a chlorine atom on C3 (**3** and **4**) when water solvation was included. The latter supported a possible implication of radical species of these molecules in their antiproliferative activity. Moreover, the effect of chlorine atom decreases the SD on C3 for molecules **3** and **4** when water solvation was considered, which led to a more delocalized SD distribution and can explain the lower BDE for these molecules. Besides, local electrophilic reactivity results showed that the presence of chlorine atom, which modifies the local electrophilic reactivity in electrophilic atoms, could also account to explain the difference in their biological activities. The results of this study have potential implications for the design of chromone analogues as anticancer compounds.

Competing Interests

The authors declare that they have no competing interests.

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