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Nuclear Magnetic Resonance (NMR), Infrared (IR) and Mass Spectrometry (MS) study of keto-enol tautomerism of isobenzofuran-1(3H)-one derivatives

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ABSTRACT

The keto-enol tautomerism of 3-(2-hydroxy-4,4-dimethyl-6-oxo-cyclohexen-1-yl)isobenzofuran-1(*3H*-one (**1**), 3-(2-hydroxy-6-oxocyclohex-1-enyl)isobenzofuran-1(*3H*)-one (**2**), 3-(2-hydroxy-4-methyl-6-oxocyclohex-1-enyl)isobenzofuran-1(*3H*)-one (**3**), 3-(2-hydroxy-5-oxocyclopent-1-enyl) isobenzofuran-1(*3H*)-one (**4**) and 2-(3-oxo-1,3-dihydroisobenzofuran-1-yl)-*1H*-indene-1,3(*2H*)-dione (**5**) were investigated. We noticed that for compounds **1** to **4** only the enol form is observed in solid, in solution or in the gas phase. Their tautomeric equilibria are not affected by the solvent, temperature or physical state. Compound **5** was observed in its keto form in solution (NMR) and solid state (IR). The enol species of **5** was also observed upon Mass Spectrometry analysis. These findings were supported by NMR, IR, MS/MS and molecular modeling analyses.

1. Introduction

The keto-enol tautomerism is important in many fields of chemistry and biochemistry. The investigation of this phenomenon allows us to learn about the physicochemical properties and biological activities of the substances in which it occurs. Several factors, such as intra and intermolecular interactions, the hydrogen bond formation, solvents effects, temperature, physical state, and others, can influence the keto-enol equilibrium, favoring the keto or the enol species [1–6].

Isobenzofuran-1(*3H*)-ones (phtalides) are heterocyclic compounds characterized by the presence of a benzene ring fused to a γ-lactone one. Secondary metabolites containing the phtalide nucleus and presenting relevant biological activities have been isolated from plants, fungi, bacterias and liverworts [7]. In particular, the C-3 functionalized phtalides display several significant medicinal properties [8–13]. It stands out the success story of 3-butylisobenzofuran-1(*3H*)-one (also known as *n*-butylphthalide), a compound which nowadays has been clinically used as antiplatelet drug for ischemia-cerebralapoplexy [14,15]. It also deserves comments the importance of isobenzofuran-1(*3H*)-ones as building blocks in organic synthesis [16,17].

Our own interest in this class of compounds led us to synthesize a series of twelve C-3 functionalized isobenzofuran-1(*3H*)-ones possessing alicyclic and aromatic groups. These compounds were evaluated *in vitro* for their antiproliferative activity against leukemia cell lines [18]. The obtained biologically data revealed that the antiproliferative activity of two of the evaluated compounds was superior than Etoposide (VP-16), an anticancer drug used in several chemotheraphy regime, including leukemia. In another investigation, we also assessed the effect of the aforementioned series of isobenzofuranones on the photosynthetic machinery. It was found

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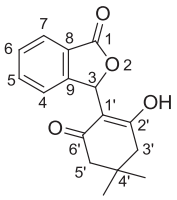
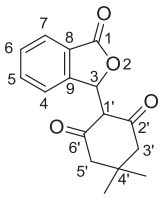
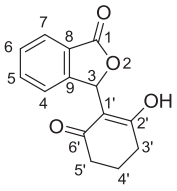
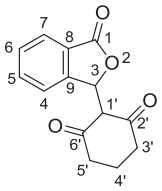
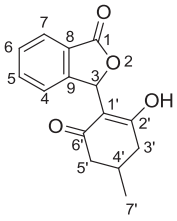
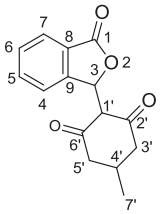
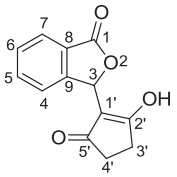
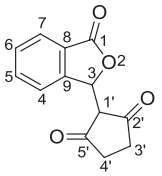
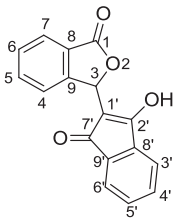
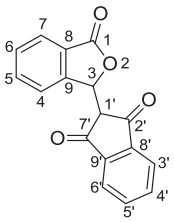
0022-2860/

Table 1
The keto and the enol structures of the investigated compounds.

Compound	Enol form	Keto form
1		
2		
3		
4		
5		

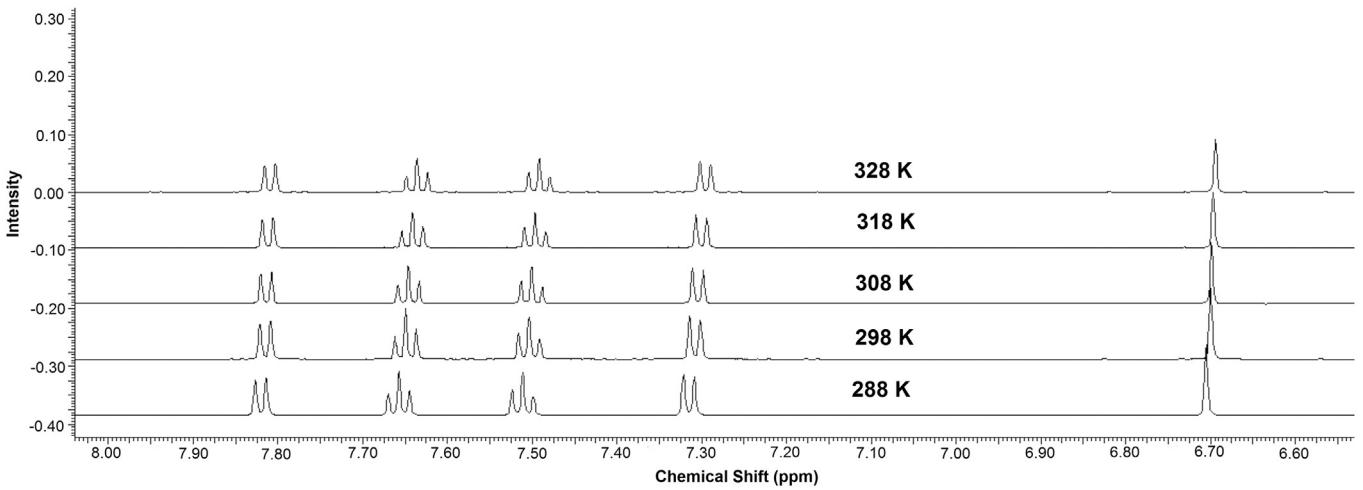


Fig. 1. ^{1}H-NMR spectra of compound **1** in methanol-d_4 at different temperatures. The H-3 signal is observed near 6.70 ppm.

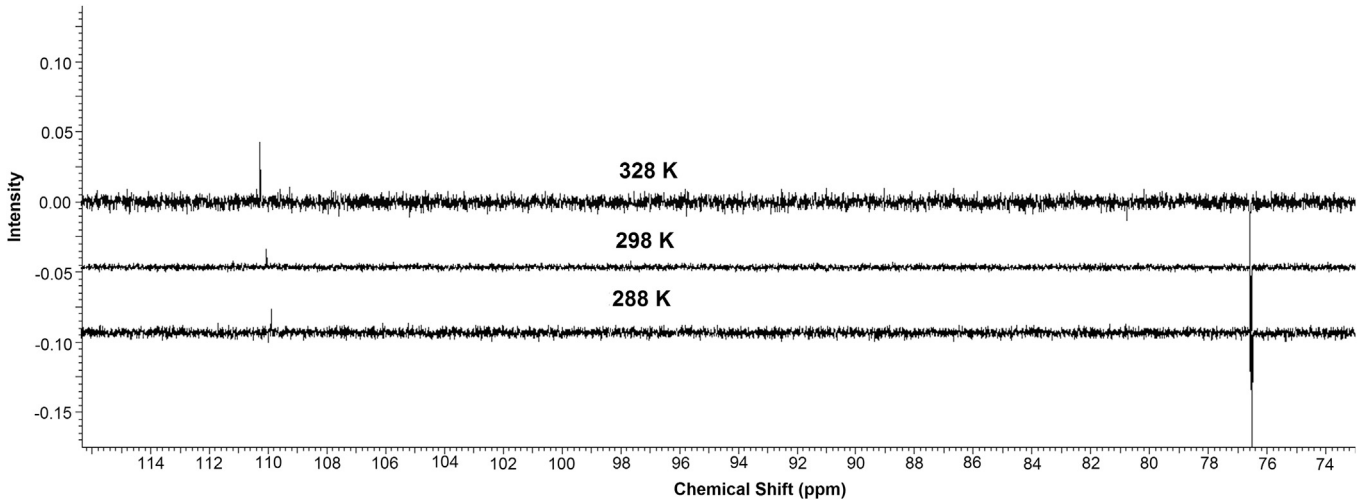


Fig. 2. APT spectra of compound **1** in methanol-d_4 at different temperatures. The C-1′ signal is observed at 110.1 ppm.

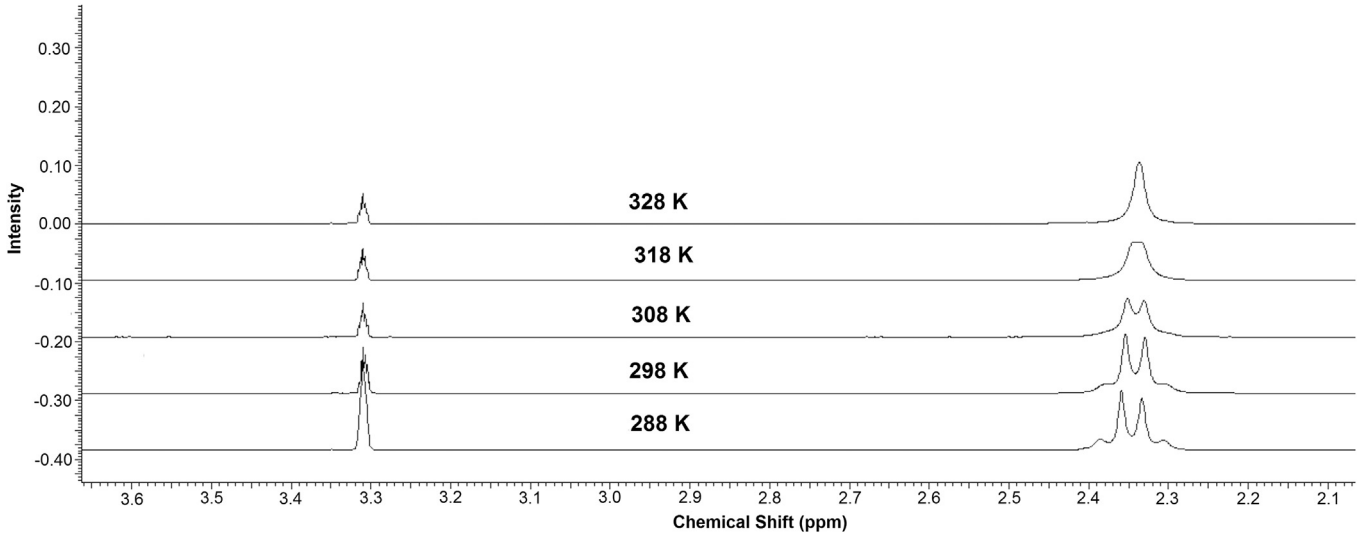


Fig. 3. ^{1}H-NMR spectra of compound **1** in methanol-d_4 at different temperatures. The H-3′ and H-5′ signals are observed around 2.34 ppm.

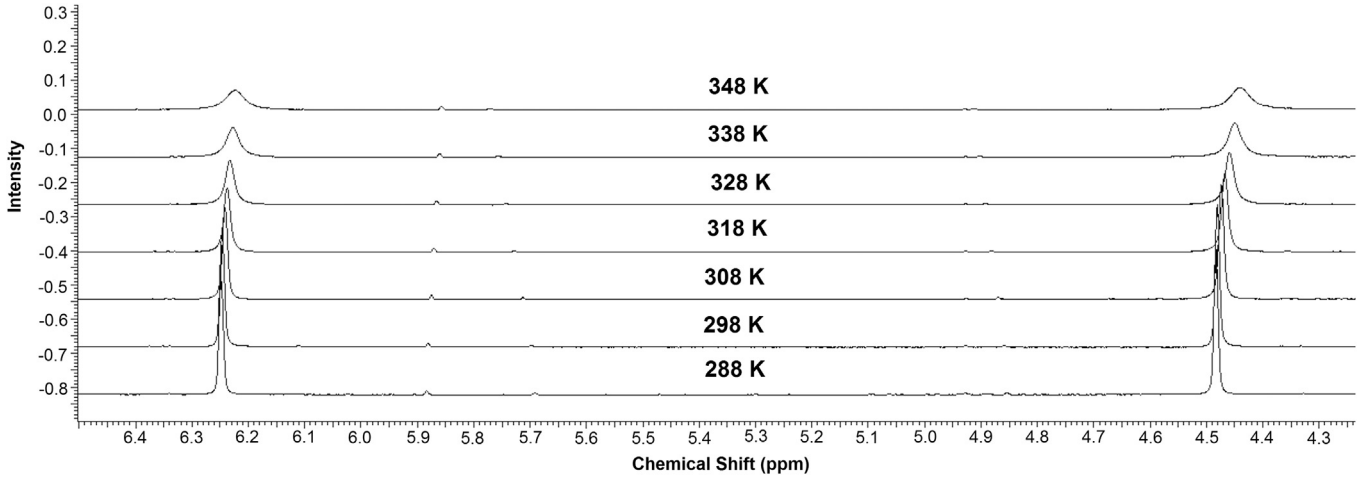


Fig. 4. ^{1}H-NMR spectra of compound **5** in DMSO-d_6 at different temperatures. The H-3 signal is observed at 6.25 ppm and the H-1′ signal at 4.48 ppm.

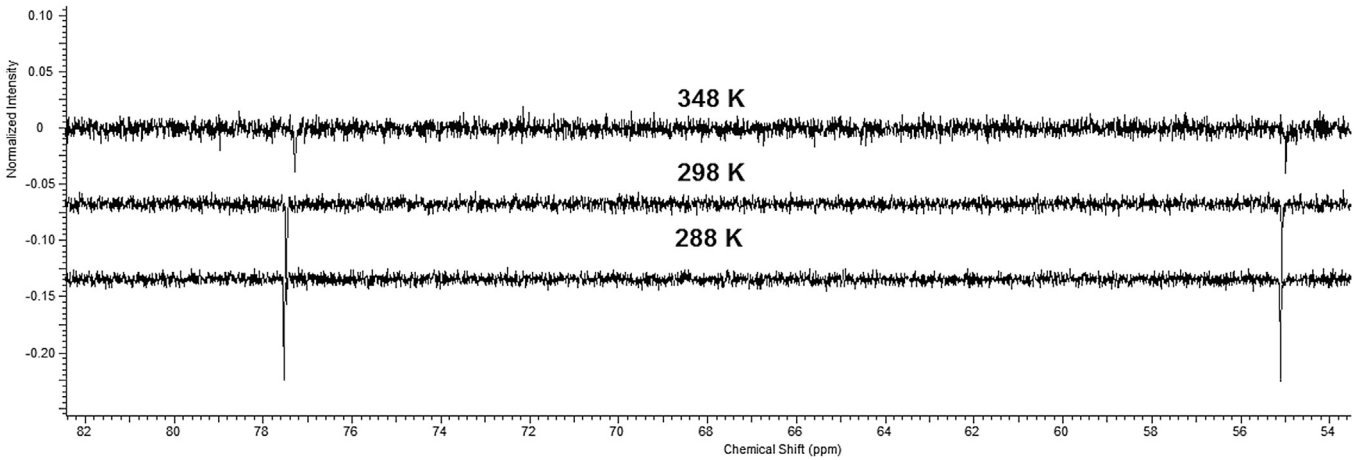


Fig. 5. APT spectra of compound **5** in DMSO-d_6 at different temperatures. The C-1′ signal is observed near 55.1 ppm and C-3 signal is near 77.5 ppm.

that the compounds are capable of interfering with electron transport chain in spinach chloroplast *via* two different mechanisms [19].

This paper is concerned about the investigation of keto-enol tautomerism of isobenzofuran-1(*3H*)-ones containing different appendages at C-3 position of isobenzofuranone nucleus (see Table 1 for numbering). The structures of the investigated compounds are presented in Table 1. The results herein described were supported by the NMR and IR spectroscopy as well as Mass Spectrometry analyses of the species involved in the tautomerism equilibria.

2. Experimental

2.1. Study of the keto-enol tautomerism

The NMR studies were performed in a 600 MHz spectrometer (Premium Compact, Varian) with a 5 mm probe and with

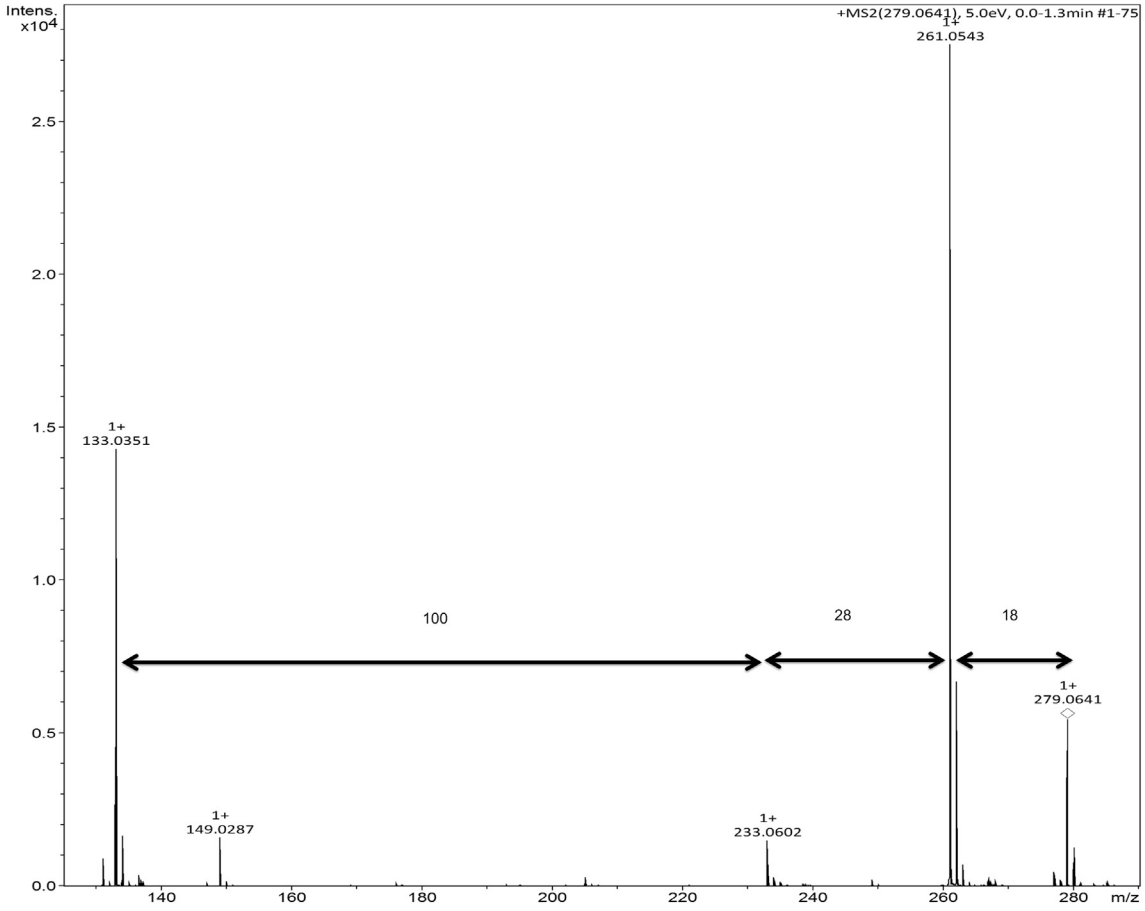


Fig. 6. MS/MS spectrum of the compound **5**, showing the fragmentation of 18 Da that indicates the presence of an OH group.

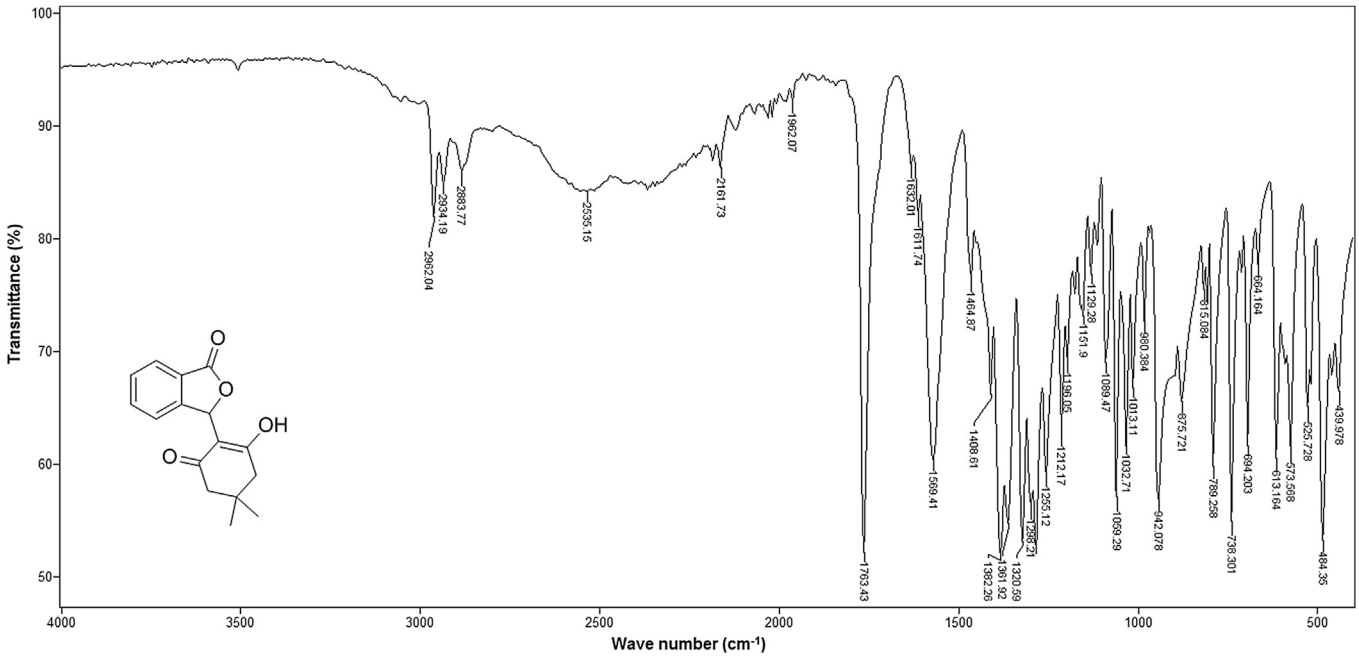


Fig. 7. IR spectrum (ATR) of the compound **1** (a broad band in the range 3200–2400 cm^{-1} was associated with O–H stretching).

isobenzofuranone solutions that presented the concentration of 16.67 mg mL^{-1}. ^{1}H-NMR (32 scans) experiments were acquired in acetone-d_6 (273–318K), methanol-d_4 (288–328 K) and DMSO-d_6 (288–348 K). APT (3000 scans) and ^{13}C-NMR (2000 scans) spectra were acquired in acetone-d_6 (288–318K), methanol-d_4 (288–328 K) and DMSO-d_6 (288–348 K).

The Mass spectra were acquired in a micrOTOF – QII (Bruker) spectrometer in the positive mode of acquisition. The samples were solubilized in acetonitrile (1% of formic acid), with a 4.5 kV voltage at the capillary and a flow rate of 2.0 μL min^{-1}. MS and MS/MS experiments were performed.

The IR analyses were conducted in solid state in a Varian 660-IR with the GladiATR accessory.

The molecular modeling calculation procedures for the compounds **1** to **5** were done using the DFT/B3LYP method and 6-31G* using the Spartan'06 program [20]. The keto-enol and the diketo conformations of all compounds were calculated with the Energy Profile method in vacuum and in the presence of solvents (acetone, DMSO and methanol).

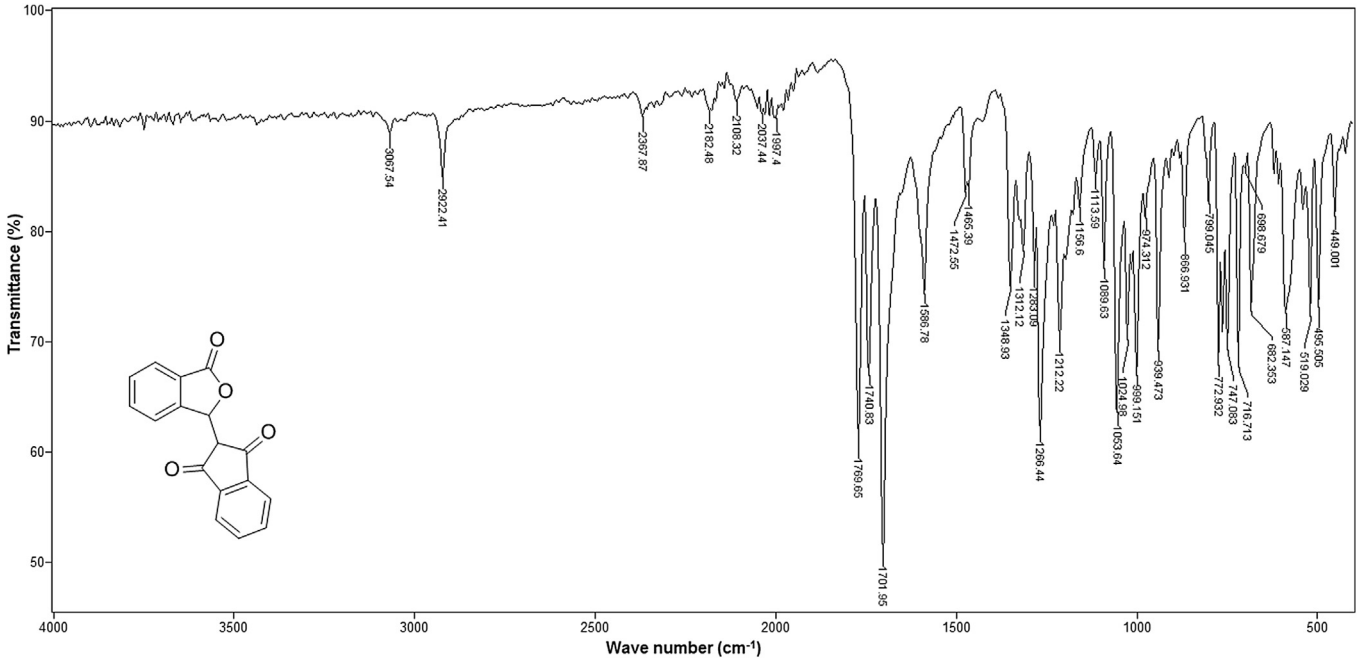


Fig. 8. IR spectrum (ATR) of compound **5** (signals in 1769, 1740 and 1702 cm^{-1}: C=O stretching).

Table 2
Energy (minima) difference of the keto and the enol forms for each compound calculated by DFT (B3LYP)/6-31G[a] in vacuum and in the presence of solvents (acetone, DMSO and methanol).

Compound		ΔE_{vacuum} (kJ/mol)	$\Delta E_{acetone}$ (kJ/mol)	ΔE_{DMSO} (kJ/mol)	$\Delta E_{methanol}$ (kJ/mol)
1	Enol[a]	14.43	6.12	5.19	7.09
	Keto				
2	Enol[a]	18.13	2.86	2.53	4.58
	Keto				
3	Enol[a]	18.75	3.71	3.22	5.16
	Keto				
4	Enol[a]	22.56	24.61	25.02	24.87
	Keto				
5	Enol	23.37	23.29	22.78	25.10
	Keto[a]				

[a] The most stable form.

3. Results and discussion

As previously reported [18,19], the synthesis of compounds **1** to **5** (Table 1) were achieved *via* DBU-promoted condensation between phtalaldehydic acid and appropriated 1,3-diketones. For all synthesized compounds, their tautomeric equilibrium was investigated by NMR, IR spectroscopy, Mass spectrometry, as well as Molecular Modeling.

The study of the keto-enol equilibria by ^{1}H-NMR was performed observing the multiplicity of the H-3 signal (singlet for the enol form and duplet for the keto form). It was also observed the presence (for the keto form) or the absence (for the enol form) of a signal due to H-1′ (see Table 1 for numbering). Furthermore, the C-1′ signal in APT spectra was also used to study the tautomeric equilibria, observing that it is a quaternary carbon in the enol form and a −CH in the keto form. The complete assignments of compounds **1** to **5** in acetone, DMSO and methanol can be seen in the Supplementary material.

The expansion of the aromatic region of the ^{1}H-NMR spectrum for compound **1** in methanol, at different temperatures, is shown in Fig. 1. The H-3 signal is observed as a singlet, and the integration refers to a single proton. Additionally, both the multiplicity and the integration of the signals do not vary with the temperature increase.

The APT spectra of compound **1** in methanol at different temperatures show the signal concerning to the C-1′ being a quaternary carbon at all temperatures (Fig. 2). A similar trend is also observed for the spectra acquired in acetone and DMSO solvents (see Supplementary Material). Therefore, the enol form of compound **1** is favored regardless the solvent, even at higher temperatures.

It is interesting to observe the variation in the multiplicity of H-3′ and H-5′ (Fig. 3) with increasing temperature. This variation can be explained by the faster rotation around C1′-C3 bond, which leads to the coalescence of the signals at higher temperatures. This coalescence was also observed for compound **1** in acetone and DMSO (see Supplementary Material). This faster rotation also affects the signals for C-3′ and C-5′ (Supplementary Material)

Compounds **2**, **3** and **4** showed similar behavior to that described for compound **1**. For all of them, the enol form is favored in different solvents (methanol, acetone and DMSO) as well as at different temperatures (see Supplementary Material). Therefore, for these compounds, solvent and temperature did not interfere in the keto-enol equilibria. The presence of methyl groups (one or two) attached to alicyclic portion also did not affect the equilibrium. Moreover, the replacement of the six-membered ring by a five-membered one did not favor the keto form (see Supplementary Material).

Compound **5** was insoluble in methanol, which precluded NMR analyses using this solvent. The ^{1}H-NMR spectrum of compound **5** in DMSO (Fig. 4), showed the signal for the H-3 as compounds **1** to **4**. However, it was also observed one signal related to H-1′ (characteristic of keto form). The same behavior was observed for substance **5** in acetone (see Supplementary Material). The temperature did not influence the tautomeric equilibrium of **5**.

The APT spectra of **5** in DMSO showed a signal at 55.1 ppm, related to C-1′ (Fig. 5), indicating the presence of a −CH group. The quaternary C-1′ was not observed and, consequently, confirmed the keto form for this compound. Similarly to the proton spectrum, temperature did not affect the APT spectrum. The same behavior is observed for compound **5** in acetone (see Supplementary Material).

It is worth to mention that ^{13}C-NMR showed two different peaks for ketone carbonyl groups (Supplementary Material). This observation is in agreement with the keto form of **5**. The same result was observed in acetone. The carbon spectra for compounds **1** to **4** showed only the presence of one ketone carbonyl signal.

By comparing the NMR data of compounds **4** and **5**, it can be inferred that the presence of an aromatic ring fused to the 1,3-diketone five membered ring changes the tautomeric equilibrium so that the keto form is observable in the case of compound **5**.

Mass spectrometry experiments can provide insight about the species involved in tautomeric equilibria. Fragmentation of 18 Da can be related to the dehydration of the OH group in the form of H_2O^+, and fragmentation of 28 Da can be related to the C═O group in the form of CO^+ [21].

The MS/MS analysis of compound **5** revealed the presence of the enol form as the sole species in the equilibrium. This statement is corroborated by the observation of a fragmentation of 18 Da of the OH group in the form of H_2O^+ as shown in Fig. 6 for compound **5** [21,22].

The MS/MS spectra of compounds **1**–**4** (see Supplementary Material) also showed that fragmentation (18 Da), indicating that the enol form of these compounds is favored in the equilibria in the gas phase.

The Mass spectrometry, as well as the NMR experiments for compounds **1** to **4,** revealed only the presence of enol species in the tautomeric equilibria. On the contrary, while the NMR experiments showed the presence of keto form in the equilibrium for compound **5**, the mass experiments results showed exclusively the presence of the enol form of compound **5**. Thereby, the physical state of the compound, gas phase (analyzed by Mass spectrometry) and solution (analyzed by NMR) can affect the keto-enol equilibrium of the investigated compound.

We also sought information about the tautomeric equilibrium of substances **1** to **5** analyzing them in solid phase by IR spectroscopy. Regarding compounds **1** to **4**, the IR spectra present a broad band which can be associated to the O–H group of the enol form, as it is shown in Fig. 7 for compound **1** (IR spectra for compounds **2** to **4** are shown in the Supplementary Material). Thus, the enol form is

favored in solid phase for compounds **1** to **4**.

The inspection of IR spectrum of the compound **5** (Fig. 8) showed only bands related to the C=O stretching; it was not observed any band related to the O–H stretching, characterizing this compound in the keto form in the solid phase.

The IR experiments (solid state) for compounds **1** to **4** showed only the enol form as observed with the NMR (liquid state) and Mass spectrometry (gas state) experiments. Solvent, temperature and physical state did not change their equilibrium. However, for compound **5,** the keto form was observed when it was analyzed by IR (solid state) and NMR (in solution), and enol form was noticed by Mass spectrometry (gas state). The physical state could be the responsible for the changes in the keto-enol equilibrium for the compound **5**.

The keto and the enol forms of all compounds were submitted to the geometry and energy optimization by molecular modeling and had their energy calculated for each form (Table 2).

According to the molecular modeling data, it was observed that solvent didn't interfere in the predominant form of the compounds in the keno-enol equilibrium when compared to the vacuum calculations. For compounds **1** to **4**, the molecular modeling shows the enol form as the most stable for all the calculations, giving support to the NMR, MS and IR findings previously described. For compound 5, the molecular modeling showed the keto form as the most stable, which is in total agreement with the IR and NMR experimental data. The MS results for compound 5 (which shows only the enol form) were the only one not supported by the molecular modeling results.

4. Conclusion

It was showed that the keto-enol equilibrium for compounds **1** to **4** is not affected by changes in solvent, temperature and physical state. Only the enol form was observed for these compounds by NMR, Mass Spectroctrometry, IR and Molecular Modeling. For compound **5**, it was observed the enol form by Mass Spectroscopy. Interestingly, the observation of only enol form of compound **5** by Mass Spectroscopy and exclusively the keto form by IR, NMR and Molecular Modeling, indicates that the physical state can influence the tautomerism for this compound.

Comparing the structures of compounds **4** and **5,** and looking at the NMR, IR and Molecular Modeling results, it can be inferred that the presence of an aromatic ring changes the tautomeric equilibrium.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.molstruc.2016.02.015.

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