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Dedicated to Professor Dr. Cozar Onuc on His 70th Anniversary

UV-VIS PH DEPENDENCE OF DACARBAZINE: EXPERIMENTAL AND TD-DFT INVESTIGATIONS

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ABSTRACT. Dacarbazine (DTIC) is one of the most used chemotherapeutic drugs in the treatment of patients with metastatic malignant melanoma. Depending on the pH of the solution, different conformers or tautomers of the drug are expected to exist, eventually with different biological activities. In this study the UV-Vis absorption spectroscopy coupled with quantum chemical calculations were used to get new insights about the molecule's structure and the pH influence on the absorption spectra of dacarbazine. The attention was focused on the possible conformers and tautomers of the molecule, on their stability and energetic order in gas-phase as well as in liquid state. Correlating the experimental UV-Vis data and Time dependent density functional theory (TD-DFT) computational results the conformers of DTIC, which are responsible for the absorption spectrum in water at different pH values, were identified. Subtle features of the absorption spectrum of DTIC were reliably explained using proper computational models and techniques. By using convergent approximations the experimental UV-Vis spectrum of dacarbazine was reproduced within the experimental errors. The changes that appear in the absorption spectra as a result of a pH change, have been attributed to the presence of protonated and deprotonated species of DTIC present in acidic and alkaline solutions, respectively.

Keywords: *dacarbazine, UV-Vis, pH, TD-DFT*

1. INTRODUCTION

Dacarbazine (5-(3,3-dimethyltriazeno)imidazol-4-carboxamide, DTIC), is one of the most researched chemotherapeutic drugs used in the treatment of patients with metastatic malignant melanoma [1]. Dacarbazine's mode of action is still uncertain, but

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most sources state that this drugs works similar to other cytotoxic drugs classified as „alkylating agents". These agents stop the cancer cells from multiplying by binding together the strands of the cell's genetic material. This makes the strands unable to uncoil and separate, which is necessary in DNA replication. As a result, the cells can no longer divide and therefore they die. The faster the cells are dividing, the more likely it is that chemotherapy will kill the cells, causing the tumor to shrink [2].

Considering the importance of this drug in chemotherapy, in the present study we used UV-Vis absorption spectroscopy coupled with quantum chemical calculations based on density functional theory (DFT) to get new insights about the molecule's structure.

It is known that depending on pH value of the solution, different conformers or tautomers of a drug are expected to have different biological activities [3]. Moreover, a study by Saunders et al. [4] shows that dacarbazine's toxicity is dependent on illumination and also greatly affected by the pH of the environment. Therefore, the pH influence on the absorption spectra of DTIC was further investigated. The lowest energy conformers of DTIC have been identified by DFT calculations. Our attention was focused on the possible conformers and tautomers of the molecule, on their stability and energetic order in gas phase as well as in liquid state. Subsequently, the corresponding optimized geometry was used for electronic structure calculations. Quantum chemical calculations performed in the framework of DFT enabled us to explain in detail the experimentally derived data.

2. EXPERIMENTAL

Materials and Methods

For the experimental measurements, dacarbazine powder was purchased from Sigma Aldrich, and used as received without any further purification.

The UV-Vis spectra were firstly recorded for aqueous solutions at different concentrations (ranging from 10^{-4} M to 10^{-6} M) and at normal pH value, and secondly at a concentration of 10^{-5} M for different pH values in the range between 2 and 13.

The desired concentrations for the measurements were obtained from an initial stock solution with a concentration of 10^{-2} M. For preparing the aqueous solutions, ultrapure water was used. The samples were stored at room temperature in plastic recipients wrapped in aluminum foil. The pH of the solutions was changed by adding small amounts of 1% solutions of hydrochloric acid (HCl) or sodium hydroxide (NaOH).

The UV-Vis spectra were recorded using a Jasco V630 UV-Vis double beam spectrophotometer with a silicon photodiode detector. The samples were placed in 1 cm path length quartz cuvettes, in a distilled water solution. The spectra were recorded in the 190-1100 nm range.

Computational Details

Density functional theory (DFT) calculations were employed in order to obtain the optimized geometries of the investigated compounds. As starting geometries for energy minimizations, we used the X-ray structures derived from crystallographic data reported by Freeman and Hutchinson [5]. The calculations on monomers and dimers were performed at B3LYP/6-31+G(2d,2p) level of theory, with and without dispersion correcting potentials (DCPs) on the atoms of molecule, according to the procedure proposed by DiLabio's group [6].
The absorption spectra were calculated using the TD-DFT formalism and the solvent effects were accounted for by using the Polarizable Continuum Model (PCM) [7]. The conformer energies, molecular structures and the absorption spectra calculations were performed by using the Gaussian package [8].

3. RESULTS AND DISCUSSION

3.1. Tautomers and conformers stability

When investigating a drug, the dissimilarities in biological activities arising from different conformers or tautomers of the same drug must be taken into consideration. Combining experimental techniques and quantum chemical calculations is an adequate approach to answer for the high interest in structural and spectroscopic characterization of a given drug [3].

Thus, our first interest was to investigate the stability of the possible tautomers and conformers of dacarbazine. The starting geometries used for the structure optimizations, were derived from the X-ray diffraction data reported by Freeman and Hutchinson [5]. This data shows the existence of two tautomers in the asymmetric unit (Fig. 1). The difference between them is related to the position of the second hydrogen in the imidazole ring. In one molecule, the protonated nitrogen of the imidazole ring is adjacent to the triazene group, while in the other one it is adjacent to the carboxamide group.

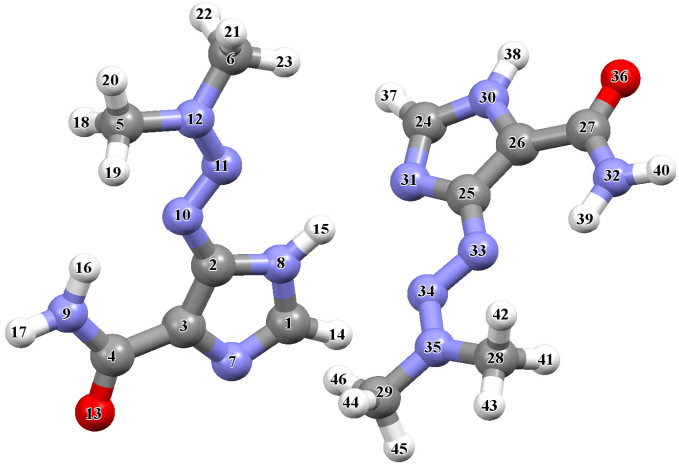

Figure 1. Optimized dimeric structure of DTIC as obtained from the crystallographic data.

We started by investigating the energetic stability of the possible tautomers and conformers of dacarbazine. For this purpose, we used a B3LYP exchange-correlation functional and the 31+G(2d,2p) basis set. The theoretical simulations for geometry optimization were carried out on both tautomers revealed by the crystallographic data (m1_cx and m2_cx), two conformers and one tautomer of m1 (denoted m1_c2, m1_c3 and m1_c2_t2), as well as two conformers of m2 (denoted m2_c2 and m2_c3). The geometry for the conformers is dependent on the orientation of the carboxamide or the triazene groups around the imidazole ring connective bond (Fig. 2).

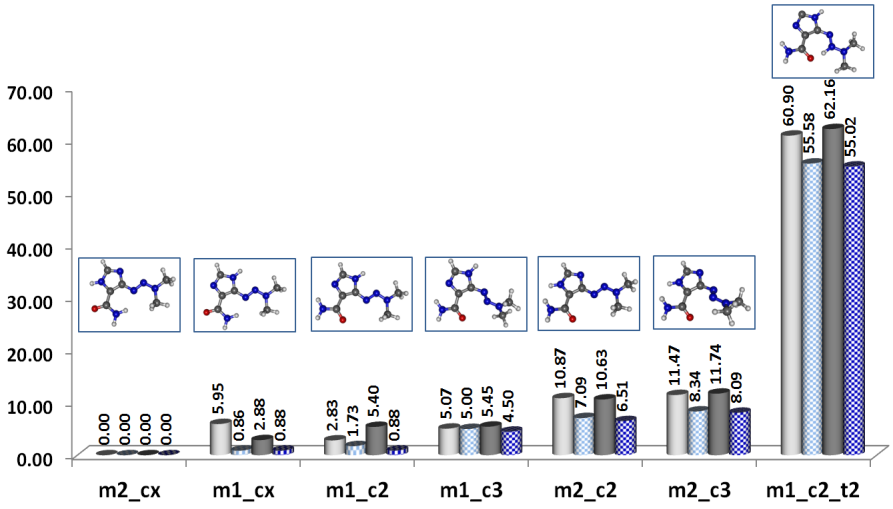

Figure 2. B3LYP/6-31+G(2d,2p) optimized geometries and relative energies (ΔE – light color, ΔG – dark color) of the DTIC monomers in vacuo (full fill) and water (squared fill).

All the optimizations were performed firstly in gas-phase, and then, by considering water as a solvent. To account for the interaction between the drug molecule and water, we used the Polarizable Continuum Model (PCM) [7].

In order to find the most stable conformer, in other words for the energetic order characterization, we used the relative energies (ΔE) but also the relative Gibbs free energies (ΔG), the last quantity being very recently recommended for such analyses and for calculating the relative Boltzmann populations [9].

Our results suggest that m2_cx monomer is more stable than the other structures in both gas-phase and in water. Structure optimizations show that water considerably stabilizes all geometries and an important difference is noticed between the gas-phase and the liquid state of m1_cx and m2_cx. The ΔG values of the three most stable geometries, with respect to the m2_cx are identical in water (0.88 kcal mol^{-1}) with relative Boltzmann populations of 15.4 %, while that of m2_cx is 69.1 %. All the other investigated conformers and tautomers have negligible populations with respect to the three most stable ones (Table 1).

Table 1. Relative free Gibbs energies and Boltzmann populations at room temperature for all conformers

Conformer	Relative Gibbs free energy in water (kcal/mol)	Boltzmann population at RT (%)
m2_cx	00.00	69.14
m1_cx	0.88	15.41
m1_c2	0.88	15.41
m1_c3	4.5	0.03
m2_c2	6.51	0.00
m2_c3	8.09	0.00
m1_c2_t2	55.02	0.00

The small relative Gibbs energy of the m1 and m2 tautomers in water points to the possibility of coexistence of these structures in water solutions since their structural interconversion could be activated at room temperature. Thus, the spectroscopic response of DTIC in water solution must be explained considering the contribution coming from both m2_cx and m1_cx tautomers.

3.2. Absorption spectrum of dacarbazine

Next, the absorption spectrum of dacarbazine was investigated by correlating the experimental results with the theoretical ones. For this purpose the calculations were performed by using the TD-DFT approach. By using convergent approximations, we were able to reproduce the experimental UV-Vis spectrum of DTIC within the experimental errors.

As observed in Fig. 3, the experimental absorption spectrum shows two peaks: one at 328 nm, representing the electronic transition between the ground and the first allowed excited state, and the second one at 236, assigned to a transition between the ground and the second allowed excited state. Both absorption peaks preserve their shape or position, when changing the concentration of DTIC in solution.

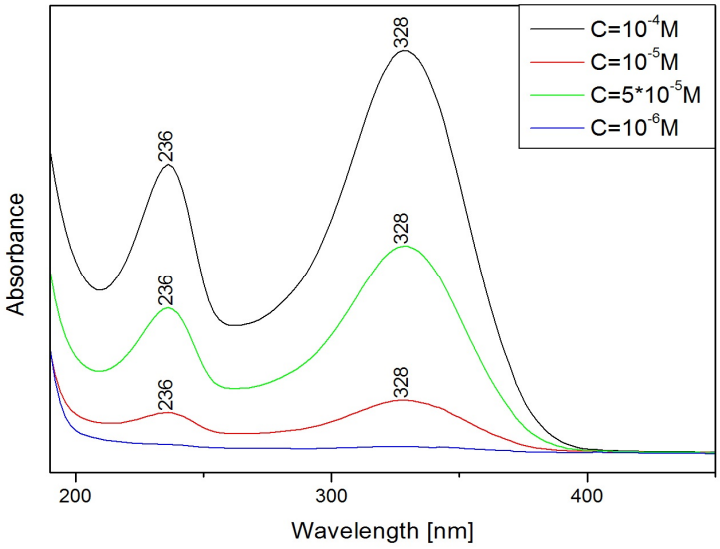

Figure 3. UV-Vis absorption spectrum of dacarbazine in water at different concentrations as indicated.

Subsequently, we investigated the absorption peaks in the UV-Vis spectrum and analyzed the excited states involved in these transitions. The necessary calculations were performed at B3LYP/6-31+G(2d,2p) level of theory on both tautomers found in solid state and the m1_c2 conformer, whose stability has been discussed in the previous section.

The obtained results reproduce the excitation energies values for all three monomers. Particularly, we were able to obtain an excellent agreement between the experimental energy of the first excited state at 328 nm and the calculated one at 324 nm. For the second transition, observed at 236 nm the calculated bands are found at 219, 224 and 231 ((228+233)/2) nm for the gas-phase m1_cx, m1_c2 and m2_cx monomers, respectively (Table 2). Therefore, for the gas-phase, we can state that a better agreement between experiments and TD-DFT calculations is obtained for m1_cx for the first excited state, and for m2_cx for the second excited state.

Table 2. Calculated electronic transition of dacarbazine in gas-phase and water at B3LYP/6-31+G(2d,2p) level of theory. Theoretical values (λ_{teor}) compared to experimental values (λ_{exp})

Gas phase					
System	**λ_{teor} (nm)**	**λ_{exp} (nm)**	**f**	**Transitions**	**Orbital Contributions**
m2_cx	298	328	0.53	H-L	98%
	233	236	0.11	H-4-L	61%
	228	236	0.18	H-3-L	40%
Water					
System	**λ_{teor} (nm)**	**λ_{exp} (nm)**	**f**	**Transitions**	**Orbital Contributions**
m1_cx	323	328	0.61	H-L	90%
	224	236	0.20	H-4-L	73%
m1_c2	320	328	0.68	H-L	99%
	224	236	0.18	H-4-L	76%
m2_cx	310	328	0.62	H-L	99%
	232	236	0.28	H-3-L	79%

The theoretical data obtained for the two monomers in water shows that the solvent affects very slightly the energy of the first excited state for m1_cx. However, for m2_cx we can observe a better agreement with the experiment, the new calculated transition energy being 310 nm. This is also true for the second transition whose calculated energy (232 nm) is also in better agreement with the experimental value (236 nm).

The calculated transition energies for both excited states are influenced more by considering the solvent effects in case of m2 monomer than for m1. It is also worth noting that the molecular orbitals involved in the transition corresponding to the first excited state are HOMO and LUMO, for both m1 and m2 monomers. The second excited state involves basically the transitions HOMO->LUMO+4, HOMO-4->LUMO or HOMO-LUMO+1. These orbitals are depicted in Fig. 4.

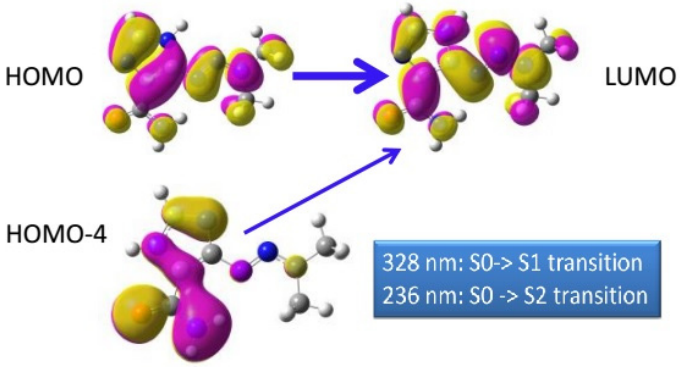

Figure 4. Molecular orbitals involved in the transition corresponding to the first and second electronic excited states of dacarbazine.

3.3. Dacarbazine – UV-Vis spectrum – pH dependence

The UV-Vis spectra of DTIC water solutions at different pH values were recorded in the 2-13 pH range (Fig. 5). With increasing the pH values the spectra exhibit considerable changes. We can easily observe the appearance of a new band located at 204 nm (pH 13) in addition to a red shift of the bands associated to the first and second excited states. Thus, the first band shows a displacement from 322 nm at pH=2 to 342 nm at pH=13. The second band shifts from 222 to 248 nm.

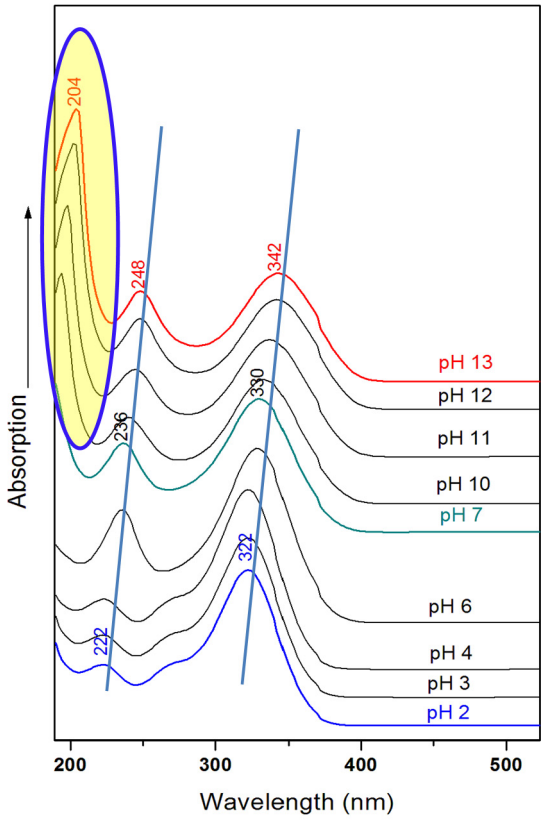

Figure 5. pH dependent UV-Vis spectra of dacarbazine in water.

We assume that this shift can be due to the presence of different molecular species of dacarbazine depending on the pH of the solution. Thus, by decreasing the pH value of the solution the protonated species are predominant, while after increasing the pH value the deprotonated species become predominant (Fig. 6).

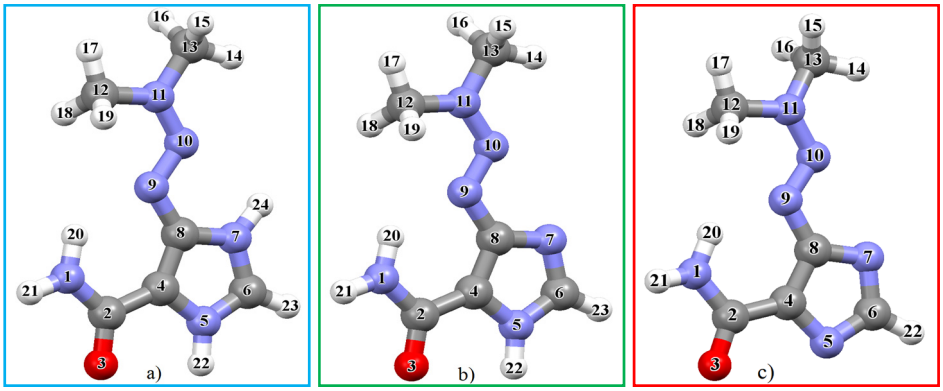

Figure 6. Species of the m2 conformer of dacarbazine found at different pH values: a) protonated; b) neutral; c) deprotonated.

To verify this hypothesis, we calculated the UV-Vis spectra for both protonated and deprotonated species. The results completely explain the experimental data (Fig. 7). Thus, we can observe a red shift of the entire spectrum for the deprotonated species, and a blue shift for the protonated ones. Moreover, as one can see from Fig. 7 the calculations reproduce not only the shifts of the transitions but also their relative intensities.

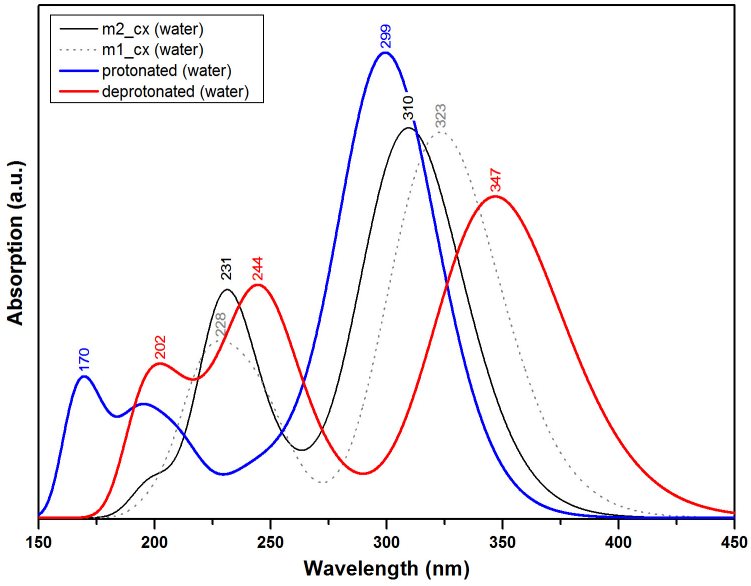

Figure 7. Calculated absorption spectra of pH dependent species of dacarbazine.

4. CONCLUSIONS

The herein presented results clearly demonstrate that there is need for a careful analysis of all possible conformers and tautomers of a drug's molecule as different conformations can give different spectroscopic responses. Special attention must be paid especially before any analysis of the liquid spectra.

The UV-Vis absorption measurements of aqueous solutions at several pH values correlated to TD-DFT calculations enabled us to give an assignment for the electronic transitions corresponding to the observed bands. Excellent agreement between the experimental UV-Vis data and TD-DFT results has been obtained considering the Boltzmann populations averaged molecular properties. In order to achieve a reliable assignment of UV-Vis spectra, it is necessary to take into consideration the solvent effects and the selection of the corresponding molecular models. Additionally, we identified the species that appear as a result of pH changes of the solution, namely protonated species at low pH values, and deprotonated species at high pH values.

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