**Theoretical study of two coumarin derivatives photosensitivity for possible use in photo-dynamic therapy**

**1Lamoussa Ouattara, 2Mamadou Guy-Richard Koné, 3Ouattara W. Patrice, 4Kafoumba Bamba**

*1Unité de formatin et de recherche des sciences de la mer, Université Polytechnique de San Pédro, BP 1800 Côte d’Ivoire*

*2,3,4Laboratoire de Thermodynamique et Physico-Chimie du Milieu, Université Nangui Abrogoua, Abidjan, 02 B.P. 801 Abidjan 02 Côte d’Ivoire*

\*Corresponding author: [kafoumba2001@yahoo.fr](mailto:kafoumba2001@yahoo.fr)

[okafile@gmail.com](mailto:okafile@gmail.com)

[wawohin@gmail.com](mailto:wawohin@gmail.com)

[guyrichardkone@gmail.com](mailto:guyrichardkone@gmail.com)

Abstract

The dynamism of cancer and its side effects related to different treatments are real questions for mankind to solve. Thus, this manuscript aims to explore the photochemical and photo-physical properties of two coumarin molecules due to their multiple biological and spectroscopic activities [1] in the framework of photodynamic therapy as a photosensitizer. For our aim fulfillment, quantum chemical methods such as DFT and TD-DFT at the B3LYP/6-31G(d,p) level were used in different media [2] in order to determine the parameters quoted above. The obtained results show that photosensitivity of the compounds are influenced by the nature of the solvent [3]. Thus, both compounds M1 and M2 produce charged radicals. Moreover, compound M1 presents the lowest values of VIP and the energy of the excited state ET necessary for the production of charged radicals. Therefore, it is assumed to be the most photosensitive and this photosensitivity is more accentuated in polar solvents. In sum, studied coumarins, in addition to the fact of being used in chemotherapy, they can also be used in photodynamic therapy as photosensitizers. However, the theoretical improvement of the studied parameters would be a significant advance for the experimenter.

Keywords: DFT, TD-DFT, photo-sensitizer, coumarin

1 Introduction

Coumarins are heterocyclic compounds with a wide range of biological activities [1] that, in addition to treating cancer, effectively fight against the side effects caused by radiotherapy [2].

Cancer is a worldwide public health problem. A cell is considered normal when it has a well-defined life cycle, including a programmed death called apoptosis. A series of genetic mutations in at least one cell is at the origin of the carcinogenesis process, as the cell develops an insensitivity to apoptosis and is no longer able to repair this error in the DNA [2]. Despite all the efforts made in terms of treatment, cancer continues to make desasters. The recurrence of metastases remains the main cause of failure of the various treatments [3], as well as the side effects which are often very devastating. These include mastectomy, resurgence of the pathology in other organs, anorexia, hair loss, etc. [4].

Photo-dynamic therapy (PDT) was discovered for the first time in the years 1900s. This method of treament is highly promising therapy with multiple advantages. PDT uses photoreactive molecule or photo-sensitizer which is combined with light of an appropriate wavelength to destroy tumour cells [5]. This method of treament is based on the selective destruction of tumors induced by photo-oxidation [8]. Photo-dynamic therapy has been juged to be effective in the treament of various types of cancer. Particularly which are superficially localized, as this intervention brings a significant improvement in both the patient's quality of life and its efficacy compared with palliative surgery or palliative chemotherapy treatments [6] [7]. As photo-dynamic therapy (PDT) does not compromise other treatment options. It reduces long-term morbidity compared with chemotherapy or radiotherapy. It also appears to be a promising treatment for the control of malignant diseases [7]. Photo-dynamic therapy (PDT) offers an emerging alternative to major surgical procedures. This is why this method was chosen for this study. The aim is to demonstrate the photosensitivity of two coumarin molecules synthesized by Morsy et al [9]. Figure 1 shows the 2D structures of the studied molecules.

 

M1 M2

Figure 1: 2D structure of compounds M1 and M2

2 Methods

2.1 Study of photo-physicochemical properties

M1 and M2 molecules undergo an optimization followed by a frequency calculation in different media. Optimized structures are then used to perform a single-point calculation in order to determine the anion and cation energies of these compounds at the B3LYP/6-31G (d,p) level. For the exploration of excited states, the time-dependent density functional (TDDFT) has been chosen. It is an effective approach for determining the excited state properties of photo-sensitizers in vacuum and solvents. Determined properties allow to understand photo-dynamic mechanisms [10]. Various selected solvents such as: water, which is a polar protic solvent, dimethylsulfoxide (DMSO), which is a polar aprotic solvent, and finally diethylether which is an apolar aprotic solvent.

The effects of the different solvents were taken into account by carrying out optimization and single-point calculations in each medium using the polarizable continuum model (CPCM) developed by Tomasi et al. [11].

**2.2 Vertical electron affinity and vertical ionization potential**

Vertical electron affinity and vertical ionization potential are very useful parmeters for chemists and biochemists, as they enable them to understand biological and chemical phenomena such as the donor or acceptor nature of DNA or RNA [12].

Vertical electron affinity is determined by the following expression

VEAso= Ea - Ep

VIP is defined as the difference between the electronic energies of the cationic form of concerned compound (Ec) and that of the neutral molecule (Ep). Vertical ionization potential (VIP) [13-14] measures the tendency of a chemical system to give up its electron. It can be compared to the ionization potential

VIPso= Ec- Ep.

**2.3 Principle of photodynamic therapy**

The three (3) components necessary for photo-dynamic therapy are [15]:

-the photo-sensitizer (PS) ;

- light with appropriate wavelength ;

- dissolved oxygen in cells [16].

There are two main mechanisms of photo-dynamic reactivity, all of which are closely depending on the oxygen molecules in the cellular environment. The first step of both mechanisms is similar [15]. After emission of a photosensitizer into the cellular environment, the latter is irradiated with light. This light must have a wavelength that coincides with the absorption spectrum of the photosensitizer. Excitation of the photosensitizer by the light causes it to change from the fundamental singlet energy state S0 to the excited singlet state S1 or Sn. When the photo-sensitizer is in the Sn state, it undergoes internal conversions to return to the S1 state. From this S1 state, part of the energy can be irradiated in the form of a fluorescence quantum, and the remaining energy will promote migration of the photo-sensitizer compound to the appropriate therapeutic form, which is the excited triplet T1 or Tn state, by intersystem conversion [17] [18].

At this level, the compound can evolve according to one of the two main mechanisms of photodynamic therapy, namely type I and type II. In mechanism I, the compound reacts directly with the target substrate, notably DNA or RNA. This reaction can occure in the compound's excited triplet form or in its cationic form. In mechanism II, the photosensitizer reacts with the target substrates via reactive oxygen species (ROS). These reactive oxygen species are generated by the interaction of the photo-sensitizer with the fundamental triplet oxygen 3O2. During this interaction, the photosensitizer either transfers energy to the fundamental triplet oxygen 3O2 to produce oxygen 1O2 or electrons to generate other radicals important for photo-dynamic activity.

**3 Results and discussion**

**3.1 Absorption spectra in different media**

Oscillation strengths and excitation energies of the singlet and triplet states of the two studied molecules were determined using TD-DFT calculations. All these parameters are listed in Table I. These calculations were carried out in different media, namely vacuum, water, DMSO and diethylether. These four types of medium are samples that reflect both the polarity-related character of a solvent and its protonic effect. In addition, the choice of these solvents is also linked, on the one hand, to the fact that some of them are widely used in the medical field and, on the other, to the fact that they are used in the laboratory for the synthesis and purification of organic molecules [19].

**3.1.1 Singlet excited states**

Table I: Excitation energies of the singlet and triplet states of M1 and M2 at B3LYP/6-31G (d, p).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | S1 | S2 | S3 | S4 | S5 | S6 | T1 | T2 | T3 | T4 | T5 | T6 |
| M1-vide | E | 3,3029 | 3,6315 | 3,6778 | 3,8535 | 3,9487 | 4,0066 | 0,5196 | 0,6052 | 0,7182 | 0,9853 | 1,0354 | 1,1328 |
| L | **375,38** | **341,41** | **337,12** | **321,75** | **313,98** | **309,45** | 2386,1 | 2048,7 | 1726,3 | 1258,3 | 1197,4 | 1094,5 |
| f | 0,0012 | 0,3832 | 0,014 | 0,0895 | 0,0006 | 0,0110 | 0,0001 | 0,0002 | 0,0000 | 0,0007 | 0,0006 | 0,0002 |
| M1- eau | E | 3,1563 | 3,4171 | 3,6994 | 3,7273 | 3,8380 | 3,9668 | 0,3397 | 0,6433 | 0,8722 | 0,9294 | 1,1140 | 1,2359 |
| L | **392,82** | **362,83** | **335,15** | **332,64** | **323,04** | **312,56** | 3649,8 | 1927,2 | 1421,5 | 1334,1 | 1112,9 | 1003,2 |
| f | 0,0010 | 0,4283 | 0,0139 | 0,0000 | 0,1135 | 0,0213 | 0,0002 | 0,0005 | 0,0056 | 0,0000 | 0,0266 | 0,0009 |
| M1-DMSO | E | 3,1583 | 3,4117 | 3,7022 | 3,7266 | 3,8323 | 3,9662 | 0,3430 | 0,6411 | 0,8764 | 0,9265 | 1,1085 | 1,2327 |
| L | **392,57** | **363,41** | **334,89** | **332,70** | **323,53** | **312,60** | 3614,4 | 1933,9 | 1414,6 | 1338,1 | 1118,5 | 1005,8 |
| f | 0,0010 | 0,4421 | 0,0142 | 0,0000 | 0,1197 | 0,0215 | 0,0002 | 0,0006 | 0,0062 | 0,0000 | 0,0291 | 0,0009 |
| M1- ether | E | 3,2041 | 3,4667 | 3,7158 | 3,7699 | 3,8412 | 3,9772 | 0,4110 | 0,6164 | 0,8656 | 0,9707 | 1,0841 | 1,1742 |
| L | **386,95** | **357,64** | **333,66** | **328,88** | **322,78** | **311,74** | 3016,9 | 2011,3 | 1432,3 | 1277,2 | 1143,6 | 1055,9 |
| f | 0,0011 | 0,4443 | 0,0000 | 0,0282 | 0,1003 | 0,0178 | 0,0002 | 0,0005 | 0,0000 | 0,0074 | 0,0065 | 0,0008 |
| M2- vide | E | 3,099 | 3,400 | 3,546 | 3,717 | 3,750 | 3,926 | 0,5578 | 0,5804 | 0,9293 | 1,2033 | 1,5828 | 1,5977 |
| L | **400,10** | **364,67** | **349,65** | **333,55** | **330,67** | **315,79** | 2222,5 | 2136,3 | 1334,2 | 1030,4 | 783,34 | 776,01 |
| f | 0,005 | 0,425 | 0,081 | 0,002 | 0,007 | 0,002 | 0,0009 | 0,0179 | 0,0001 | 0,0282 | 0,0011 | 0,0000 |
| M2- eau | E | 2,927 | 3,429 | 3,644 | 3,703 | 3,760 | 3,778 | 0,3435 | 0,6525 | 1,0950 | 1,3705 | 1,6153 | 1,7067 |
| L | **423,59** | **361,53** | **340,25** | **334,86** | **329,78** | **328,18** | 3609,3 | 1900,1 | 1132,2 | 904,65 | 767,55 | 726,46 |
| f | 0,003 | 0,173 | 0,275 | 0,071 | 0,009 | 0,011 | 0,0371 | 0,0034 | 0,0401 | 0,0039 | 0,0052 | 0,0002 |
| M2- DMSO | E | 2,928 | 3,424 | 3,641 | 3,702 | 3,759 | 3,782 | 0,3422 | 0,6510 | 1,0920 | 1,3699 | 1,6141 | 1,7058 |
| L | **423,50** | **362,07** | **340,53** | **334,88** | **329,83** | **327,87** | 3623,6 | 1904,5 | 1135,4 | 905,08 | 768,13 | 726,82 |
| f | 0,004 | 0,181 | 0,304 | 0,064 | 0,009 | 0,011 | 0,0378 | 0,0036 | 0,0427 | 0,0041 | 0,0056 | 0,0002 |
| M2- ether | E | 3,099 | 3,400 | 3,546 | 3,717 | 3,750 | 3,926 | 0,1106 | 0,4543 | 0,5630 | 0,8282 | 0,8474 | 0,8894 |
| L | **400,10** | **364,67** | **349,65** | **333,55** | **330,67** | **315,79** | 11205 | 2728,8 | 2202,1 | 1496,9 | 1463,0 | 1393,9 |
| f | 0,005 | 0,425 | 0,081 | 0,002 | 0,007 | 0,002 | 0,0139 | 0,0003 | 0,0000 | 0,0004 | 0,0012 | 0,0016 |

Table I contains the oscillation strengths, excitation energies of the six first singlet and triplet Excited states of studied coumarin derivatives. These parameters are determined in vacuum, water, DMSO and ether. Considering the oscillation strengths of these excited states, which reflect their realization probability, we can say that all states of these two compounds exist, excepted two states of compound M1 namely S4 and S3 respectively in DMSO and in ether. The wave-lengh of these states are located at 332.64 nm in water, 332.70 nm in DMSO and 333.66 nm in ether respectively. They reflect the same absorption band of compound M1, which is affected by polarity. There is a bathochromic effect when polarity is decreasing. First excited states (S1) are decisive in elucidating the mechanism of photodynamic therapy. These first states (S1) are found in compound M1 at 375.38 in vacuum, 392.82 in water; 392.57 nm in DMSO and 386.95 nm. Concerning compound M2 the same states are found at 400.10 nm in vacuum, 423.59 nm in water, 423.50 nm in DMSO and 410.10 nm in ether. For the first states, we find that λS1(M1) < λS1(M2) in all considered media. This finding is certainly linked to the substitution of hydrogen H in M1 by the methyl group to obtain compound M2. Concerning absorption wavelengths of excited states of molecules M1 and M2 have absorption bands which belong to UVA range. This shows that these compounds can be used to treat superficial tumors. In addition, UVA light can penetrate the skin as far as the dermis.

**3.1.1 Triplet excited states**

Triplet states are less stable than singlet states, but they have much longer lifetimes than singlet states [20]. For this reason, they play a decisive role in photo-dynamic activity. This long lifetime favors photochemical processes. Table I contains the oscillation strengths and excitation energies of the six first triplet excited states of coumarin M1 and M2 determined in vacuum, water, DMSO and ether. The T3 states in vacuum, T4 in water, T4 in DMSO and T3 in ether of compound M1 have zero as oscillation strengths. Concerning M2, the states such as T6 in vacuum and T3 in ether have their oscillator strength which are nul. The fact that oscillator strength is nul means that these states cannot be realized. Consequently, they will not be taken into account in elucidating the mechanism of photo-dynamic therapy. The ET energies of these triplet states will be used to determine the vertical electronic affinities in the triplet state (VEAT) and the vertical ionization potentials in the triplet state (VIPT), as well as to assess the ability of these compounds to produce singlet oxygen.

**3.2 Vertical Electronic Affinity (VEA) and Vertical Ionization Potential (VIP) of coumarin M1 and M2.**

Energies values of VEAS0, VIPS0 and the different energies of the neutral molecule and the cationic and anionic forms are given in Table II. In the ground state, compounds can behave as electron donors or acceptors during the photosensitization reaction. Quantities such as vertical electron affinity (VEA) and vertical ionization potential (VIP) can help to understand electron movements between the photosensitizer and other molecules, notably DNA or RNA molecules. The subscript Sn indicates the nth-order excited singlet state, while the subscript Tn is used for the nth-order excited triplet state.

Table II: Cation and anion molecule energies and VEAs0 and VIPs0

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Composés | Milieu | Ep | Ec | Ea | VEAs0 | VIPs0 |
| M1 | Vide | -1995,852 | -1995,583 | -1995,895 | -1,185 | 7,323 |
| DMSO | -1995,874 | -1995,655 | -1995,975 | -2,757 | 5,955 |
| Eau | -1995,874 | -1995,656 | -1995,976 | -2,774 | 5,941 |
| Ether | -1995,867 | -1995,635 | -1995,953 | -2,357 | 6,301 |
| M2 | Vide | -1670,322 | -1670,025 | -1670,374 | -1,437 | 8,079 |
| DMSO | -1670,343 | -1670,107 | -1670,451 | -2,939 | 6,422 |
| Eau | -1670,344 | -1670,108 | -1670,452 | -2,939 | 6,414 |
| Ether | -1670,338 | -1670,087 | -1670,431 | -2,531 | 6,83 |

With Ep: energy of the neutral molecule, Ec: energy of the cationic form, Ea: energy of the anionic form, VEAS0: vertical electronic affinity in the ground state, and VIPS0: vertical ionization potential in the ground state.

In Table II values analysis allows to say that the compound M1 is more ionisable than compound M2 in all the media because the following inequality VIPso(M1) < VIPso(M2) is observed in all media According to polarity table II shows that polar solvents are the media in which VIP and VEA values are lowest. This means that studied compounds are more susceptible to donate or accept electrons in polar media. Consequently, we can conclude that solvent polarity is an amplifying factor in the reactivity of these compounds. Benzocoumarin (M1) has the lowest VIPso values, making it the best electron donor. The VEAso values show that the compound M2 can easily accept electrons, as it has the lowest VEAso values whatever considered medium.

**3.3 Vertical electron affinity and ionization potential of DNA or RNA bases**

All DNA and RNA bases have undergone the same types of calculations as M1 and M2 molecules. Parameters such as the vertical electron affinity VEAso and the vertical ionization potential VIPso of DNA and RNA bases were determined in the different media like compound M1 et M2. The values are given below in Table III.

Table III: Vertical electron affinity and vertical ionization potential of DNA and RNA bases expressed in eV.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Base** | **Milieu** | **En** | **Ec** | **Ea** | **VEAS0** | **VIPS0** |
| **Adénine** | Eau | -467,345 | -467,121 | -467,369 | -0,664 | 6,105 |
| Vide | -467,331 | -467,037 | -467,276 | 1,519 | 8,001 |
| DMSO | -467,345 | -467,120 | -467,369 | -0,645 | 6,122 |
| Ether | -467,342 | -467,102 | -467,348 | -0,163 | 6,532 |
| **Cytosine** | Eau | -394,962 | -394,728 | -394,991 | -0,789 | 6,368 |
| Vide | -394,941 | -394,633 | -394,892 | 1,345 | 8,39 |
| DMSO | -394,962 | -394,727 | -394,991 | -0,776 | 6,384 |
| Ether | -394,957 | -394,706 | -394,968 | -0,313 | 6,823 |
| **Guanine** | Eau | -542,59 | -542,378 | -542,598 | -0,218 | 5,759 |
| Vide | -542,565 | -542,283 | -542,499 | 1,799 | 7,675 |
| DMSO | -542,59 | -542,377 | -542,597 | -0,207 | 5,775 |
| Ether | -542,583 | -542,356 | -542,573 | 0,281 | 6,191 |
| **Thymine** | Eau | -454,163 | -453,924 | -454,197 | -0,925 | 6,504 |
| Vide | -454,149 | -453,828 | -454,107 | 1,142 | 8,721 |
| DMSO | -454,163 | -453,923 | -454,196 | -0,894 | 6,527 |
| Ether | -454,159 | -453,902 | -454,176 | -0,445 | 7,015 |
| **Uracile** | Eau | -414,841 | -414,591 | -414,879 | -1,034 | 6,803 |
| Vide | -414,826 | -414,489 | -414,786 | 1,075 | 9,159 |
| DMSO | -414,841 | -414,59 | -414,878 | -1,009 | 6,821 |
| Ether | -414,837 | -414,567 | -414,857 | -0,551 | 7,341 |

All the VEA values in Table III are negative, with the exception of those obtained in vacuum and that of guanine in ether. Furthermore, for a given medium, DNA or RNA bases are ordered according to VEAso values as follows: VEAso (uracil) ˂ VEAso (thymine) ˂ VEAso (cytosine) ˂ VEAso (adenine) ˂ VEAso (guanine). This order indicates a decreasing attractive power of these bases, whatever the considered medium

. Uracil is the best attractor.

On the other hand, ranking according to VIPso values gives the following order VIPso (Guanine) ˂ VIPso (Adenine) ˂ VIPso(Cytosine) ˂ VIPso (Thymine) ˂ VIPso (uracil). This order is the same in all media. From this ranking, guanine emerges as the best electron donor. Furthermore, water is the solvent with the lowest VEA and VIP values, followed by DMSO, ether and vacuum. Ultimately, polar solvents favor the donor and acceptor characteristics of DNA and RNA bases.

**3.4 Elucidation of photosensitization mechanisms for coumarin molecules**

In photo-dynamic therapy, there are essentially two different mechanisms quafied by type I and type II. The ES1 energies of the first singlet state and the ET energies of the lowest triplet excited states are essential for the elucidation of these mechanisms.

**3.4.1 Type I mechanism**

The triplet state of each coumarin derivative is achieved by bringing energy (hν) from the ground state to the excited singlet state. Once in the singlet state, the compound is converted into triplet state by inter-system conversion.

In this triplet state, the coumarin molecule can attack DNA or RNA bases directly by removing them of an electron. This is possible if the sum VEAT of the coumarin molecule and VIP of the DNA or RNA bases is negative. This attack is characterized by equation I.

Equation **1 : *PS* (T1) + B  *PS*.- + B.+  (I)**

With B: DNA or RNA base

According to Jablonski's diagram [21], the energy of the excited triplet state (ET) must be less than the energy of the excited singlet state (S1) [22]. For this reason, all ET energies lower than that of the first excited singlet state will generate VEAT and VIPT values that can be used to determine the conditions for carrying out the various photochemical reactions. Thus, the ET energy values, as well as the corresponding sums of the VEAT values of the coumarin molecule and VIP values of the DNA or RNA bases as a function of solvent, are given in Table IV.

Table IV: Direct attack values for DNA and RNA compounds and bases

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Et1 | M-A | M-C | M-G | M-T | M-U |
| M1 | vide | 2,828 | 3,988 | 4,377 | 3,662 | 4,708 | 5,146 |
| eau | 2,838 | 0,493 | 0,756 | 0,147 | 0,892 | 1,191 |
| DMSO | **2,838** | 0,527 | 0,789 | 0,180 | 0,932 | 1,226 |
| éther | **2,325** | 1,850 | 2,141 | 1,509 | 2,333 | 2,659 |
| M2 | vide | 3,270 | 3,294 | 3,683 | 2,968 | 4,014 | 4,452 |
| eau | **2,851** | 0,315 | 0,578 | **-0,021** | 0,714 | 1,013 |
| DMSO | **2,850** | 0,333 | 0,595 | **-0,014** | 0,738 | 1,032 |
| éther | 3,024 | 0,977 | 1,268 | 0,636 | 1,460 | 1,786 |

Avec:

C-A : VEAT1 (coumarine) +VIP(Adenine); C-C : VEAT1 (coumarine) +VIP(Cytosine) ;

C-G: VEAT1 (coumarine) +VIP(Guanine); C-T : VEAT1 (coumarine) +VIP(Thymine);

C-U: VEAT1 (coumarine) +VIP (Uracil)

Compound M1 values are all positive in all media, suggesting that compound M1 does not react with DNA and RNA bases. Concerning molecule M2, it reacts only with guanine in polar media. The sum values for this reaction are -0.021 and -0.014 in water and DMSO respectively, for ET energies of 2.851 eV and 2.850 eV. This means that compound M2 can ionize guanine from DNA or RNA when the triplet state energy reached after intersystem conversion is of the order of 2.851 eV in water and 2.850 eV in DMSO.

These compounds can interact with DNA or RNA bases via a cation resulting from auto-ionization. Auto-ionization step is characterized by equations (2) and (3). Once the cationic species is formed, the DNA base, via equation (4), will transfer its electrons to the cationic species.

Equation 2 : *PS* (T1) + *PS* (S0)  *PS***.**+ + *PS***.**-

Equation 3 : *PS* (T1) + *PS* (T1)  *PS***.**+ + *PS*

Equation 4 : *PS* + + B  *PS* (S0) + B**.**+

Equations 2 and 3 lead to the production of anionic and cationic radical species, which are powerful oxidizing agents used in photo-dynamic theory. The realization of these equations is linked to the signs of the following sums VEAT1+VIP, VIPT1+VEA and VEAT1+VIPT1. These sums have been calculated and reported in Table V while exploring all energies of the excited triplet state that are lower than that of the first excited singlet state.

Table V: Sum of VEAT1 + VIP, VIPT1 + VEA and VEAT1+VIPT1 reflecting auto-ionization reactions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Milieu | Et1 | VEAT1+VIP | VIPT1+VEA | VEAT1+VIPT1 |
| M1 | Vide | 2,828 | 3,31 | 3,31 | 0,483 |
| Ether | 1,986 | 1,958 | 1,958 | -0,028 |
| DMSO | 1,614 | 1,584 | 1,584 | -0,03 |
| Eau | 1,615 | 1,551 | 1,551 | -0,064 |
| M2 | Vide | 3,27 | 3,373 | 3,373 | 0,103 |
| Ether | 2,234 | 2,065 | 2,065 | -0,169 |
| DMSO | 1,823 | 1,66 | 1,66 | -0,163 |
| Eau | 1,836 | 1,639 | 1,639 | -0,197 |

Values in Table V indicate that for molecules M1 and M2, the relationships VEAT1+VIP, VIPT1+VEA and VEAT1+VIPT1 are all positive, whatever the energy value of the triplet excited state under consideration, suggesting that equations (2), (3) and (4) are thermodynamically impossible in a vacuum. The type I mechanism cannot take place in a vacuum, as there is no production of ionic radicals in coumarins.

The first two relationships, VEAT1+VIP and VIPT1+VEA, are all positive for molecules M1 and M2. These positive values mean that reaction (2) is thermodynamically impossible in all solvents. Consequently, there can be no production of coumarin cationic radicals following this reaction. This implies that reaction (4) cannot take place in any of the different media using this process [13]. According to VEAT1+VIPT1 relationship, it shows negative values in all solvents. For compound M1, negative values are obtained with the following ET energies 1.986 eV; 1.614 eV and 1.615 eV in ether, DMSO and water respectively. As far as compound M2 is concerned, negative values are obtained with the following ET energies 2.234 eV; 1.823 eV and 1.836 eV in ether, DMSO and water respectively.

Thus, in polar solvents, the value of required energy for the triplet excited state is lower than that needed in apolar solvent. Molecule M1 is also more reactive than M2 molecule under these conditions because ET energy required for M1 compound in reaction (3) is lower than that needed in M2 compound. For both studied compounds, auto-ionization can allows the production of anionic coumarin radicals via equation (3) for precise values of triplet state energy. The formation of these cationic radicals is also accompanied by the formation of anionic radicals. Ultimately, studied compounds are photosensitive for given triplet energies in solution. This photosensitivity is much more pronounced in water and DMSO than in ether.

3.5 Type II photosensitization mechanism

In the type II mechanism, the photosensitizer must be in the triplet state, so it can react directly with the oxygen in its fundamental triplet state (3O2), transferring him excess energy or electrons which will allow to bring it back to the fundamental state. In the case of energy transfer from the photo-sensitizer to oxygen in its triplet state, the oxygen passes from triplet state (3O2) to its singlet state (1O2). Singlet oxygen 1O2 is a powerful oxidizing agent that can react with many cellular constituents, such as saturated glycerol triacyls, membrane cholesterol, phospholipids, amino acids (histidine, tryptophan, methionine) and nucleic acids [19]. Singlet oxygen 1O2 is generated by a triplet-triplet energy transfer between the triplet ground state of oxygen and the triplet excited state of the photo-sensitizer, which is formed by inter-system conversion ISC [24]. This inter-system conversion can take place between two energy levels, such as from S1 to Tn or from T1 to S0 [23] [25] [24]. Due to its short lifetime and high reactivity, the produced singlet oxygen (1O2) reacts at its place of formation in the cell [19]. The reaction operates by destroying the target and surrounding cells. It is expressed as

l’équation 5 ***PS* (T1) + 3O2   *PS* (S0) + 1O2**

Equation 5 is highly oxygen-dependent. The required condition for the realization of this reaction depends on the energy ET value of the triplet excited state of the photosensitizer. On the one hand, this energy must be higher than the excitation energy of singlet oxygen, whose theoretical value is estimated at 1.06 eV [13] [23]. On the other hand, it must also be lower than that corresponding to the first excited singlet state ES1, in order to promote intersystem conversion [23]. For this reason, we have evaluated the energies of the lowest triplet states of the coumarin derivatives M1 and M2. Table VI shows the energies corresponding to the first singlet state S1, as well as those for the first six triplet states of each molecule below that of the S1 state.

Table VI: ES1 and ET energy values for M1 and M2 molecules in vacuum and solvents (water, DMSO and ether).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | M1 | | | | M2 | | | |
|  | Vide | Eau | DMSO | Ether | Vide | Eau | DMSO | Ether |
| *S1* | *3,099* | *2,927* | *2,928* | *2,945* | *3,303* | *3,156* | *3,158* | *3,204* |
| T1 | 0,558 | 0,344 | 0,342 | 0,111 | 0,52 | 0,34 | 0,343 | 0,411 |
| T2 | 0,58 | 0,653 | 0,651 | 0,454 | 0,605 | 0,643 | 0,641 | 0,616 |
| T3 | 0,929 | 1,095 | 1,092 | 0,563 | 0,718 | 0,872 | 0,876 | 0,866 |
| T4 | 1,203 | 1,371 | 1,37 | 0,828 | 0,985 | 0,929 | 0,927 | 0,971 |
| T5 | 1,583 | 1,615 | 1,614 | 0,847 | 1,035 | 1,114 | 1,109 | 1,084 |
| T6 | 1,598 | 1,707 | 1,706 | 0,889 | 1,133 | 1,236 | 1,233 | 1,174 |

Energy values in Table VI show that the two coumarin derivatives M1 and M2 have their energies ET1 of first excitation which are less than 1.06eV. However, these coumarin derivatives also possess other triplet states with energies which are above 1.06eV. Compound M1, for example, has three triplet states in vacuo that fulfill this superiority condition. In solution, two trendancy are observed. Firstly, in polar solvents (water and DMSO), compound M1 has four triplet states with energies which are above 1.06eV. Secondly, in ether, compound M1 has no triplet states among its lowest states which fulfills this criterion. As far as compound M2 is concerned, it has one triplet state in vacuum and two triplet states in solution which are satisfying this criterion. According to what has been written above, we can conclude that these two coumarin derivatives possess photodynamic activity according to mechanism II. Taking into account energy values given in Table VI, these compounds are likely to produce singlet oxygen 1O2 by energy transfer [19]. Highest energy values of these triplet states fulfilling the selection criteria for these two coumarin derivatives are obtained in aqueous solution. These values are 1.095eV; 1.371eV; 1.615eV and 1.707eV for compound M1; 1.114eV and 1.236eV for compound M2. This finding indicates that photodynamic activity is more intense in polar protic solvents.

In the case of electron transfer, superoxide anion (O2-) is produced. This anion can be generated according two different ways: either by interaction between the triplet-state of the photosensitizer and triplet-state of oxygen (3O2), or by interaction between the radical anion of coumarin resulting from auto-ionization and triplet-state of oxygen (3O2).

Thus, equation 6 translates the production reaction of superoxide anion (O2-) by the interaction of the photosensitizer with oxygen (3O2), both members of the equation 6 are in their triplet state. For Equation 6, to be thermodynamically favorable, the sum of the vertical ionization potential (VIPT) of coumarin molecules and the adiabatic affinity of oxygen (AEA(O2)) must be negative. The value of adiabatic affinity differs from one medium to another: -0.59 in vacuum, -3.91 in water, -3.65 in DMSO and -3.14 in diethyl ether [23] [13] [10].

Equation 6 *PS* (T1) + 3O2   *PS* **·+ + O2·-**

Equation 7 shows the reaction of anionic oxygen production (O2-) by transferring an electron from coumarin radical anion issued from equation (3) to the triplet oxygen .

Equation 7 *PS* **·-** + 3O2  *PS* (S0) **+ O2·-.**

Equation 7 is governed by the sign of the following difference (AEA(O2)-VEAso ). If this sign is negative so the reaction is thermodynamically favorable.

The formation of anionic oxygen radical species will generate the production of different reactive species of oxygen (ROS ) that will be formed during these last photochemical reactions (H2O2, O2•-, •OH). These superoxide compounds possess potent oxidative power for a wide variety of biomolecules such as cholesterol or the side chains of certain amino acids (tryptophan, histidine, and methionine) [26]. In Table VII Sum of AEA(O2)-VEAso and VIPT1+AEA(O2) reflecting superoxide radical anion production

Table VII : Superoxide radical anion production parameters

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Milieu | Et1 | *AEA(O2)-VEAso* | | *VIPT1+AEA(O2)* |
| M1 |  |  |  | |  |
| Vide | 2,828 | 0,595 | 3,906 | |
| Ether | 1,986 | -0,783 | 1,175 | |
| DMSO | 1,614 | -0,893 | 0,69 | |
| Eau | 1,615 | -1,136 | 0,675 | |
| M2 | Vide | 3,27 | 0,847 | 4,219 | |
| Ether | 2,234 | -0,609 | 1,456 | |
| DMSO | 1,823 | -0,711 | 0,949 | |
| Eau | 1,8 36 | -0,971 | 0,668 | |

In vacuum, all quantities are positive, which confirms that reactions 6 and 7 are not thermodynamically favourable. This is true for the two studied molecules.

In Table VII, all the VIPT+AEA(O2) sums are positive for all the studied compounds, whatever the medium considered. Thus, superoxide radical anion production is not favorable according to equation 6. On the other hand, some values of the difference (AEA(O2)-VEAso ) are negative for molecules M1 and M2. For the molecule M1, the negative values are -0.783; -0.893 and -1.136 these values are obtained respectively for the following ET energies 1.986 eV in ether; 1.614 eV in DMSO and 1.614 eV in water. As far as M2 molecule is concerned, negative values are -0.609; -0.711 and -0.971 these values are obtained respectively for the following values of ET energies 2.234 eV in ether; 1.823 eV in DMSO and 1.836 eV in water. As the auto-ionization leads to the formation of the anionic radical of each of these two compounds occurs in solvents , then the production of superoxide radical anion (O-2) by photo-irradiation of these two molecules is favorable. Furthermore, the ET required energy for superoxide radical anion production for a given compound is lower in polar solvents than in apolar ones. Furthermore, the required energy ET for superoxide radical anion production for the M1 molecule is lower than that required for the M2 compound. This could be explained by the different geometries and the natur of subtituents of these two molecules.

4 Conclusion

Photo-physical and photochemical properties, such as the energies of triplet states that are lower than the energy of the excited singlet state, vertical electronic affinities and vertical ionization potentials, of coumarin molecules, in polar and non-polar solvents were examined by TDDFT method. These parameters were used to elucidate the mechanisms of photosensitivity of these compounds in the context of photodynamic therapy. As a result of these analyses, these coumarin compounds were juged to be susceptible to develop photo-sensitizing activity in the theory of photo-dynamic therapy, according to the two described mechanisms. On the one hand, photo-dynamic activity can occur according to mechanism I. Under these conditions, studied coumarin derivatives can generate damage to DNA bases or target tissues in a direct manner for a well-defined ET1 energy via guanine. On the other hand, photo-dynamic activity can take place according to mechanism II. In this case, these compounds will react indirectly with DNA or RNA bases through the production of singlet oxygen in water, diethyl ether and DMSO, or through the production of the anionic superoxide radical (O2-) in water, DMSO and diethyl ether. These radicals can act directly or indirectly on these substrates. Solvent polarity is a factor that amplifies the photo-sensitizing properties of the studied coumarins.

**References**

[1] L. Ouattara *et al.*, “Predictive Modeling of Breast Anticancer Activity of a Series of Coumarin Derivatives using Quantum Descriptors,” *Chem. Sci. Int. J.*, vol. 26, no. 4, pp. 1–10, 2019, doi: 10.9734/csji/2019/v26i430098.

[2] E. K. Akkol, Y. Genç, B. Karpuz, E. Sobarzo-Sánchez, and R. Capasso, “Coumarins and coumarin-related compounds in pharmacotherapy of cancer,” *Cancers (Basel).*, vol. 12, no. 7, pp. 1–25, 2020, doi: 10.3390/cancers12071959.

[3] L. L. Dos Santos A F, de Almeida D R Q, Terra L F, Baptista M S, “Photodynamic therapy in cancer treatment - an update review,” *J. Cancer Metastasis Treat.*, vol. 25, no. 5, pp. 1–20, 2019.

[4] P. W. Yss *et al.*, “PHOTODYNAMIC THERAPY OF LOCOREGIONAL BREAST CANCER RECURRENCES USING A CHLORIN-TYPE PHOTOSENSITIZER,” *Int. J. Cancer*, vol. 93, pp. 720–724, 2001.

[5] S. O. Gollnick, L. Vaughan, and B. W. Henderson, “Generation of effective antitumor vaccines using photodynamic therapy,” *Cancer Res.*, vol. 62, no. 6, pp. 1604–1608, 2002.

[6] A. K. D’Cruz, M. H. Robinson, and M. A. Biel, “mTHPC-mediated photodynamic therapy in patients with advanced, incurable head and neck cancer: A multicenter study of 128 patients,” *Head Neck*, vol. 26, no. 3, pp. 232–240, 2004, doi: 10.1002/hed.10372.

[7] A. F. Dos Santos, D. R. Q. De Almeida, L. F. Terra, M. S. Baptista, and L. Labriola, “Photodynamic therapy in cancer treatment - an update review,” *J. Cancer Metastasis Treat.*, vol. 25, no. 5, pp. 1–20, 2019.

[8] T. J. Dougherty, “Hematoporphyrin derivative for detection and treatment of cancer,” *J. Surg. Oncol.*, vol. 15, pp. 209–210, 1980.

[9] S. A. Morsy, A. A. Farahat, M. N. A. Nasr, and A. S. Tantawy, “Synthesis, molecular modeling and anticancer activity of new coumarin containing compounds,” *Saudi Pharm. J.*, vol. 25, no. 6, pp. 873–883, 2017, doi: 10.1016/j.jsps.2017.02.003.

[10] L. Shen, H. F. Ji, and H. Y. Zhang, “A TD-DFT study on triplet excited-state properties of curcumin and its implications in elucidating the photosensitizing mechanisms of the pigment,” *Chem. Phys. Lett.*, vol. 409, no. 4–6, pp. 300–303, 2005.

[11] M. Cossi, V. Barone, R. Cammi, and J. Tomasi, “Ab initio study of solvated molecules: A new implementation of the polarizable continuum model,” *Chem. Phys. Lett.*, vol. 255, no. 4–6, pp. 327–335, 1996, doi: 10.1016/0009-2614(96)00349-1.

[12] Y. Valadbeigi, H. Farrokhpour, and M. Tabrizchi, “G4MP2, DFT and CBS-Q calculation of proton and electron affinities, gas phase basicities and ionization energies of hydroxylamines and alkanolamines,” *J. Chem. Sci.*, vol. 126, no. 4, pp. 1209–1215, 2014, doi: 10.1007/s12039-014-0668-y.

[13] X. Zhao, Z. Zheng, S. Feng, Z. Shi, and D. Chen, “A TD-DFT study on the photo-physicochemical properties of chrysophanol from rheum,” *Int. J. Mol. Sci.*, vol. 10, no. 7, pp. 3186–3193, 2009.

[14] C. M. N. Yow *et al.*, “Photocytotoxic and DNA damaging effect of Temoporfin ( mTHPC ) and merocyanine 540 ( MC540 ) on nasopharyngeal carcinoma cell,” *Toxicol. Lett.*, vol. 115, pp. 53–61, 2000.

[15] S. Kwiatkowski *et al.*, “Photodynamic therapy – mechanisms, photosensitizers and combinations,” *Biomed. Pharmacother.*, vol. 106, pp. 1098–1107, 2018.

[16] R. R. Allison and K. Moghissi, “Photodynamic therapy (PDT): PDT mechanisms,” *Clin. Endosc.*, vol. 46, pp. 24–29, 2013.

[17] C. A. Robertson, D. H. Evans, and H. Abrahamse, “Photodynamic therapy (PDT): A short review on cellular mechanisms and cancer research applications for PDT,” *J. Photochem. Photobiol. B Biol.*, vol. 96, pp. 1–8, 2009.

[18] A. P. Castano, T. N. Demidova, and M. R. Hamblin, “Mechanisms in photodynamic therapy: Part two - Cellular signaling, cell metabolism and modes of cell death,” *Photodiagnosis Photodyn. Ther.*, vol. 2, no. 1, pp. 1–23, 2005.

[19] W. P. Ouattara *et al.*, “Theoretical Studies of Photodynamic Therapy Properties of Azopyridine δ-OsCl2(Azpy)2 Complex as a Photosensitizer by a TDDFT Method Wawohinlin,” *Comput. Chem.*, vol. 09, no. 01, pp. 64–84, 2021.

[20] L. Shen, H. F. Ji, and H. Y. Zhang, “A TD-DFT study on photo-physicochemical properties of hypocrellin A and its implications for elucidating the photosensitizing mechanisms of the pigment,” *J. Photochem. Photobiol. A Chem.*, vol. 180, no. 1–2, pp. 65–68, 2006.

[21] R. C. Evans, P. Douglas, and H. D. Burrows, *Applied photochemistry*, vol. 9789048138. 2014.

[22] S. Perun, J. Tatchen, and C. M. Marian, “Singlet and triplet excited states and intersystem crossing in free-base porphyrin: TDDFT and DFT/MRCI study,” *ChemPhysChem*, vol. 9, no. 2, pp. 282–292, 2008, doi: 10.1002/cphc.200700509.

[23] J. Llano, J. Raber, and L. A. Eriksson, “Theoretical study of phototoxic reactions of psoralens,” *J. Photochem. Photobiol. A Chem.*, vol. 154, no. 2–3, pp. 235–243, 2003.

[24] W. Wu, X. Shao, J. Zhao, and M. Wu, “Controllable Photodynamic Therapy Implemented by Regulating Singlet Oxygen Efficiency,” *Adv. Sci.*, vol. 4, no. 7, pp. 1–21, 2017.

[25] W. Wu, X. Shao, J. Zhao, and M. Wu, “Controllable Photodynamic Therapy Implemented by Regulating Singlet Oxygen Efficiency,” *Adv. Sci.*, vol. 4, no. 7, pp. 1–21, 2017.

[26] B. Halliwell, “Antioxidant defence mechanisms: From the beginning to the end (of the beginning),” *Free Radic. Res.*, vol. 31, no. 4, pp. 261–272, 1999.