

Theoretical study of two coumarin derivatives photosensitivity for possible use in photodynamic therapy

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Abstract: The dynamism of cancer and its side effects related to different treatments are real questions for humankind 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 (PDT) as a photosensitizer (PS). 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 to determine the parameters quoted above. The obtained results show that the solvent's nature influences the compounds' photosensitivity ³. Thus, both compounds M1 and M2 are coumarins. M1 and M2 belong to benzocoumarin and simple coumarin families, respectively. In other words, the coumarin ring of M1 is attached to a benzene ring. Apart from this difference, compound M1 contains a triazol ring, and compound M2 contains an oxadiazol ring. These compounds produce charged radicals.

Moreover, compound M1 presents the lowest values of VIP and the energy of the excited state E_T necessary for producing 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 being used in chemotherapy, can also be used in PDT as PS. 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 with 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 can no longer repair this error in the DNA ². Cancer continues to produce disasters despite all the efforts made in terms of treatment. The recurrence of metastases remains the leading cause of failure of the various therapies 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. PDT was discovered for the first time in the years 1900s. This treatment method is a highly promising therapy with multiple advantages, such as

showing minimal side effects, and one of the significant advantages is long-term effects without harm to non-cancerous cells ⁵⁻⁷. PDT uses a photoreactive molecule or PS combined with light of an appropriate wavelength to destroy tumor cells ⁸. This treatment method is based on the selective destruction of tumors induced by photooxidation. PDT has been judged to be effective in the treatment of various types of cancer. Mainly those superficially localized, this intervention significantly improves the patient's quality of life and efficacy compared with palliative surgery or chemotherapy treatments ^{9,10}. As 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 ³. 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. ¹⁰. Figure 1 shows the 2D structures of the studied molecules.

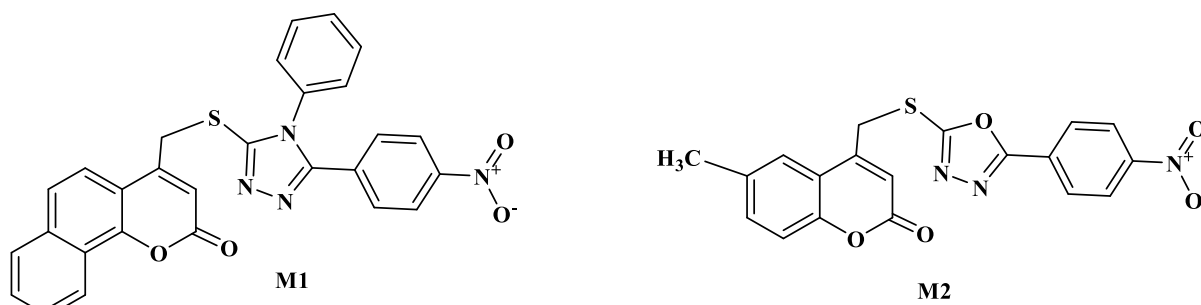


Figure 1. 2D structure of compounds M1 and M2

PDT has three main components, namely PS, light, and oxygen, which are necessary. Two different mechanisms exist, but the first step of both mechanisms is similar ^{11,12}.

After the emission of a PS into the cellular environment, the latter is irradiated with light with an appropriate wavelength. They are coinciding with the absorption spectrum of the PS. The excitation of the PS by the light leads it from the fundamental singlet energy state S_0 to the excited singlet state S_1 or S_n . When the PS is in the S_n state, it undergoes internal conversions to return to the S_1 state. From this S_1 state, part of the energy can be irradiated in the form of a fluorescence quantum, and the remaining energy will promote migration of the PS compound to the excited triplet T_1 or T_n state by intersystem conversion ^{13,14}. At this level, the compound can evolve according to one of the two main mechanisms of PDT, namely type I or type II. In mechanism I, the compound reacts directly with the target substrate, DNA or RNA. This reaction can occur in the compound's excited Triplet or cationic form. In mechanism II, the PS reacts with the target substrates via ROS). Those reactive oxygen species are generated by the interaction of PS and the fundamental triplet oxygen 3O_2 . During this interaction, the PS transfers energy to the fundamental triplet oxygen 3O_2 to produce singlet oxygen 1O_2 or electrons to generate other radicals necessary for photodynamic activity.

2. Methods

2.1. Study of photo-physicochemical properties

M1 and M2 molecules undergo optimization and frequency calculations in different media. Each studied molecule has no imaginary frequency. Optimized structures are then used to perform a single-point calculation to determine these compounds' anion and cation energies at the B3LYP/6-31G (d,p) level. The time-dependent density functional (TDDFT) has been chosen to explore excited states. It is a practical approach for determining the excited state properties of PS in vacuum and solvents. Determined properties allow to understand PDT mechanisms ¹⁵. Various selected solvents include water, a polar protic solvent; dimethylsulfoxide (DMSO), a polar aprotic solvent;

and diethyl ether, 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. ¹⁶.

2.2. Vertical electron affinity and vertical ionization potential

Vertical electron affinity and vertical ionization potential are beneficial parameters 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 ¹⁷. The following expression determines vertical electron affinity

$$VEA_{so} = E_a - E_p$$

VIP is defined as the difference between the electronic energies of the cationic form of the concerned compound (E_c) and that of the neutral molecule (E_p). Vertical ionization potential (VIP) ^{18,19} measures the tendency of a chemical system to give up its electron. It can be compared to the ionization potential.

$$VIP_{so} = E_c - E_p$$

3. Results and discussion

3.1. Absorption spectra in different media

The two studied molecules' oscillation strengths and excitation energies of the singlet and triplet states were determined using TD-DFT calculations. All these parameters are listed in Table 1. These calculations were carried out using different media: vacuum, water, DMSO, and diethyl ether. 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 ²⁰.

3.1.1. singlet excited states

Table I contains the oscillation strengths and excitation energies of the six first singlet and triplet Excited.

Table 1. 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- vacuum	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- water	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- vacuum	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- water	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 1 contains the oscillation strengths excitation energies of the six first singlet and triplet Excited states of studied coumarin derivatives. The subscript Sn indicates the nth-order excited singlet state, while the subscript Tn is used for the nth-order excited triplet state. 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, except two states of compound M1, namely S₄ and S₃, respectively, in DMSO and ether. These states' wavelengths are 332.64 nm in water, 332.70 nm in DMSO, and 333.66 nm in ether. 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 PDT. 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 $\lambda_{S_1}(M1) < \lambda_{S_1}(M2)$ in all considered media. This finding is undoubtedly 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 that ring 300nm and 500nm. These compounds can be used as PS to

treat superficial tumors according to wavelength values. Coumarins can efficiently generate reactive oxygen species, which are helpful in PDT ²¹.

3.1.2. triplet excited states

Triplet states are less stable but have much longer lifetimes than singlet states ²². For this reason, they play a decisive role in photodynamic activity. This extended lifetime favors photochemical processes. Table 1 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 a vacuum, T₄ in water, T₄ in DMSO, and T₃ in ether of compound M1 have zero oscillation strengths. Concerning M2 states such as T₆ in vacuum and T₃ in ether have null oscillator strength. The fact that oscillator strength is null means that these states cannot be realized. Consequently, they will not be considered in elucidating the PDT mechanism. The E_T energies of

these triplet states will be used to determine the vertical electronic affinities in the triplet state (VEA_T) and the vertical ionization potentials in the triplet state (VIP_T), 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 VEA_{S0}, VIP_{S0}, and the different energies of the neutral molecule, as well as the cationic and anionic forms, are given in Table 2. Compounds can behave as electron donors or acceptors during the photosensitization reaction in the ground state. Quantities such as vertical electron affinity (VEA) and vertical ionization potential (VIP) can help to understand electron movements between the PS and other molecules, notably DNA or RNA molecules.

Table 2. Cation and anion molecule energies and VEA_{S0} and VIP_{S0}

Composés	Milieu	Ep	Ec	Ea	VEA _{S0}	VIP _{S0}
M1	Vacuum	-1995,852	-1995,583	-1995,895	-1,185	7,323
	DMSO	-1995,874	-1995,655	-1995,975	-2,757	5,955
	Water	-1995,874	-1995,656	-1995,976	-2,774	5,941
	Ether	-1995,867	-1995,635	-1995,953	-2,357	6,301
M2	Vacuum	-1670,322	-1670,025	-1670,374	-1,437	8,079
	DMSO	-1670,343	-1670,107	-1670,451	-2,939	6,422
	Water	-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, VEA_{S0}: vertical electronic affinity in the ground state, and VIP_{S0}: vertical ionization potential in the ground state.

In Table 2, values analysis allows us to say that compound M1 is more ionizable than compound M2 in all the media because the following inequality VIP_{S0}(M1) < VIP_{S0}(M2) is observed in all media. According to polarity, Table 2 shows that polar solvents are the media in which VIP and VEA values are lowest. This means studied compounds are more susceptible to donating or accepting 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 VIP_{S0} values, making it the most ionizable compound and the best electron donor ²³. The VEA_{S0} values show that the compound M2 can easily accept electrons, as it has the lowest VEA_{S0} values, whatever is considered medium.

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

All DNA and RNA bases have undergone the same calculations as M1 and M2 molecules. Parameters such as the vertical electron affinity VEA_{S0} and the vertical ionization potential VIP_{S0} of DNA and RNA bases were determined in the different media like compound M1 et M2. The values are given below in Table 3.

Table 3. Vertical electron affinity and vertical ionization potential of DNA and RNA bases expressed in eV

Base	Milieu	En	Ec	Ea	VEA _{so}	VIP _{so}
Adénine	Water	-467,345	-467,121	-467,369	-0,664	6,105
	Vacuum	-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	Water	-394,962	-394,728	-394,991	-0,789	6,368
	Vacuum	-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	Water	-542,59	-542,378	-542,598	-0,218	5,759
	Vacuum	-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	Water	-454,163	-453,924	-454,197	-0,925	6,504
	Vacuum	-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	Water	-414,841	-414,591	-414,879	-1,034	6,803
	Vacuum	-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 3 are negative, except those obtained in vacuum and that of guanine in ether. Furthermore, for a given medium, DNA or RNA bases are ordered according to VEA_{so} values as follows: VEA_{so} (Uracil) < VEA_{so} (thymine) < VEA_{so} (cytosine) < VEA_{so} (adenine) < VEA_{so} (guanine). This order indicates a decreasing attractive power of these bases; whatever medium is considered Uracil is the best attractor.

On the other hand, ranking according to VIP_{so} values gives the following order VIP_{so} (Guanine) < VIP_{so} (Adénine) < VIP_{so} (Cytosine) < VIP_{so} (Thymine) < VIP_{so} (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 PDT, two mechanisms are qualified: type I and type II. The E_{S1} energies of the first singlet state and the E_T energies of the lowest triplet excited states are essential for elucidating 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 a triplet state by inter-system conversion.

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



With B: DNA or RNA base.

According to Jablonski's diagram²⁴, the energy of the excited triplet state (E_T) must be less than that of the excited singlet state (S_1)²⁵. For this reason, all E_T energies lower than that of the first excited singlet state will generate VEA_T and VIP_T values that can be

used to determine the conditions for carrying out the various photochemical reactions. Thus, the E_T energy values, as well as the corresponding sums of the VEA_T values of the coumarin molecule and VIP values of the DNA or RNA bases as a function of solvent, are given in Table 4.

Table 4. Direct attack values for DNA and RNA compounds and bases.

		Et1	M-A	M-C	M-G	M-T	M-U
M1	vacuum	2,828	3,988	4,377	3,662	4,708	5,146
	Water	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
	ether	2,325	1,850	2,141	1,509	2,333	2,659
M2	vacuum	3,270	3,294	3,683	2,968	4,014	4,452
	Water	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
	ether	3,024	0,977	1,268	0,636	1,460	1,786

With:

C-A : VEA_{T1} (coumarine) + VIP (Adenine); C-C : VEA_{T1} (coumarine) + VIP (Cytosine);

C-G: VEA_{T1} (coumarine) + VIP (Guanine); C-T : VEA_{T1} (coumarine) + VIP (Thymine);

C-U: VEA_{T1} (coumarine) + VIP (Uracil)

Compound M1 values are positive in all media, suggesting that compound M1 does not react with DNA and RNA bases^{20,23}. 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 E_T 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 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. The 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.



Equations 2 and 3 lead to the production of anionic and cationic radical species, which are powerful oxidizing agents used in photodynamic theory. The realization of these equations is linked to the signs of the following sums $VEA_{T1}+VIP$, $VIP_{T1}+VEA$, and $VEA_{T1}+VIP_{T1}$. These sums have been calculated and reported in Table 5 while exploring all energies of the excited triplet state lower than that of the first excited singlet state.

Table 5. Sum of $VEA_{T1} + VIP$, $VIP_{T1} + VEA$ and $VEA_{T1}+VIP_{T1}$ reflecting auto-ionization reactions

	Milieu	Et1	$VEA_{T1}+VIP$	$VIP_{T1}+VEA$	$VEA_{T1}+VIP_{T1}$
M1	Vacuum	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
	Water	1,615	1,551	1,551	-0,064
M2	Vacuum	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

	Water	1,836	1,639	1,639	-0,197
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Values in Table 5 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 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 occur in any media using this process¹⁸. According to the VEAT1+VIP_{T1} relationship, it shows negative values in all solvents. For compound M1, negative values are obtained with the following E_T 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 E_T energies: 2.234 eV, 1.823 eV, and 1.836 eV in ether, DMSO, and water, respectively.

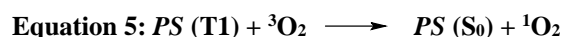
Thus, in polar solvents, the value of required energy for the Triplet excited state is lower than that needed in apolar solvent. Molecule M1 is also more reactive than M2 molecule under these conditions because E_T energy required for M1 compound in reaction (3) is lower than that needed in M2 compound.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

Table 6. E_{S1} and E_T energy values for M1 and M2 molecules in vacuum and solvents (water, DMSO, and ether)

	M1				M2			
	Vacuum	water	DMSO	Ether	Vacuum	water	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

3.5. Type II photosensitization mechanism

In the type II mechanism, the PS must be in the triplet state so it can react directly with the oxygen in its fundamental triplet state (³O₂), transferring excess energy or electrons, which will allow it to bring it back to the primary state. In the case of energy transfer from the PS to oxygen in its triplet state, the oxygen passes from the 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 acids¹⁹. 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 PS, which is formed by inter-system conversion ISC²⁴. This inter-system conversion can occur between two energy levels, S1 to Tn or T1 to S0²⁶⁻²⁷. Due to its short lifetime and high reactivity, the produced singlet oxygen (¹O₂) reacts at its place of formation in cell²⁰. The reaction operates by destroying the target and surrounding cells. It is expressed as



Equation 5 is highly oxygen-dependent. The required condition for the realization of this reaction depends on the energy E_T value of the triplet excited state of the PS. 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^{18,26}. On the other hand, it must also be lower than that corresponding to the first excited singlet state E_{S1} to promote intersystem conversion²⁶. For this reason, we have evaluated the energies of the lowest triplet states of the coumarin derivatives M1 and M2. Table 6 shows the energies corresponding to the first singlet state S₁ and those for the first six triplet states of each molecule below that of the S₁ state.

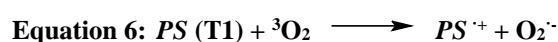
T5	1,583	1,615	1,614	0,847	1,035	1,114	1,109	1,084
T6	1,598	1,707	1,706	0,889	1,133	1,236	1,233	1,174

Energy values in Table 6 show that the two coumarin derivatives M1 and M2 have their energies E_{T1} of the first excitation, which are less than 1.06eV. However, these coumarin derivatives also possess other triplet states with energies above 1.06eV. Compound M1, for example, has three triplet states in a vacuum that fulfill this superiority condition. In solution, two tendencies are observed. Firstly, in polar solvents (water and DMSO), compound M1 has four Triplet states with energies above 1.06eV. Secondly, compound M1 has no triplet states among its lowest states in ether, which fulfills this criterion. As far as compound M2 is concerned, it has one triplet state in a vacuum and two triplet states in solution, which satisfy this criterion. According to what has been written above, we can conclude that these two coumarin derivatives possess photodynamic activity according to mechanism II. Considering the energy values in Table 6, these compounds will likely produce singlet oxygen 1O_2 by energy transfer²⁰. The highest energy values of these triplet states fulfilling the selection criteria for these two coumarin derivatives are obtained in an 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 ($O_2^{\cdot-}$) is produced. This anion can be generated in two different ways: either by the interaction between the triplet-state of the PS and triplet-state of oxygen (3O_2), or by the interaction between the radical anion of coumarin resulting from auto-ionization and triplet-

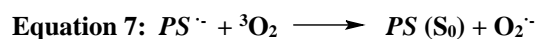
state of oxygen (3O_2).

Thus, equation 6 translates the production reaction of superoxide anion ($O_2^{\cdot-}$) by the interaction of the PS with oxygen (3O_2); both members of equation 6 are in their triplet state. For Equation 6, to be thermodynamically favorable, the sum of the vertical ionization potential (VIP_T) of coumarin molecules and the adiabatic affinity of oxygen ($AEA(O_2)$) must be negative. The value of adiabatic affinity differs from one medium to another: -0.59 in a vacuum, -3.91 in water, -3.65 in DMSO, and -3.14 in diethyl ether^{26,18,15}.



Equation 7 shows the reaction of anionic oxygen production ($O_2^{\cdot-}$) by transferring an electron from the coumarin radical anion issued from equation (3) to the triplet oxygen.

Equation 7 is governed by the sign of the following difference ($AEA(O_2)-VEA_{so}$); if this sign is negative, the reaction is thermodynamically favorable.



The formation of anionic oxygen radical species will produce different reactive species of oxygen (ROS) that will be formed during these last photochemical reactions (H_2O_2 , $O_2^{\cdot-}$, $\cdot OH$). These superoxide compounds possess potent oxidative power for various biomolecules, such as cholesterol or the side chains of specific amino acids (tryptophan, histidine, and methionine)²⁸. In Table 7, the sum of $AEA(O_2)-VEA_{so}$ and $VIP_{T1}+AEA(O_2)$ reflect superoxide radical anion production.

Table 7. Superoxide radical anion production parameters.

	Milieu	Et1	$AEA(O_2)-VEA_{so}$	$VIP_{T1}+AEA(O_2)$
M1	Vacuum	2,828	0,595	3,906
	Ether	1,986	-0,783	1,175
	DMSO	1,614	-0,893	0,69
	Water	1,615	-1,136	0,675
M2	Vacuum	3,27	0,847	4,219
	Ether	2,234	-0,609	1,456
	DMSO	1,823	-0,711	0,949
	Water	1,8 36	-0,971	0,668

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

In Table 7, 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)-VEA_{so}) 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 E_T 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 E_T 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 occurring in solvents, producing superoxide radical anion (O₂⁻) by photo-irradiation of these two molecules is favorable.

Furthermore, the E_T required energy for superoxide radical anion production for a given compound is lower in polar solvents than in apolar ones. Moreover, the energy necessary E_T 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 nature of the substituents 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 PDT. As a result of these analyses, these coumarin compounds were judged to be susceptible to developing photo-sensitizing activity in the theory of PDT, according to the two described mechanisms. On the one hand, photodynamic activity can occur according to mechanism I. Under these conditions, studied coumarin derivatives can damage DNA bases or target tissues directly for a well-defined E_{T1} energy via guanine.

On the other hand, photodynamic 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 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. As a perspective, a theoretical study in which studied compounds will be combined with ruthenium to explore their photophysicochemical properties.

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