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Photoreactivity of Furocoumarins and DNA in PUVA Therapy: Formation of Psoralen−Thymine Adducts

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The mechanism of the [2 + 2] cycloaddition photoreaction of psoralen and a DNA nucleobase, thymine, cornerstone of the furocoumarin-based PUVA (psoralen + UVA radiation) phototherapy, has been studied by the quantum-chemical multiconfigurational CASPT2 method. Triplet- and singlet-mediated mono- and diadduct formations have been determined to take place via singlet−triplet crossings and conical intersections, correlated with the initially promoted triplet or singlet states in different possible reactive orientations. Pyrone-side monoadducts are suggested to be formed in the triplet manifold of the system, and to be less prone to yield diadducts because of the properties of the monoadduct lowest triplet state and the minor accessibility of its excited singlet states. Furan-side monoadducts are better produced in the singlet manifold after reaching a conical intersection with the ground state of the system. From there, the absorption of a second photon would in this case trigger the formation of the diadduct. The proposed mechanisms enable rationalizing the phototherapeutic behavior of several furocoumarins.

1. Introduction

Knowledge on the photosensitizing ability of furocoumarins (psoralens), in which the widely employed PUVA (psoralen + UVA) phototherapy relies,[1−4] has provided successful clinical treatments against different diseases, from skin disorders like psoriasis or vitiligo to promising anticarcinogenic uses.[5] The interaction of furocoumarins and UVA light (400−320 nm, 3.10−3.87 eV) in the affected tissues triggers photoreactions which are the source of the therapeutic properties of the procedure. The most important mechanism implies direct photobinding between the photosensitizer and DNA base monomers and the formation of furocoumarin−nucleobase mono- and diadducts that prevents the division of the injured cell.[5] In another set of oxygen-dependent photoreactions, the activated furocoumarin can transfer energy to molecular oxygen to generate reactive radicals or excited $^1\Delta_g$ oxygen which becomes prone to damage the membrane of the harmed cell. The latter technique is known as photodynamic therapy (PDT), and its participation has been considered of minor impact in the PUVA technique.[6−10]

The interaction of the furocoumarins with DNA that exerts the photosensitizing action takes place through the pyrimidine nucleobases, as it was evidenced by the modification of the pyrimidine nucleobase fluorescence spectra in aqueous solutions containing psoralen, pyrimidine, and purine nucleobases.[11] Thymine is the most frequent target, in accordance with its predominance in other similar photoreactions such as the formation of cyclobutane thymine dimers (T <> T) in UV-irradiated DNA.[12−15] The key step of the mechanism is known to be a [2 + 2] cycloaddition photoreaction of one of the two reactive ethylenic bonds of psoralen (C_3═C_4 or $C_{4'}$═$C_{5'}$) and the reactive C_5═C_6 double bond of thymine (see Figure 1), leading to a cyclobutane structure linking both molecules (see Figure 2). The process begins in the dark with the furocoumarin molecule inserting into the DNA double strand. Recent molecular dynamics computations show that psoralen photoreacts preferably in $[AT]_n$ sequences.[16] As in other DNA−drug interactions, getting the proper geometric alignment is a relatively low-efficiency process because of the existence of two competitive noncovalent interaction mechanisms: on the one hand, the intercalation of the furocumarin between two nucleobases that gives rise to the photoreaction, and, on the other hand,

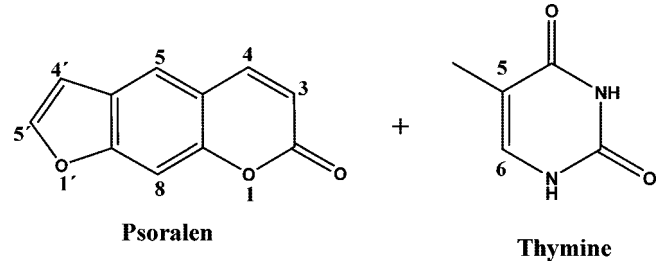

Figure 1. Labeling and structure of psoralen and thymine.

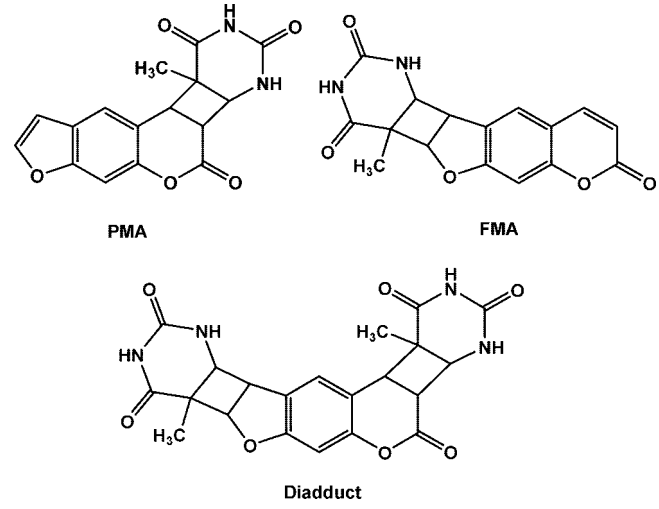

Figure 2. Psoralen−thymine pyrone (PMA) and furan (FMA) monoadducts and diadduct.

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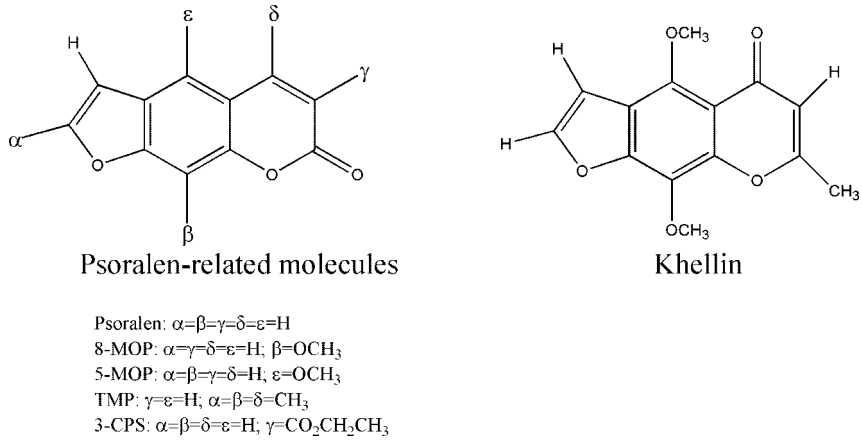

Figure 3. Molecular structures of the psoralen family.

its association with the minor groove binding of DNA.[16,17] Following irradiation with UVA light, photoadditions occur either at the $C_{4'}$═$C_{5'}$ bond of the furan moiety of the furocoumarin (to form furan monoadducts, FMA) or at the C_3═C_4 bond of the pyrone moiety (to form pyrone monoadducts, PMA)[18] (see Figure 2). The monoadduct may absorb another photon, inducing the other photoreactive C═C double bond to interact with a thymine on the opposite DNA strand and producing a diadduct that cross-links the DNA helix. The existence of the photoadducts furocoumarin−thymine has been analyzed in different media and with various techniques.[19−23] The poly-[dA−dT]•poly[dA−dT] sequence region appears to be the most favorable site for the photocycloaddition reactions of furocoumarins.[24]

Both double bonds $C_{4'}$═$C_{5'}$ and C_3═C_4 are able to react with thymine, although several proposals indicate that the one belonging to furan is the most active biologically,[25] a question still under debate. Formation of cross-links was thought to be extremely relevant for the therapeutic effectiveness, but it is also reported that diadducts cause adverse side effects such as carcinogenesis, mutagenesis, and immunosuppression.[5] Only furocoumarins with bifunctional groups (see Figure 3), for example, psoralen or the widely used 8-methoxypsoralen (8-MOP), can form diadducts. Certain monofunctional furocoumarins such as khellin or 3-carbethoxypsoralen (3-CPS) have been proved to yield as efficient phototherapy as bifunctional furocoumarins but with less phototoxicity. As a result, several studies of 3-CPS photoadducts have been performed.[26−28]

Despite the extended use of the PUVA technique and the characterization of the furocoumarin−thymine complexes, the underlying formation mechanism of the mono- and diadducts is far from being known.[5] According to the Woodward−Hoffmann rules,[29−31] [2 + 2] cycloadditions are pericyclic thermally forbidden reactions, that, upon conservation of the orbital symmetry, are allowed photochemically. For instance, in the simplest model reaction of two ethene molecules, their lowest singlet excited state correlates directly with that of cyclobutane. Consequently, there is no symmetry-imposed barrier to this transformation and the reaction is named as symmetry-allowed. On quantum-chemical grounds, this reaction, that involves 4π electrons, is excited-state-allowed because the surface topology of S_1 possesses a minimum that corresponds to a diradicaloid character as the antiaromatic transition state on S_0.[32−35] Therefore, the key point in the mechanism of such type of pericyclic reaction is the existence of a suitable surface crossing, a conical intersection (CI), $(S_1/S_0)_{CI}$, that behaves as a funnel allowing the occurrence of a radiationless jump, that is, an internal conversion (IC) from S_1 to S_0. Within the same framework, the formation of furocoumarin−thymine adducts would in principle involve population of the S_1 state of the supermolecule, either localized in the furocoumarin or in the thymine moiety, and subsequent evolution toward the corresponding CI, $(S_1/S_0)_{CI}$. This is not, however, the only possibility. It is believed that the triplet state of the furocoumarin is involved in the formation of the DNA cross-linked adducts. Furocoumarins are known to efficiently populate their lowest triplet state[36] and act as photosensitizers.[4] Relatively high intersystem-crossing quantum yields (0.076) have been also established in the production of FMA.[37] If the T_1 state of the supermolecule participates in the photoreaction, this will proceed from the populated T_1 state toward a singlet−triplet crossing (STC) with the ground state, $(T_1/S_0)_{STC}$, finally leading to the formation of the adduct in S_0. This mechanism relies on the efficient population of the lowest triplet state of the furocoumarin from the initially irradiated singlet excited state, a process that has been recently rationalized on theoretical grounds for the family of furocoumarins.[38−41]

The overall framework of the reaction is complex.[42,43] The most common furocoumarins (psoralen, 8-MOP, 5-methoxypsoralen (5-MOP), 4,5′,8-trimethylpsoralen (TMP), khellin, and 3-CPS; see Figure 3 and the Supporting Information for their structures)[40,41] produce monoadducts. Both PMA and FMA adducts are formed by irradiation at 365.5 nm (3.39 eV) and are nonfluorescent and fluorescent, respectively.[44−46] Once the monoadduct is formed, a second absorption of a photon can trigger the interaction of another thymine molecule with the opposite reactive double bond of the monoadduct, giving rise to a diadduct. The involvement of one or other monoadduct in the cross-link process is unclear. Some studies suggest that diadducts are formed only from FMA upon irradiation at 360 nm (3.44 eV).[22,47] A significant amount of PMA is, however, observed in certain derivatives.[22,23,48] Adduct distribution in DNA samples differs depending on the wavelength of the second photon. Whereas absorption at 341.5 nm (3.63 eV) seems to favor diadducts, that at 397.9 nm (3.12 eV) yields FMA as the primary photoproduct.[48] On the other hand, monoadducts and diadducts can be split into original monomers under irradiation with short wavelength UV light.[18,20] For instance, FMA monoadducts have been reported as decomposing to the original moieties after irradiation with UV light at 253.7 nm (4.89 eV).[44] With respect to the behavior of the different furocoumarins, it can be concluded that only psoralen and TMP show a very strong ability to build diadducts, that 5-MOP and 8-MOP do not have such a pronounced trend, and that diadducts are not obtained from 3-CPS and khellin.[6,49] Taking into account that

3-CPS is also considered a productive source of singlet oxygen for PDT therapy[6−10] and that khellin has been recently suggested as the most efficient photosensitizer among a number of furocoumarins,[41] both 3-CPS and khellin are plausible candidates to substitute in the PUVA therapy to the widely employed 8-MOP.[50]

From the theoretical standpoint, studies have been only focused on the intercalation of the photosensitizer between the π-stacked nucleobases, using classical mechanics approaches,[16] and in the determination of the ground-state structures of the adducts, at the semiempirical[51−55] or ab initio single-reference RHF, DFT, MP2, and CCSD(T) levels.[56] The complexes have been determined much more stable in the noncoplanar *trans* than in the stacked *cis* arrangements (see Figure 2).[56] In the present paper, we address, for the first time theoretically, the photochemical mechanisms of formation of monoadducts psoralen−thymine, both acting on the furan (FMA) and the pyrone (PMA) side of the photosensitizer. Psoralen, the parent furocoumarin compound, has been selected as the most representative compound in the family of substances. In order to interpret some of the measured trends, the formation of monoadducts has been analyzed both in the singlet and in the triplet manifold. Excited-state energies, oscillator strengths, state minima, conical intersections, singlet−triplet crossings, and charge and spin populations will be computed using a highly accurate ab initio multiconfigurational method, CASPT2//CASSCF. The study of the properties of FMA and PMA monoadducts and their mechanism of formation will enable us to identify the most important reactive centers and elucidate the participation of each one of the two moieties, psoralen and thymine, in the photoreaction, determining also the prevalence of the singlet and triplet manifold in the overall process and relating them to the phototherapeutic technique.

2. Computational Details

Initially, the ground state of reactives, psoralen and thymine, and products, monoadducts PMA and FMA, were optimized at the DFT/B3LYP/6-31G(d) level of theory. At the optimized geometries of the species, several singlet and triplet excited states were computed using CASSCF multiconfigurational wave functions as reference and second-order perturbation theory, the CASPT2 method, to obtain electronic energies, always employing the 6-31G(d) basis set. The CASPT2 approach, employed with the standard zeroth-order Hamiltonian[57] and an imaginary level shift of 0.3 au to prevent the presence of intruder states,[58] is an accurate procedure to compute excited states, as proved repeatedly.[59−64] An active space of eight electrons in eight active orbitals (8/8) was employed in the CASSCF procedure, both to compute vertical excited states of the monoadducts and to optimize CIs and STCs in the different hypersurfaces. In order to make a straightforward comparison, the corresponding singlet and triplet excited states of the reactives, isolated psoralen and thymine, were optimized at the CASSCF level with the spaces (6/6) and (2/2), respectively, which constitute approximately equivalent active spaces that each moiety possesses in the supermolecule (FMA or PMA). It is important to emphasize that the so-obtained CASPT2 excitation energies can be considered accurate enough if compared to our previous results on the isolated systems (psoralen[38−40] and thymine[65,66]) performed at higher levels of calculation, that is, larger ANO-type basis sets and active spaces, with the differences being less than 0.2 eV. No symmetry or geometry constraints were used in the final calculations. Energies, displayed relative to the ground states of the separated reactives, include in all cases the basis set superposition error (BSSE) corrected through the counterpoise procedure,[67] as described elsewhere.[68] All calculations have been performed using the MOLCAS 6.0 suite of programs.[69−71] Further computational details can be found in the Supporting Information.

3. Results and Discussion

In the present research, we aim to the determination of the basic mechanistics in the formation of the furan- and pyrone-side psoralen−thymine monoadducts, FMA and PMA, respectively (see Figure 2). Both the singlet and the triplet manifold will be explored in order to characterize the most favorable conditions for the photoreaction to take place. Initially, we consider the isolated psoralen and thymine molecules in their ground-state equilibrium geometry. As shown in our previous studies on the furocoumarin family (psoralen, 8-MOP, 5-MOP, TMP, 3-CPS, and khellin),[41] in the range of absorption energies in which the psoralen-based phototherapy is operative, that is, UVA light (400−320 nm, 3.10−3.87 eV), only the low-lying singlet excited $S_1(\pi\pi^*)$ state of the furocoumarin molecule can be efficiently populated by direct absorption. The state was computed vertically in the range 3.52−3.98 eV for the different furocoumarins, and the related transition had noticeable oscillator strength. Considering that the lowest singlet state of thymine lies vertically near 4.9 eV,[65] it is expected that the direct phototherapeutic process begins with the absorption of one photon, leading to the $S_1(\pi\pi^*)$ state of the furocoumarin. As the overall process corresponds to a [2 + 2] cycloaddition photoreaction, two possible paths may lead from the activation of S_1 in the furocoumarin (psoralen hereafter) toward the monoadduct: (i) in the singlet manifold the $S_1(\pi\pi^*)$ evolves following an efficient pathway toward a CI with the ground state, $(S_1/S_0)_{CI}$, which behaves as a funnel for IC toward the monoadduct in S_0; (ii) in the triplet manifold, and including a proper ISC process, there is a switch from $S_1(\pi\pi^*)$ toward low-lying triplet states of psoralen leading to $T_1(\pi\pi^*)$, $(S_1/T_1)_{STC}$, and from there, the system evolves following an efficient path to the STC $(T_1/S_0)_{STC}$, that is, the structure connecting the T_1 hypersurface with the ground state of the monoadduct. We still shall add one more mechanism as a possible source of adduct formation. As the lowest triplet state of thymine can be populated by energy transfer from some other endogenous or exogenous photosensitizers,[72,73] as psoralen itself, it is possible to envisage a photoreaction leading from the $T_1(\pi\pi^*)$ state of the thymine to a proper STC $(T_1/S_0)_{STC}$ connecting with the ground state of the monoadduct. The three types of mechanisms shall be explored here.

3.1. Reactivity in the Triplet Manifold. The most plausible and probably major mechanism in the formation of psoralen−thymine monoadducts involves the participation of the lowest triplet state of psoralen, $T_1(\pi\pi^*)$. It was already determined, experimentally and theoretically, that there is a very efficient pathway to populate $T_1(\pi\pi^*)$ in psoralen from the initially singlet excited $S_1(\pi\pi^*)$ state through a mechanism that combines the intermediacy of the lowest $S_n(n\pi^*)$ and $T_n(n\pi^*)$ excited states with the direct ISC switch from $S_1(\pi\pi^*)$ to $T_1(\pi\pi^*)$, depending on the environmental conditions.[38−41,74,75] The reported phosphorescence/fluorescence ratio in psoralen, 7.1, similarly as in other furocoumarins,[36] confirms the existence of a populated T_1 state, whereas its long computed lifetime[38−41] anticipates the properties of the molecule as an efficient triplet photosensitizer. In our previous CASPT2//CASSCF study on psoralen, we calculated the T_1 state at 3.27 eV, vertically from the ground-state mininum. At the optimized T_1 structure, one

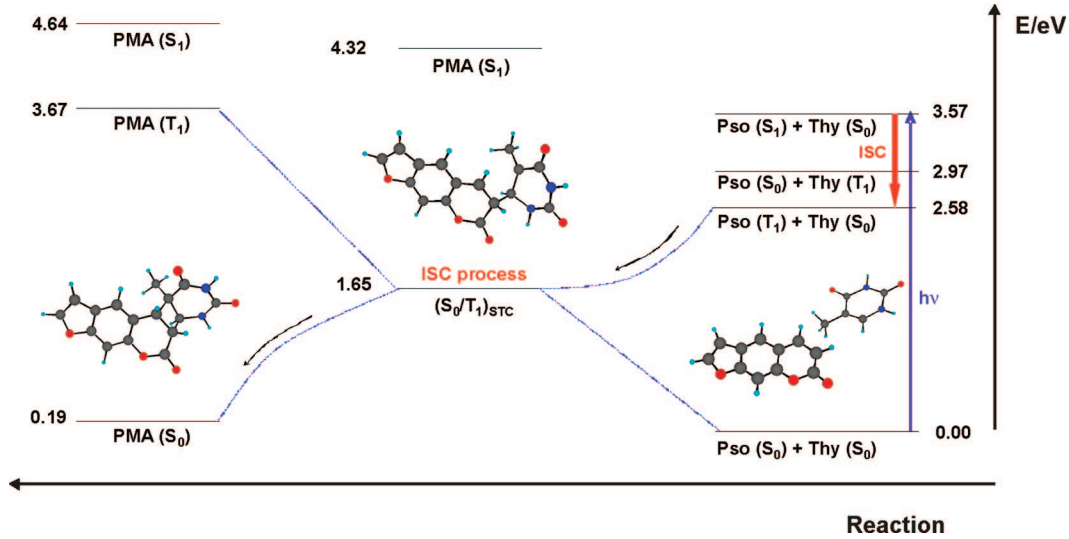

Figure 4. Photochemical mechanism proposed for the formation of psoralen (Pso)−thymine (Thy) pyrone monoadducts (PMA) in the triplet manifold via a singlet−triplet hypersurface crossing (STC). The photoreaction starts upon population of the T_1 state of psoralen after an ISC process from the initially activated S_1 state of the molecule.

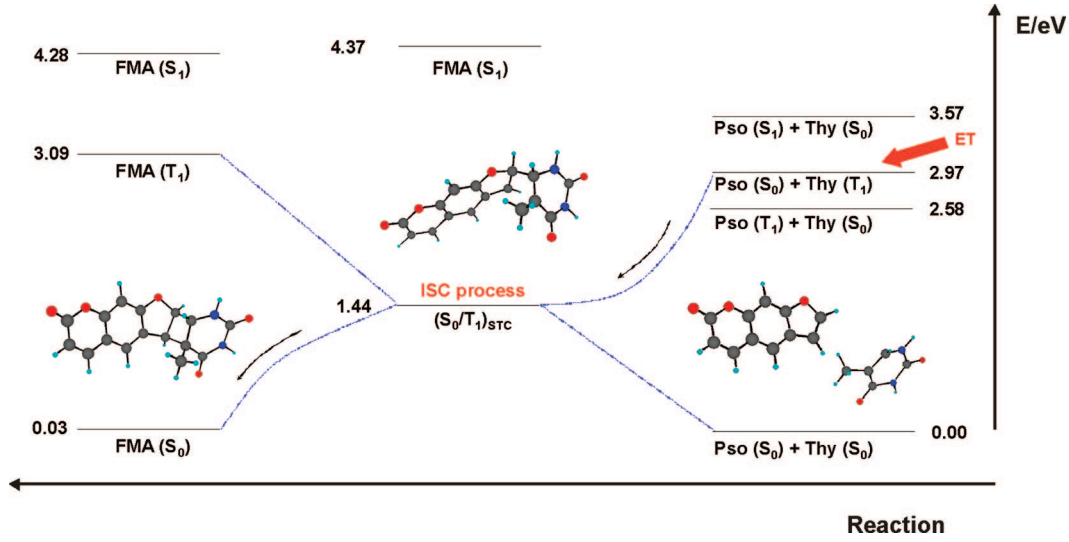

Figure 5. Photochemical mechanism proposed for the formation of psoralen (Pso)−thymine (Thy) furan monoadducts (FMA) in the triplet manifold via a singlet−triplet hypersurface crossing (STC). The photoreaction starts upon population of the T_1 state of thymine after an energy transfer (ET) process from an endogenous or exogenous photosensitizer.

geometric feature has to be emphasized: the bond length of the pyrone double bond C_3═C_4 enlarged from 1.342 Å in the ground state to 1.469 Å in T_1. Additionally, the spin population in the triplet state was located essentially on atoms C_3 and C_4, turning the corresponding bond into a very reactive feature. This property is shared by the other mentioned furocoumarins, except khellin, whose π-ring structure is quite different (see the Supporting Information). It is therefore clear that the T_1 state of psoralen is a good candidate to react with thymine through a [2 + 2] cycloaddition photoreaction in the side of the pyrone moiety, leading to PMA. Less clear is the formation of FMA, considering that the corresponding furan $C_{4'}$═$C_{5'}$ bond remains in T_1 (1.344 Å), as in the ground state of psoralen (1.348 Å), and no spin density is found in the bonding atoms. However, as mentioned above, thymine can also populate its lowest triplet T_1 state by proper energy transfer. As we computed earlier for thymine,[66] in the optimized $T_1(\pi\pi^*)$ state, its double C_5═C_6 bond (see Figure 1) has enlarged up to 1.513 Å (from a ground-state optimal value of 1.346 Å), and therefore, it behaves as the pyrone double bond of psoralen, with a near diradicaloid and elongated structure becoming prone to react with psoralen.

Two schemes of the most plausible mechanisms for the formation of PMA and FMA adducts within the triplet manifold are displayed in Figures 4 and 5, respectively. As reactants, we have the two isolated molecules, psoralen (Pso) and thymine (Thy), optimized in the corresponding state. Therefore, excitation energies from the ground state, Pso(S_0) + Thy(S_0), are displayed as adiabatic transitions. As mentioned above, direct UVA radiation basically populates the psoralen $S_1(\pi\pi^*)$ state, adiabatically placed at 3.57 eV, and by means of an efficient ISC process, the system can transfer its population to the molecule lowest triplet $T_1(\pi\pi^*)$ state, adiabatically at 2.58 eV from the ground-state minimum. The enlargement of the psoralen pyrone C_3═C_4 bond and the localized spin population in C_3 and C_4 inform us about the reactive character of such a bond and strengthens the hypothesis of an efficient reactivity at the pyrone side of psoralen with the thymine C_5═C_6 bond to form PMAs. The mechanism is displayed in Figure 4, and comprises the evolution of the system from the isolated systems Pso(T_1) + Thy(S_0), constituting an overall triplet state in the supermolecule, toward a singlet−triplet crossing (S_0/T_1)STC connecting with the ground state of PMA. At the STC, there is a reaction

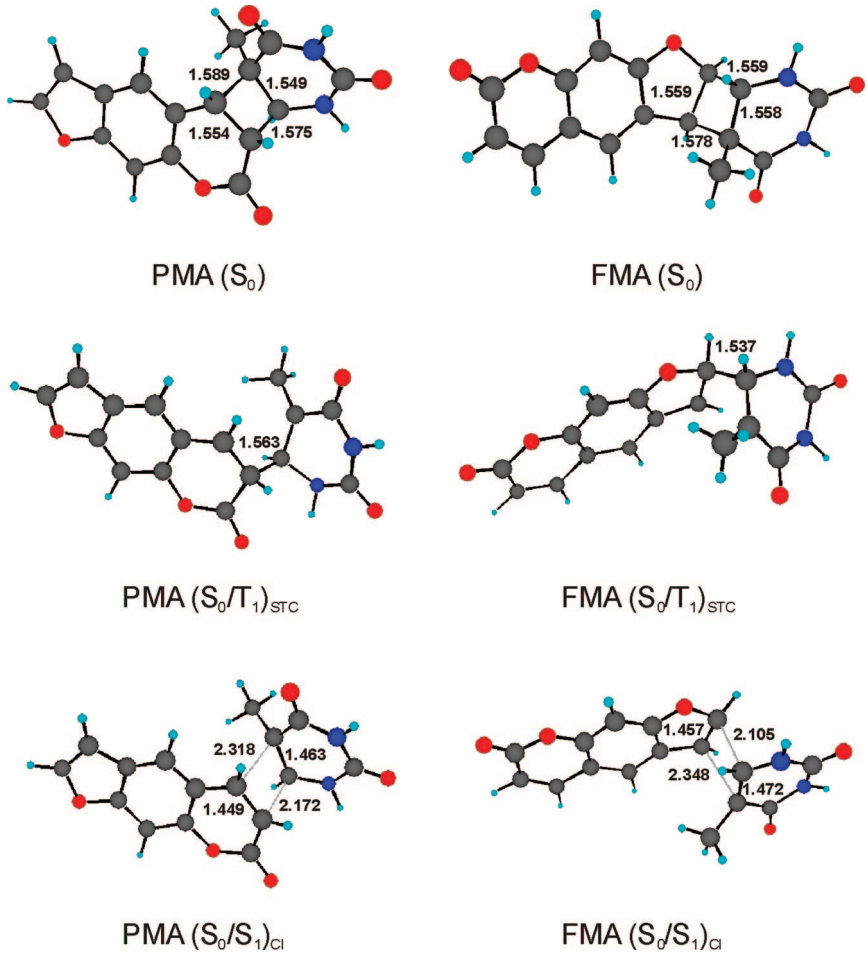

Figure 6. Optimized structures for PMA and FMA S_0 minima, $(S_0/T_1)_{STC}$ singlet−triplet crossings, and $(S_0/S_1)_{CI}$ conical intersections. The most relevant C−C distances are displayed in angstroms.

intermediate in which a covalent bond has been formed between two of the carbon atoms of the reactive bonds, C_3 from psoralen and C_6 from thymine, displaying an optimized bond length of 1.563 Å, whereas the other two atoms, C_4 and C_5, respectively, remain far apart, at 2.814 Å (see Figure 6). Such a type of intermediate, as well as the presence of a STC crossing, is typical of triplet-mediated [2 + 2] cycloaddition photoreactions in the case of an asymmetric chemical environment at the double bonds. For instance, whereas the photocycloaddition of two ethene molecules leads to an avoided crossing and absence of a STC between S_0 and T_1, the feature shows up in fluorethene, where the fluoride atom breaks the symmetric environment of the double bond.[73] The path toward the STC intermediate from the initial products, located energetically almost 0.9 eV below, will be probably barrierless in most cases, as proved in the photocycloaddition of nucleobases,[73] although it would ultimately depend on the favorable insertion of the drug between the strand of nucleobases, on the diffusion of the two species, and on the inherent flexibility of the DNA structure to provide reactive orientations. From the STC intermediate, and after a subsequent ISC process, the system will evolve to the ground state of PMA, which is placed 0.19 eV above the initial reference. At the STC structure, the computed electronic spin−orbit coupling term is somewhat low, ~0.1 cm^{-1}, but the effect can be expected to enhance by vibronic coupling and environmental effects.[74,75] Figure 6 displays the computed geometries for the STC intermediates and final monoadducts. The cyclobutane structure in the monoadducts is similar to those found in other biological photocycloadditions, like those leading to the pyrimidine nucleobase dimers thymine−thymine (T <> T) and cytosine−cytosine (C <> C).[73] A noncoplanar *trans* conformation is, however, preferred in PMA and FMA,[56] unlike for the bipyrimidine dimers, in which a sandwich-type *cis* structure is more stable, probably due to the favorable π-stacked nucleobase structures.[73]

As regards the formation of FMA monoadducts via a triplet manifold, it is unlikely that it can take place by the absorption of a photon from psoralen and further population of the molecule T_1 state. The furan fragment of psoralen is barely involved in the lowest triplet state, and therefore, the corresponding $C_{4'}$=$C_{5'}$ cannot be considered reactive, having a bond length of 1.344 Å and no spin population. Still, a probably minor mechanism may participate depending on the external conditions. The thymine S_1 state is too high in energy to be populated at the phototherapeutic wavelengths, but as it has been observed, the thymine T_1 state can be directly activated by an energy transfer process from an endogenous, e.g., other nucleobases, or exogenous, different photogenotoxic substances or psoralen itself, which is known to be an efficient triplet photosensitizer.[72] The psoralen−thymine system can then evolve from the initial conditions, $Pso(S_0)$ + $Thy(T_1)$, toward a STC FMA intermediate corresponding to the $(S_0/T_1)_{STC}$ structure, and, after an ISC process, reach the FMA ground state. The covalent bond is formed as $C_{5'}$—C_6, with a bond length of 1.537 Å, while the distance $C_{4'}$—C_5 becomes 2.964 Å. The structure of the STC intermediate is similar in both cases, PMA and FMA, and involves formation of a covalent bond at C_6, precisely the thymine

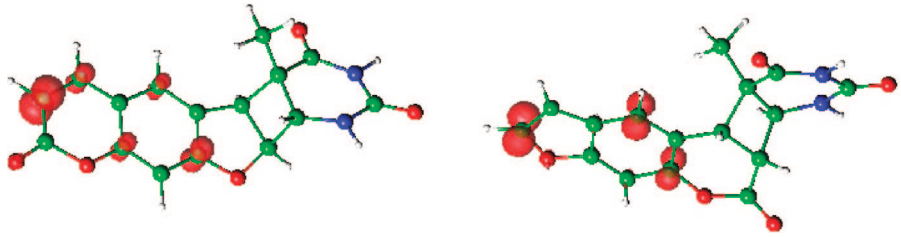

Figure 7. Spin population in the optimized T_1 excited state of FMA (left) and PMA (right). FMA has density on both carbon atoms of the elongated C_3═C_4 pyrone double bond.

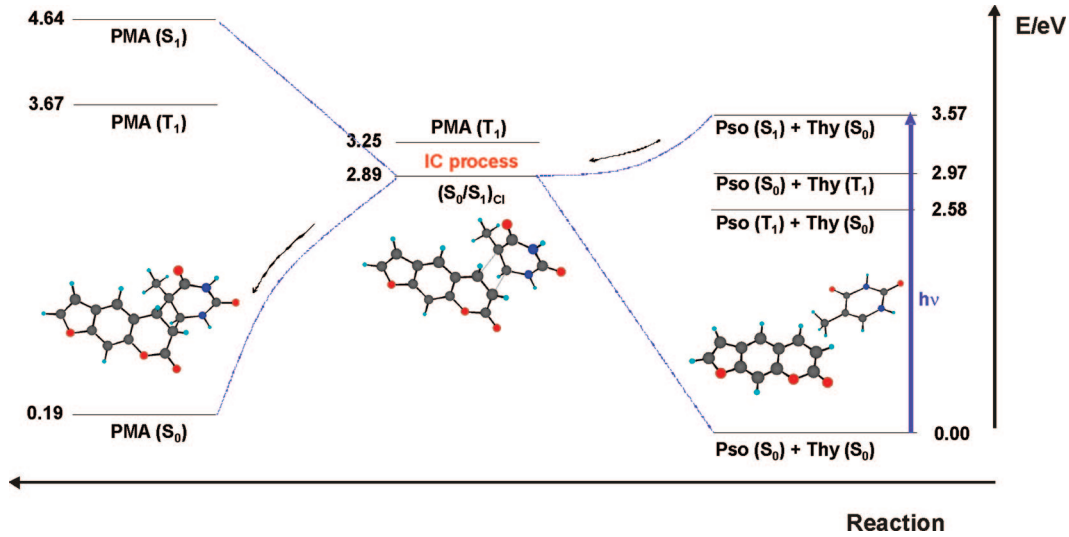

Figure 8. Photochemical mechanism proposed for the formation of psoralen (Pso)−thymine (Thy) pyrone monoadducts (PMA) in the singlet manifold via a conical intersection hypersurface crossing (CI). The photoreaction starts upon direct population of the S_1 state of psoralen by UVA light.

double bond carbon atom without the methyl group, preferred instead of C_5 probably by steric reasons. At the STC structure, the spin−orbit coupling term is 3 times smaller for FMA than for PMA, pointing to a less efficient ISC process. All together, it can therefore be concluded that, in the triplet manifold, the formation of PMA monoadducts is more favored than that of FMA monoadducts.

The electronic structure of both monoadducts differs. Whereas FMA has vertical excitation energies for its lowest excited triplet and singlet states at 3.06 and 4.25 eV, respectively, the corresponding energies in PMA rise to 3.48 and 4.45 eV, respectively. Both excitations involve the psoralen fragment. The first conclusion we obtain is that it is the $S_1(\pi\pi^*)$ state of FMA and not of PMA, located too high in energy, which is more favored to absorb the second photon that triggers the formation of diadducts with a new thymine molecule. The hypothesis is further supported by the analysis of the T_1 state properties. By optimizing the lowest triplet state of both monoadducts at the CASSCF level, we identified that the spin population in this state is basically localized in the C_3═C_4 bond of the pyrone moiety in FMA and somewhat delocalized on the psoralen ring in PMA (see Figure 7). Therefore, the formation of diadducts with a thymine in the opposite DNA strand is favored in FMA, whose T_1 state has an elongated and reactive C_3═C_4 pyrone double bond. This conclusion is supported by experimental estimations, which determined that PMA, unlike FMA, could not give rise to diadducts.[22,47] The production of FMA diadducts may be diminished by some photoreversibility from FMA toward the separated subsystems. In fact, several experiments support this hypothesis. FMA species have been shown to decompose, yielding the original products after irradiation with middle UV light, at 4.89 eV.[44] Also, both mono- and diadducts can be split into the original monomers under irradiation with short wavelength UV light.[18,20] Among other factors, the distribution of adducts in DNA samples seems to depend also on the wavelength of the irradiation. Increasing the absorbed energy favors the diadduct vs monoadduct formation,[48] which can be understood by the higher energy of the initially populated singlet excited state in the monoadduct rather than in psoralen, as computed here.

3.2. Reactivity in the Singlet Manifold. Taking into account that the formation of FMA species does not seem to be favored in the triplet manifold involving triplet excited psoralen, it is worth exploring the alternative mechanism of formation of both monoadducts in the singlet manifold. Despite the fact that in psoralen the ISC process toward T_1, after one-photon absorption in S_1, is quite efficient, part of the population of the singlet state can evolve toward an intermediate structure representing a conical intersection with the ground state, $(S_0/S_1)_{CI}$, that will behave as a funnel for IC toward the formation of the monoadduct in its ground state. This type of photoreaction does not require a reactive double C═C bond elongated as in the triplet case. At the CI structures, however, the thymine and pyrone/furan C═C bonds enlarge near 0.1 Å from their initial S_1 structure. Figures 8 and 9 display the proposed schemes for the formation of PMA and FMA monoadducts, respectively, on the singlet manifold after direct population of the S_1 state of psoralen. The energy difference with respect to the initial channel, Pso(S_1) + Thy(S_0), is smaller than in the case of the triplet manifold, but still, the CI is clearly below the asymptotic limit (all energies are BSSE-corrected), in

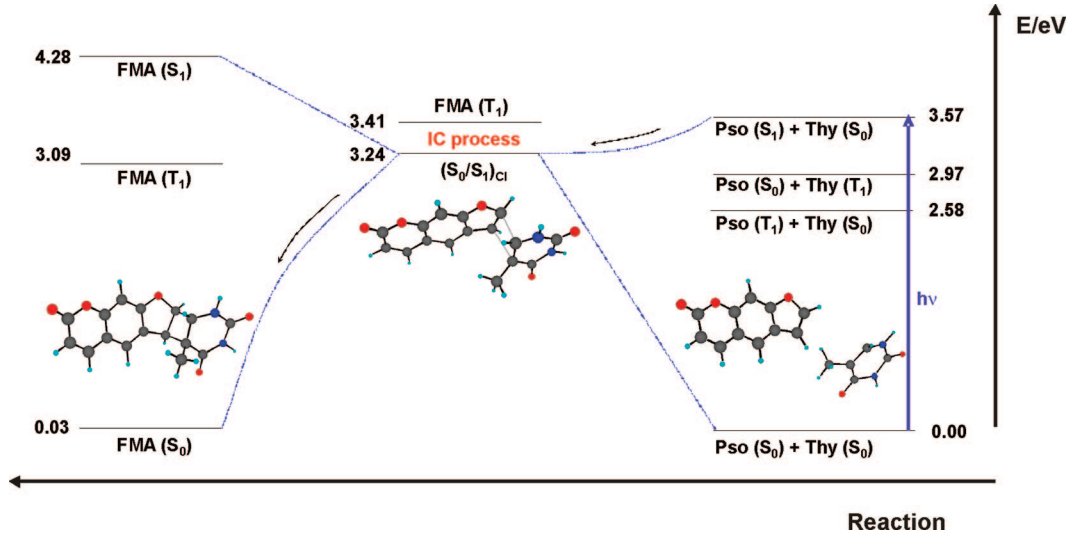

Figure 9. Photochemical mechanism proposed for the formation of psoralen (Pso)−thymine (Thy) furan monoadducts (FMA) in the singlet manifold via a conical intersection hypersurface crossing (CI). The photoreaction starts upon direct population of the S_1 state of psoralen by UVA light.

particular for the formation of the PMA CI intermediate. In the CIs, the shearing-type parallelogram structure of the system is similar to other [2 + 2] photocycloadditions,[73] with the carbon atoms of the two moieties being separated by around 2.1−2.3 Å (see Figure 6). From the CI, the system will further evolve to yield the ground-state monoadduct. Singlet-mediated [2 + 2] photocycloadditions have been proved to be efficient in other biological systems, probably even more effective than mechanisms of spin-forbidden nature. In particular, the thymine dimerization has been measured as an ultrafast photoreaction in which the dimers are formed ∼1 ps after illumination, pointing to an approximately barrierless excited-state reaction for bases that are properly oriented at the instant of light absorption.[76] Therefore, as for the triplet manifold, the efficiency of the process will also depend on the species orientation, their diffusion in the medium, and DNA flexibility. Considering the four proposed mechanisms, we suggest that PMA formation takes place mainly via the triplet manifold, whereas FMA, which is also expected to give rise to diadducts in major proportion, is probably more efficiently formed in the singlet manifold with the participation of a CI structure and the corresponding internal conversion process.

4. Summary and Conclusions

On the basis of quantum-chemical ab initio multiconfigurational CASPT2//CASSCF calculations of the psoralen−thymine system, we propose several photochemical mechanisms to elucidate the [2 + 2] photocycloaddition leading to the formation of monoadducts between the furocoumarin and the DNA nucleobase. From the two types of possible monoadducts, only those linking the thymine- and the pyrone-side psoralen reactive double C═C bonds, namely, PMAs, are suggested to be efficiently produced via the triplet manifold. The process would start by direct activation of the $S_1(\pi\pi^*)$ state of psoralen (3.9−3.6 eV) followed by rapid intersystem crossing toward the psoralen triplet manifold, finally populating the lowest triplet state $T_1(\pi\pi^*)$. Further evolution would lead the system to an intermediate species, with a single covalent bond between the two moieties, in which the lowest triplet and the singlet states are connected through a singlet−triplet crossing $(S_0/T_1)_{STC}$. Subsequent ISC would finally relax the system to the ground state of PMA. As reactivity on the furan-side double C═C bond in psoralen is not favored in the psoralen T_1 state, the only alternative to form FMA monoadducts in an equivalent mechanism via the triplet manifold is to populate the T_1 state of thymine by means of an energy transfer from another photosensitizer, that it could even be psoralen itself. It can, however, be expected that the latter mechanism is not as favorable as that for PMA, and therefore that the formation of FMA species is only minor in the triplet manifold. On the other hand, the production of both monoadducts after following a relaxation path from the initially populated psoralen S_1 state toward a conical intersection $(S_0/S_1)_{CI}$ seems equally favorable for PMA and FMA, and the predominance of one or another process will depend on the orientation effects after insertion of psoralen in the DNA strand. We can then hypothesize that the production of PMA species will take place mainly via the triplet manifold, highly favored in that case, whereas FMA is most probably formed in the singlet manifold.

Those findings allow rationalizing different experimental facts and making certain predictions. It has been found that all furocoumarins with at least one functional group, pyrone or furan double C═C bond here, produce monoadducts. As we have seen here, the formation of FMA and PMA is predicted to take place at least by one of the proposed mechanisms, although the final yield may vary upon the external conditions. Interestingly, furocoumarins show a different tendency to form diadducts, larger for psoralen and TMP and smaller for 5-MOP and 8-MOP, whereas 3-CPS and khellin seem not to yield diadducts at all.[6,49] Also, it has been suggested that only FMA species can give rise to diadducts after absorption of a photon.[22,47] Our results rationalize some of those findings. In particular, the diadduct is most probably formed upon absorption of a photon by the monoadduct in its lowest singlet state and a subsequent ISC toward T_1. In FMA, the pyrone reactive double bond concentrates the spin density, and a new photoreaction with another thymine molecule is then favored to form the diadduct. Additionally, the lowest singlet excited state of FMA is within the reach of the near UV light. On the contrary, PMA, which has a S_1 state too high in energy, in the middle UV range, delocalizes its spin density in T_1 and

the diadduct formation by the furan side can be expected to be less efficient. Furthermore, if FMA is the major source of diadducts, it is understandable that systems with a sterically hindered pyrone C=C bond like 3-CPS (see Figure 3 and the Supporting Information) or with a different π structure in the pyrone ring like khellin both yield FMA in major proportion and do not give rise to diadducts, as experimental evidence indicates. If the negative side effects of the cross-link formation of diadducts are confirmed, 3-CPS and khellin, both found as very efficient photosensitizers,[41] could be suggested as very promising photodrugs. Further experimental and theoretical studies are required to determine the contribution of the different mechanisms in the formation of the corresponding adducts in the furocoumarin family in order to design novel and highly photoreactive psoralen derivatives of photochemotherapeutical interest.

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Supporting Information Available: Calculation details, Cartesian coordinates of the optimized structures, and furocoumarin structures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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