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New insight on the photoreactivity of the phototoxic anti-cancer flutamide: photochemical pathways selectively locked and unlocked by structural changes upon drug compartmentalization in phospholipid bilayer vesicles

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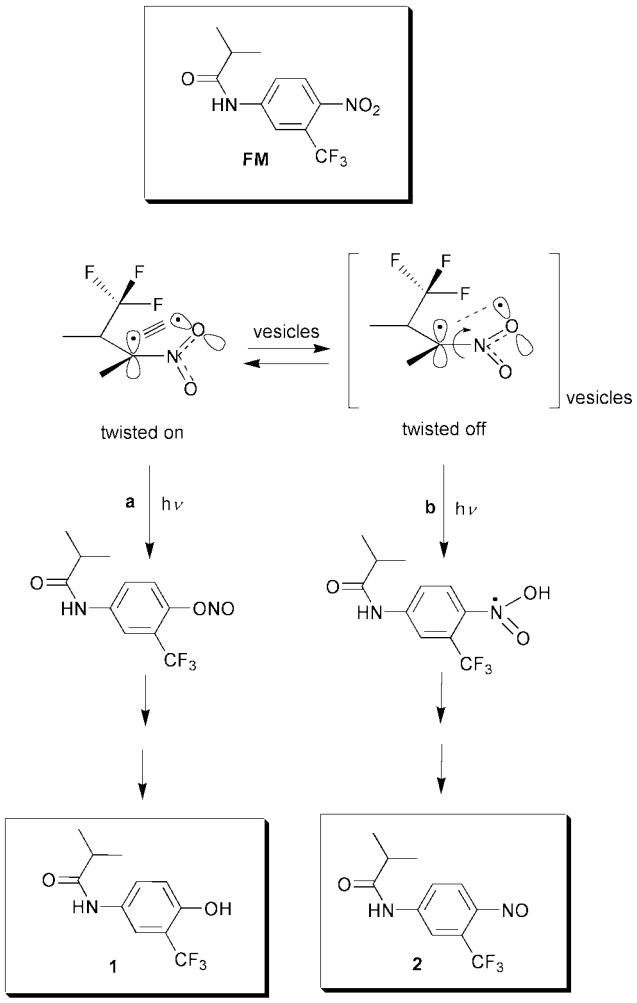
It is shown that structural changes of the phototoxic anti-cancer drug flutamide after its compartmentalization in unilamellar phospholipid bilayer vesicles lead to a highly selective modification of the photochemical outcome, locking the main photodegradation pathways observed in homogeneous media and unlocking a new and more efficient photoreactive channel.

Photochemical investigation of phototoxic drugs in organized assemblies mimicking biological systems is becoming an extremely active area of research in the wide arena of supramolecular photochemistry. Indeed, despite the knowledge of the drug photochemical behavior in homogeneous media is a first step for the understanding of molecular basis of the drug-photoinduced disorders, photoreactivity and phototoxicity are often not directly correlated to each other. The main reasons for these incongruities lie in the fact that real life photoprocesses occur at surfaces, interfaces and in multiphase heterogeneous systems. As a consequence it appears evident that a stepwise approach consisting of the investigation of the drug photobehavior in biologically mimicking systems of increasing complexity, represents an adequate strategy for a more appropriate correlation between phototoxicity and photochemical behavior. Furthermore, from a strictly photochemical point of view, studies concerning the drug photoreactivity in micromedia with particular polarity features in the presence of specific interaction and/or steric constraints provide a useful tool for the understanding of the factors influencing the molecular reactivity in order to control it.

Flutamide (FM), 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)-phenyl]propanamide, is a non-steroidal anti-androgen drug that blocks androgen receptor sites and it is widely used in advanced prostate cancer.[1] Recent reports have shown the capability of FM to induce phototoxic and photoallergic effects in patients after drug treatment.[2,3]

Our recent study performed by using UVA light excitation and dilute FM solutions[4] has shown that the photoreactivity of the drug in homogeneous solvents is almost exclusively characterized by a nitro-to-nitrite photorearrangement leading to the phenol derivative **1** as the main stable photoproduct (path **a** Scheme 1). It has been pointed out that the twisted geometry of the nitro group with respect to the aromatic plane plays a key role in triggering such a photoprocess. Indeed, such 'out of plane' geometry makes the p orbital of the oxygen atom have a constructive overlap with the adjacent p orbital of the aromatic ring in the ground state (see Scheme 1). This kind of molecular conformation determines a lowest excited state triplet state characterized by a low biradical character and, as a consequence, by a considerable inefficiency towards hydrogen abstraction (H-abstraction) even in hydrogen donating solvents[4] contrary to what is commonly observed for nitroaromatic compounds in which the nitro group is conjugated with the aromatic plane.[5,6]

In this study, the photoreactivity of FM (5×10^{-5} M) in unilamellar phospholipid bilayer vesicles (liposomes)[7] of L-α-phosphatidylcholine (10^{-3} M) was analyzed. Such organized systems are smectic mesophases of phospholipids with water interspaced among them and characterized by both high aggregation and occupancy number.[8,9] The general observation was that the self-incorporation of FM into the vesicles leads to a highly selective modification of the photochemical outcome. In fact, the chromatographic analysis† performed after 325 nm



Scheme 1

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irradiation revealed the total absence of **1** being consistent with the dramatic inhibition of the nitro-to-nitrite photorearrangement. On the contrary, the nitroso derivative **2** originated by an unexpected H-abstraction photoprocess was noticed as the sole stable photoproduct (path **b** Scheme 1). Furthermore, the photodegradation quantum yield of FM increased *ca*. 30-fold if compared to aqueous solution ($\Phi_{water} \approx 3 \times 10^{-3}$).[4]

The present scenario cannot be roughly rationalized on the basis of either the presence of the abstractable hydrogen atoms of the bilayer or on its low polarity. Actually, as outlined earlier, the irradiation of FM performed in solvents characterized by good hydrogen donating properties and polarity similar to the vesicles, interior did not activate the photonitroreductive pathway.[4]

We believe that a plausible explanation to account for the inhibition of **1** and the photogeneration of **2** may be consistent with structural changes of FM occurring upon its compartmentalization in the bilayer. In this regard, a less perpendicular geometry of the nitro group with respect to the aromatic ring, more likely caused by steric constrains and specific weak interactions (*i.e.* H-bond involving the CF_3 and/or NO_2) with the close packed lipids, would account well for the obtained results. Such changes in the perpendicularity of the nitro group would lead in fact to a less extended overlap of the p atomic orbital of oxygen with the adjacent orbital of the aromatic ring (see Scheme 1) with consequent loss of the twisted conformation. As well-documented in the literature,[10,11] such a conformation is a prerequisite for the nitro-to-nitrite photorearrangement responsible for the formation of **1**. The consequence of the loss of the twisted conformation is in turn reflected in the logical increase of the biradical character of the n,π* triplet and the consequent high ability of this latter in abstracting hydrogen in the presence of a suitable H-donor. Under these conditions, an intra-vesicles H-abstraction photoprocess involving the nitro group and the hydrogen atoms of the lipid chains might be activated, thus giving rise to the formation of **2** according to the very well known mechanistic pathways (path **b** Scheme 1).[5,6] Our hypothesis is supported well by spectroscopic data combined with theoretical calculations. In fact, from the calculated values of the dipole moments of the ground and first π,π* state, responsible for the first large absorption band (Delta μ about 10 Debyes), one would expect a blue shift of this band going from water to a less polar solvent, such as methanol and isopropyl alcohol (Fig. 1). *Vice versa*, in the vesicles (Fig. 1), where the relative permittivity is similar to that of isopropyl alcohol[9] and where in principle a blue shift should occur, one observes a marked red shift of this band (about 15 nm), consistent with a larger conjugated system which can only occur through a planarization of the NO_2 group.

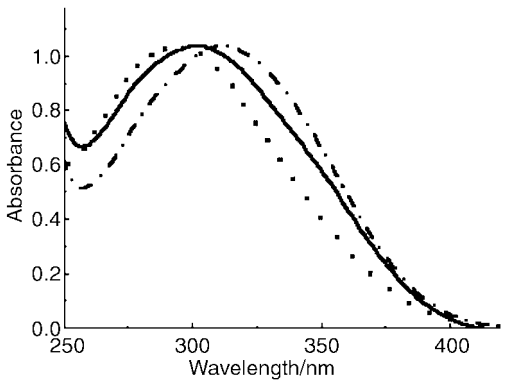

Fig. 1 Normalized absorption spectra of FM in (—) water, (···) isopropyl alcohol and (-·-·-) vesicles.

We believe that these preliminary results may be of chemical, biological and industrial relevance. From a strict chemical point of view, they represent a significant case of photochemical reactions selectively locked and unlocked by conformational changes of the molecular geometry upon substrate confinement in an organized system. From a biological point of view, given the potential of the bilayer in providing an useful model to mimic the biological membranes and by considering that the photogeneration of **2** is mediated by radical pathways[5,6] the obtained results represent a good step forward in understanding the origin of the phototoxic effects displayed by FM. Actually, the compartmentalization of the drug in particular biological sites in the presence of steric constraints and specific interactions, could lead to a relevant increase in the photoproduction of reactive radical species as a consequence of the photogeneration of a new product. Finally, from an industrial point of view, by taking into account the efforts that the scientific community has been making in the development of suitable carrier systems able to increase the FM solubility,[12] our study suggests that in light of the high photolability of the FM–liposome adduct if compared with the free molecule the use of liposome-based drug/carrier systems may not be the right approach to the aforementioned goal.

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Notes and references

† The analysis of the reaction mixture was performed by HPLC-MS on a LiChroCart RP-18 column (5 μm packing, 4 × 250 mm Hewlett Packard) eluting with a linear gradient of CH_3CN in 0.01 M phosphate buffer (pH 7) from 0 to 75% in 25 min. Both retention time and integrated area for the non-irradiated FM were the same either in the absence or in the presence of vesicles, suggesting that no complex existed during the elution. Full spectroscopic data concerning the characterization of **2** are available in ref. 4.

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