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Potential Phototoxicity of Rosuvastatin Mediated by Its Dihydrophenanthrene-like Photoproduct

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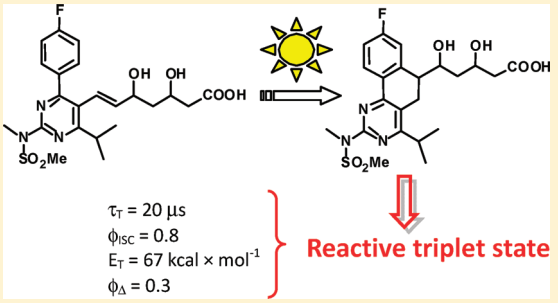
ABSTRACT: In this work, rosuvastatin has been used to gain insight into the molecular basis of statin photosensitization. This lipid-lowering drug, also known as "superstatin", contains a 2-vinylbiphenyl-like moiety and has been previously described to decompose under solar irradiation, yielding stable dihydrophenanthrene analogues. During photophysical characterization of rosuvastatin, only a long-lived transient at ca. 550 nm was observed and assigned to the primary photocyclization intermediate. Thus, the absence of detectable triplet−triplet absorption and the low yield of fluorescence rules out the role of the parent drug as an efficient sensitizer. In this context, the attention has been placed on the rosuvastatin main photoproduct (ppRSV). Indeed, the photobehavior of this dihydrophenanthrene-like compound presents the essential components needed for an efficient biomolecule photosensitizer i.e. (i) a high intersystem crossing quantum yield ($\Phi_{ISC} = 0.8$), (ii) a triplet excited state energy of ca. 67 kcal mol^{-1}, and (iii) a quantum yield of singlet oxygen formation (Φ_{Δ}) of 0.3. Furthermore, laser flash photolysis studies revealed a triplet−triplet energy transfer from the triplet excited state of ppRSV to thymidine, leading to the formation of cyclobutane thymidine dimers, an important type of DNA lesion. Finally, tryptophan has been used as a probe to investigate the type I and/or type II character of ppRSV-mediated oxidation. In this way, both an electron transfer process giving rise to the tryptophanyl radical and a singlet oxygen mediated oxidation were observed. On the basis of the obtained results, rosuvastatin, through its major photoproduct ppRSV, should be considered as a potential sensitizer.



INTRODUCTION

Photosensitizing effects of xenobiotics are of increasing concern in dermatology since modern lifestyle often combines sunlight exposure with the presence of chemical substances in the skin. In this context, an important number of compounds like perfumes, sunscreen components or pharmaceuticals as nonsteroidal anti-inflammatory agents,[1] fluoroquinolone antibiotics[2] or phenothiazine neuroleptics[3] have been reported for their photosensitizing properties. Recently, the focus has been placed on statin drugs, which are the most prescribed hypolipemiants in Europe and in the USA.[4] These lipid-lowering agents act in an early and rate-limiting step of cholesterol biosynthesis by competitive inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme that catalyzes the conversion of HMG-CoA to mevalonate. Among the adverse effects, several clinical cases of cutaneous reaction have been reported and associated with photosensivity disorders.[5] Indeed, two different mechanisms have been advanced to explain the molecular basis of the statin sensitization process. The first one has been proposed to account for the photosensitivity induced by lovastatin, which in spite of its lack of absorbance above 280 nm has been shown to be responsible for an enhancement of keratinocyte cellular damage under UVA irradiation.[6] Indeed, this HMG-CoA inhibitor produces an alteration of cell cholesterol content that results in a higher sensitivity under UVA irradiation.[6,7] In addition, photolability of UVA/UVB-absorbing statins has been shown to play a key role in their photosensitizing properties. In fact, several members of this family have been reported to decompose upon irradiation by a 6π electrocyclization process.[8] Recently, the case of atorvastatin (ATV) has been studied in detail, and it has been demonstrated that its phenanthrene-like photoproduct behaves as an efficient singlet oxygen ($^1O_2$) photosensitizer, giving rise to oxidation of biological components like tryptophan or 2′-deoxyguanosine.[9] A study on ATV *o*-hydroxy metabolite, which exhibits a slightly higher absorbance in the UVB, has drawn similar conclusions.[10] Finally, the phototoxic potential of fluvastatin has been established by *in vitro* studies with human keratinocytes and 3T3 fibroblasts. Again, its photoproduct, a benzocarbazole-like compound, has been revealed to be a more efficient photosensitizer than the parent drug.[11]

In this work, the attention has been focused on rosuvastatin (RSV, Chart 1), a synthetic statin of third generation also known as "superstatin". Photolysis of RSV has previously been studied in the literature; it leads to formation of the stable dihydrophenanthrene-like products (ppRSV and ppRSV3) and to the fully conjugated ppRSV4 compound (Chart 1).[8a] Indeed, the photoreaction mechanism has been paralleled with that established for

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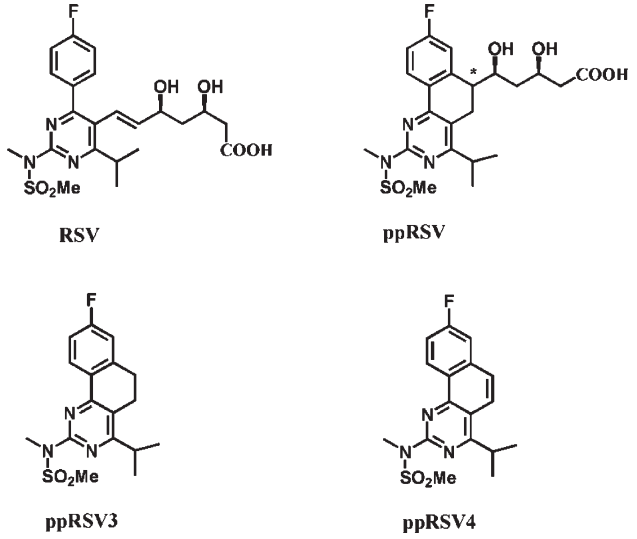
Chart 1. Structures of RSV and Its Photoproducts



2-vinylbiphenyl compounds, where photochemical electrocyclization leads to an unstable 8a,9-dihydrophenanthrene intermediate, which then undergoes a thermal sigmatropic hydrogen shift to yield dihydrophenanthrene.[8a,12] Due to the photolability of RSV, the role of its photoproducts as photosensitizing agents cannot be neglected. Here, the attention has been focused on the main photoproduct: the dihydrophenanthrene analogue ppRSV. Time-resolved and steady-state experiments have been run to determine RSV and ppRSV hotophysical properties, as well as their interaction with key biomolecule building blocks, to investigate their potential role as photosensitizers.

EXPERIMENTAL PROCEDURES

Chemicals. Rosuvastatin was purchased from KEMPROTEC Limited and was used without further purification. 2,2,6,6-Tetramethylpiperidine (TEMP) and β-carotene were purchased from Fluka. Deuterated water (D_2O), xanthone, carprofen, naphthalene (NP), naproxen, perinaphthenone, L-tryptophan (Trp), thymidine (Thd), 1-methylnaphthalene (MeNP) and benzophenone (BP) were from Sigma Aldrich.

The reagent grade solvents were obtained from Scharlau and used without further purification. Phosphate buffered saline (PBS) solutions of 0.01 M (pH = 7.4) were prepared by dissolving tablets (Sigma-Aldrich) in Milli-Q water.

Photoreactors. Irradiations were performed by means of a multilamp photoreactor equipped with lamps with a maximal output at ca. 300 nm (Hitachi, F15T8/BL). In the case of monochromatic irradiation, a Microbeam system (model L-201), including a 150 W xenon lamp coupled with a monochromator (model 101), was used.

Production of ppRSV. Compound ppRSV was isolated as described in the literature.[8a] Briefly, a dispersion of rosuvastatin calcium (75 mg in 150 mL of water) was irradiated for 20 min in Pyrex tubes using the above-described photoreactor equipped with six lamps. The irradiation mixture was dried under vacuum and separated by silica gel thin-layer chromatography eluting with CH_2Cl_2/MeOH (95:5) with drops of acetic acid (two runs) to obtain the diastereometric mixture of ppRSV. All of the experiments were conducted at room temperature and under air atmosphere. The ^{1}H and ^{13}C NMR spectral data were identical to those previously reported.[8a] Although the two diastereoisomers obtained can be chromatographically separated, the photophysical and photobiological experiments were performed with the diastereoisomeric mixture.

UV−Vis Absorption Spectroscopy. Absorbances of the samples were measured with a double beam Varian UV−vis model Cary 300 Scan spectrophotometer, using 1 cm pathway quartz cuvettes.

Laser Flash Photolysis (LFP). Two laser flash photolysis systems were employed for the studies. For 355 nm excitation, experiments were carried out using the fourth harmonic (λ_{exc} = 355 nm) of a Quantel pulsed Nd:YAG spectrum laser system instrument. The single pulses were ca. 10 ns duration, and the energy was ca.15 mJ/pulse. A pulsed Excimer Laser Systems with Xe/HCl mixture was used for excitation at 308 nm. The single pulses were ~17 ns duration, and the energy was ≪100 mJ/pulse.

For both excitation systems, a xenon lamp was employed as the detecting light source. The laser flash photolysis apparatus consisted of the pulsed laser, the Xe lamp, a monochromator, and a photomultiplier (PMT) system made up of side-on PMT, PMT housing, and a PMT power supply. The output signal from the Tektronix oscilloscope was transferred to a personal computer for study. All transient spectra were recorded using 10 × 10 mm^2 quartz cells with 4 mL capacity, and solutions were bubbled for 10 min with N_2, air, or O_2, before acquisition. The absorbance of the samples was kept in the range 0.30−0.40 at the laser wavelength. Stock solutions of quenchers were prepared so that it was only necessary to add microliter volumes to the sample cell to obtain appropriate concentrations of the quencher. A linear quenching plot was obtained, and the resulting rate constant was calculated from the slope of the Stern−Volmer plot:[13]

$$k_{obs} = k_0 + k_q[Q]$$

where k_0 is the triplet decay rate constant in the absence of quencher, k_q is the triplet decay rate constant in the presence of the quencher, and [Q] is the quencher concentration in mol L^{-1}. Photosensitization of ppRSV triplet excited state by xanthone was performed using 355 nm excitation wavelength, whereas determination of ppRSV triplet excited state properties (lifetime, intersystem crossing quantum yield, interaction with biological compounds and so on) was done exciting at 308 nm. In the case of rosuvastatin LFP was also performed with a 308 nm laser.

A modified energy transfer methodology was used to determine the molar absorption coefficient of triplet−triplet transition of ppRSV ($\varepsilon(^3$ppRSV)). It consists of the quenching of xanthone triplet excited state (λ = 633 nm) by ppRSV or 1-methylnaphthalene, used as standard. Molar absorption coefficient of ^{3}ppRSV was obtained from the ratio of ΔA of ppRSV (at λ = 400 nm) and 1-methylnaphthalene (at λ = 415 nm) when more than 95% of xanthone triplet was quenched. The following equation was used:

$$\varepsilon(^3\text{ppRSV}(400)) = \varepsilon(^3\text{MeNP}(415)) \times \Delta A(^3\text{ppRSV}(400))/\Delta A(^3\text{MeNP}(415))$$

where $\Delta A(^3$ppRSV(400)) and $\Delta A(^3$MeNP (415)) refer to the transient absorption of ppRSV and 1-methylnaphthalene triplet state (at 400 and 415 nm, respectively) at the end of the process, and $\varepsilon(^3$MeNP(415)) corresponds to the known molar absorption coefficient of the 1-methylnaphthalene triplet at 415 nm (ε = 11 200 M^{-1} cm^{-1} in acetonitrile).[14]

Then, intersystem quantum yield (Φ_{ISC}) was determined by the comparative method taking into account the amount of ^{3}ppRSV and naphthalene populated after 308 nm laser excitation. Isoabsortive degassed acetonitrile solutions of both compounds were used. So, Φ_{ISC}

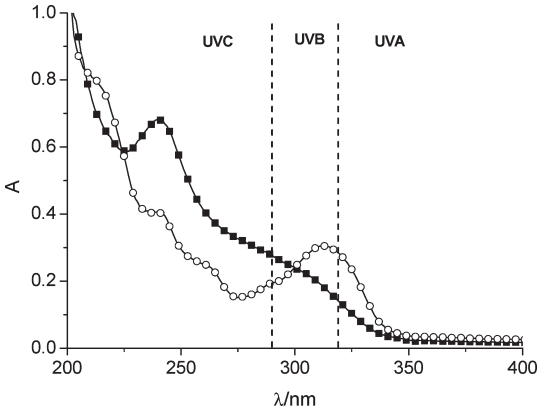


Figure 1. Absorption spectra of RSV 4.8×10^{-5} M (■) and ppRSV 1.5×10^{-5} M (○) in PBS.

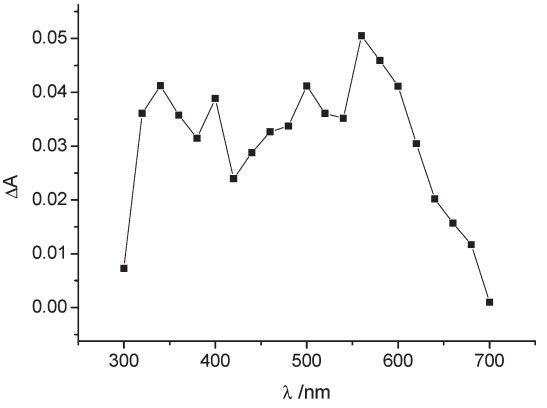


Figure 2. Transient absorption spectra of RSV (4.8×10^{-5} M) in PBS solution, recorded using a fresh solution for each laser pulse (Xe/Cl excimer, 308 nm excitation).

was obtained by using the following equation:

$$\Phi_{ISC}(^3\text{ppRSV}) = \Phi_{ISC}(^3\text{NP}) \times \Delta A(^3\text{ppRSV}(400)) \times \varepsilon(^3\text{NP}(420))/[\Delta A(^3\text{NP}(420)) \times \varepsilon(^3\text{ppRSV}(400)]$$

where Φ_{ISC} (NP) corresponds to the intersystem crossing quantum yield of naphthalene; $\varepsilon(^3\text{NP}(420))$ refers to the molar absorption coefficient of naphthalene triplet at 420 nm; $\Delta A(^3\text{ppRSV}(400))$ and $\Delta A(\text{NP}(415))$ refer to the transient absorption of ^{3}ppRSV and NP triplet at 400 and 520 nm, respectively. The values of $\varepsilon(^3\text{NP}(420))$ and Φ_{ISC}(NP) in acetonitrile were taken as 15 000 M^{-1} cm^{-1} and 0.8, respectively.[15]

Singlet Oxygen Measurements. The singlet oxygen phosphorescence decay traces after the laser pulse were registered at 1270 nm employing a Peltier-cooled (−62.8 °C) Hamamatsu NIR detector operating at 588 V, coupled to a computer-controlled grating monochromator. A pulsed Nd:YAG LS2137 V LOTIS TII was used at the excitation wavelength of 266 nm. The single pulses were ca. 10 ns duration, and the energy was lower than 10 mJ/pulse. The laser flash photolysis system consisted of the pulsed laser, a 77250 Oriel monochromator and an oscilloscope DP04054 Tektronix. The output signal from the oscilloscope was transferred to a personal computer. All measurements were made at room temperature, air atmosphere, using acetonitrile as solvents in 1 cm pathway quartz cuvettes. The absorbance of the samples was 0.30 at the laser wavelength. The singlet oxygen quantum yield (ϕ_Δ) was determined using naproxen in acetonitrile ($\phi_\Delta = 0.27$)[1] as standard. Singlet oxygen formation was calculated from the slope of the plots of signal intensity at zero time versus laser light intensity according to the following equation:

$$\phi_{\Delta(\text{sample})} = \frac{I_{\text{sample}}}{I_{\text{standard}}}\phi_{\Delta(\text{standard})}$$

where I_{sample} is the emission intensity for the sample, $I_{standard}$ is the emission intensity for the standard and $\phi_{\Delta(standard)}$ is the standard quantum yield of singlet oxygen formation.

Fluorescence. The steady-state fluorescence experiments were carried out on a Photon Technology International (PTI) LPS-220B spectrofluorometer. Fluorescence quantum yield measurements were performed with aerated methanol, acetonitrile and PBS solutions; the absorbance was adjusted to ca. 0.1 at the excitation wavelength (at λ_{ex} = 314 nm), and carprofen was used as standard (Φ_F = 0.068 in acetonitrile).[16] All measurements were recorded using 1 cm pathway quartz cells with 4 mL capacity and were carried out at room temperature.

EPR Spin Trapping Experiments. The measurements were performed in a flat cell with a Bruker EMX 10/12 EPR spectrometer, using the following parameters: microwave power, 20 mW; modulation amplitude, 1.0 G; and modulation frequency, 100 kHz. Analysis was performed recording the EPR signal of the free radical TEMPO generated by reaction of singlet oxygen with the spin trap TEMP.[17] Aerated methanolic solutions of 10 mM TEMP containing ppRSV, with an absorbance of ~0.3 at 300 nm, were irradiated in a photoreactor; EPR spectra were recorded at different irradiation times.

Tryptophan Photodegradation. PBS solution of ppRSV (2×10^{-5} M) containing an equimolar concentration of tryptophan was irradiated at 320 nm using a MicroBeam system. Tryptophan fluorescence emission intensity (λ_{ex} = 282 nm, λ_{em} = 353 nm) was recorded at increasing irradiation time. A parallel experiment was performed using deuterium oxide as the solvent under identical conditions. A solution of tryptophan in PBS (2×10^{-5} M) was used as control.

RESULTS

Photophysics of Rosuvastatin. Rosuvastatin absorption reaches the UVA region of the spectrum; it exhibits a maximum at 243 nm and a shoulder until 350 nm (Figure 1). A very weak fluorescence emission was observed at 373 nm (data not shown). This emission was increasing during measurement of successive spectra with the same sample, suggesting degradation of the compound under UV irradiation.

Photolability was further observed by laser flash photolysis (LFP) experiments performed on RSV N_2-degassed PBS solutions. Indeed, after one laser pulse, the decay monitored at 400 nm exhibited a remarkable change, suggesting formation of a new intermediate. A transient absorption spectrum of RSV, obtained using a fresh sample for each point, showed a long-lived species centered at ca. 550 nm (Figure 2). By comparison with literature data available for 2-vinylbiphenyl derivatives,[12] this transient has been assigned to the intermediate of cyclization of RSV leading to the dihydrophenanthrene-like compounds.

When LFP of an irradiated sample was run, a signal with maximum at 400 nm was detected. This species was quenched by oxygen, and thus it was tentatively assigned to the triplet excited state of RSV photoproduct(s). With this background, in view of the important photolability of the parent drug, the attention was centered on the photobehavior of the primary and main photoproduct, namely, ppRSV.

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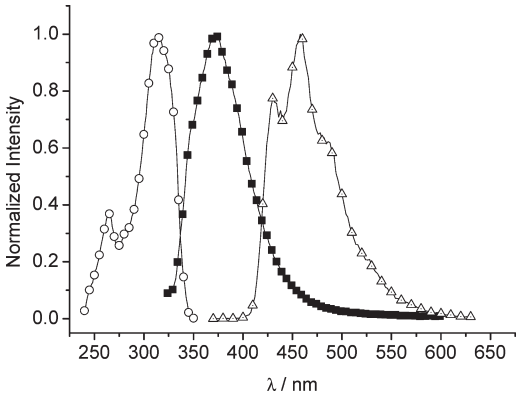

Figure 3. Fluorescence excitation (○, λ_{em} = 376 nm) and emission (■, λ_{exc} = 314 nm) of ppRSV in acetonitrile at 295 K. Phosphorescence emission spectrum in EtOH at 77 K (Δ, λ_{exc} = 312 nm).

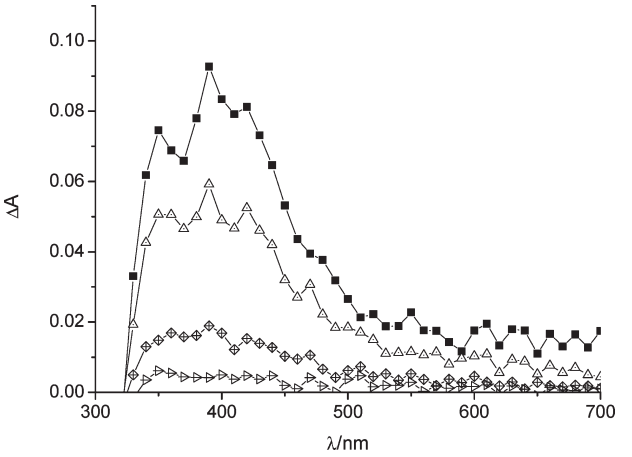

Figure 4. Transient spectrum of triplet−triplet transition obtained for a degassed acetonitrile solution of ppRSV (3.3×10^{-5} M) after 308 nm laser excitation.

Table 1. Photophysical Properties of ppRSV in Different Solvents

	λ_{em} (nm)	E_S (kcal mol^{-1})	Φ_F	λ_{T-T} (nm)	τ_T (μs)	k_q (O_2) (M^{-1} s^{-1})
PBS	368	84	0.036	400	20	1.2×10^9
methanol	370	84	0.012	400	10	2.6×10^9
acetonitrile	376	84	0.014	400	8	2×10^9

Photophysics of the Major Rosuvastatin Photoproduct. As shown in Figure 1, ppRSV exhibits a defined absorption band centered at λ_{max} = 313 nm. It is noteworthy that the photoproduct has a stronger absorption in the UVA region than RSV, which thus increases its potential to act as a photosensitizer under solar irradiation. Steady-state fluorescence of ppRSV in acetonitrile solution showed an emission spectrum with λ_{max} at 376 nm and a fluorescence quantum yield Φ_F of 0.014, obtained using carprofen as standard. Fluorescence emission was not quenched by oxygen. A singlet-state energy (E_S) of 84 kcal mol^{-1} was determined by considering the intersection between the normalized emission and excitation spectra (Figure 3). Similar results were obtained using PBS and methanol as solvent (Table 1). Low temperature emission was also performed in EtOH at 77 K. Under these conditions, ppRSV showed a well-defined emission spectrum with a maximum centered at 460 nm. A triplet excited state energy value (E_T(ppRSV)) of 67 kcal mol^{-1} was obtained from the 0−0 band of the spectrum.

Laser flash photolysis of deaerated solutions of ppRSV showed a transient at 400 nm (Figure 4) with lifetime varying from 8 μs in methanol to 20 μs in PBS. This species was efficiently quenched by oxygen, and thus it was tentatively assigned to ppRSV triplet excited state (^{3}ppRSV) (Table 1). Interestingly, this signal was similar to that observed during LFP of an irradiated sample of RSV (see above).

The nature of ppRSV transient was confirmed by further quenching experiments. In a first stage, a diffusion-controlled process was observed in the presence of β-carotene (E_T ca. 20 kcal mol^{-1}).[15] Under these conditions, the transient absorption at 400 nm disappeared concomitantly with the appearance of a new band peaking at 520 nm, which corresponds to the characteristic T−T absorption of β-carotene. Thus, a

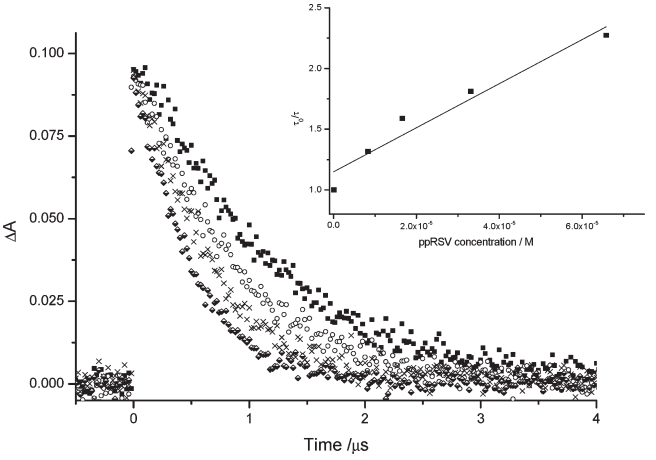

Figure 5. LFP at 355 nm excitation. Decays of xanthone (6.6×10^{-5} M in deaerated acetonitrile) monitored at 630 nm, in the presence of increasing concentration of ppRSV (from 0 to 6.6×10^{-5} M). Inset: Stern−Volmer plot.

triplet−triplet energy transfer is occurring, which agrees with the triplet state nature of the 400 nm transient species. A complementary experiment was also performed; it consisted of the quenching of the triplet excited state of a donor using ppRSV as acceptor. Taking into account the ^{3}ppRSV energy value determined above, xanthone was used as a suitable photosensitizer (E_T = 74.2 kcal mol^{-1}).[15] As shown in Figure 5, xanthone triplet−triplet absorption centered at 630 nm was quenched in the presence of ppRSV with a bimolecular rate constant of 1.6×10^{10} M^{-1} s^{-1}, thus confirming on the one hand the triplet nature of the 400 nm transient and on the other hand that E_T (^{3}ppRSV) is lower than E_T (xanthone).

Further characterization of ^{3}ppRSV was performed by determining the molar absorption coefficient of its triplet−triplet absorption in acetonitrile. The signals formed after quenching of xanthone triplet excited state by ppRSV or 1-methylnaphthalene were compared, using the latter as standard (see Experimental Procedures). In this way, a value of 8500 M^{-1} cm^{-1} at 400 nm was obtained, and the intersystem crossing quantum yield (Φ_{ISC}) was estimated at 0.8 by means of the comparative method.

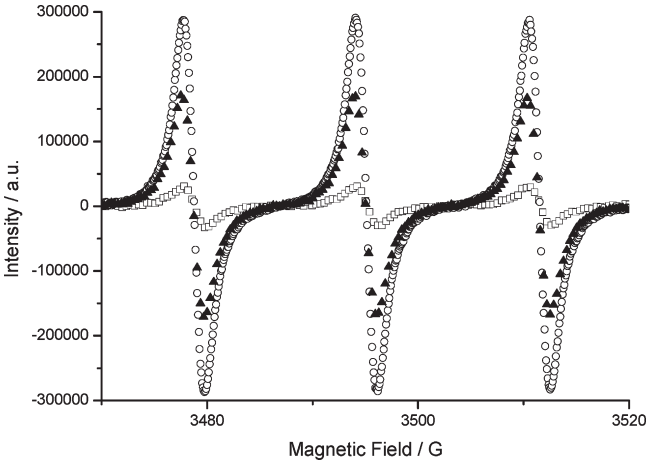

Figure 6. TEMPO signals obtained by irradiation of a methanolic solution of TEMP (10 mM) and ppRSV (3.3×10^{-5} M): without irradiation (□) and after 5 (▲) and 7 (○) min of irradiation.

Singlet Oxygen Generation. Singlet oxygen ($^1O_2$) is one of the main reactive oxygen species (ROS) involved in the oxidative damage to living systems. It is now well-established that $^1O_2$, photogenerated by endogenous or exogenous compounds, is able to react with a large number of target biomolecules, including unsaturated lipids, proteins, and nucleic acids. The efficient ^{3}ppRSV quenching by molecular oxygen observed by LFP should give rise to the formation of $^1O_2$ by a triplet−triplet energy transfer process. To confirm production of this species, spin trapping experiments were conducted using the well-known conversion of TEMP to the stable free radical TEMPO, detectable by EPR analysis. A methanolic solution of TEMP was irradiated in the presence of ppRSV. As shown in Figure 6, under these conditions, detection and increase of the typical TEMPO signal (g = 2.0054, a_N = 16.3 G) as a function of irradiation time provided clear evidence for the role of ppRSV as singlet oxygen sensitizer.

The quantum yield of $^1O_2$ formation was determined by time-resolved near-IR emission studies using naproxen as standard (Φ_Δ = 0.27 in acetonitrile).[1] The intensity of the characteristic singlet oxygen emission at 1270 nm was plotted as a function of the laser intensity for oxygenated acetonitrile solutions of ppRSV and naproxen. A quantum yield of 0.3 was obtained from the ratio of the linear plot slopes. The same value was obtained by registering steady-state emission of $^1O_2$, using perinaphthenone as standard (Φ_Δ = 1 in acetonitrile).[18]

Interaction with Biomolecules. After showing that ppRSV exhibits the appropriate properties of an efficient biomolecule photosensitizer, the next step was to check whether ^{3}ppRSV might be involved in oxidation of biological substrates. In this context, the type I mechanism proceeds via hydrogen abstraction or electron transfer mechanisms, whereas the type II oxidation is mediated by the formation and subsequent reactions of $^1O_2$.[19] Both mechanisms can be in the origin of protein or DNA photosensitization. In addition, a triplet−triplet energy transfer from a photosensitizer to thymidine (Thd) could give rise to thymidine cyclobutane dimers (Thd<>Thd), an important DNA lesion.[20] As a requirement for this process to occur, the triplet state energy of the photosensitizer has to be higher than that of the DNA base. In this context, the E_T value of ^{3}ppRSV, determined as 67 kcal mol^{-1}, is high enough to make this compound a potential Thd<>Thd photosensitizer.

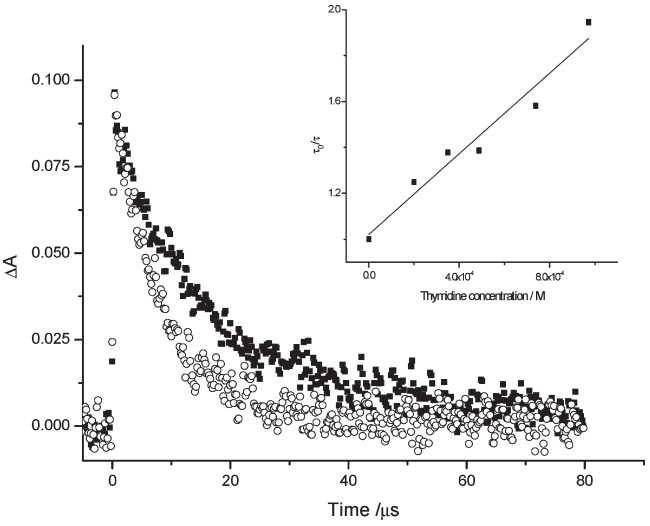

Figure 7. LFP at 308 nm excitation of ppRSV (3.3×10^{-5} M) in deaerated PBS, transient decay monitored at 400 nm without (■) and with 10^{-3} M Thd (○). Inset: Stern−Volmer plot.

In the first stage, interaction with DNA components was considered by monitoring the ^{3}ppRSV decay at 400 nm in the presence of increasing amounts of Thd. As shown in Figure 7, addition of the nucleoside results in the shortening of ^{3}ppRSV lifetime. A bimolecular quenching rate constant of 3×10^7 M^{-1} s^{-1} was determined from the Stern−Volmer plot (Figure 7, inset). Despite the relatively low value of this quenching constant, triplet−triplet energy transfer between ^{3}ppRSV and Thd, which reflects the potential Thd<>Thd dimer formation, is feasible. Moreover, the obtained value is in agreement with expectations from the isoenergetic states of the donor and the acceptor.[20]

Related studies were also performed with a protein building block. Specifically, tryptophan (Trp) represents an interesting probe as it can be oxidized both by type I and type II mechanisms. In this context, the oxidizing potential of ^{3}ppRSV has been evaluated by means of time-resolved and steady-state experiments. Direct interaction of ^{3}ppRSV with Trp has been studied by LFP, and quenching by this amino acid (Figure 8A) was found to occur with a bimolecular rate constant of 5×10^9 M^{-1} s^{-1}, leading to formation of the long-lived tryptophanyl radical (λ_{max} at 310 and 520 nm).[21] This is in agreement with a mechanism involving electron transfer from Trp to ^{3}ppRSV, followed by deprotonation of the resulting radical cation at pH = 7.4, to form the tryptophanyl radical.[21]

In addition, the ppRSV photoreactivity was investigated by steady-state photolysis, by irradiation of ppRSV at 320 nm in the presence of equimolar amounts of Trp. The disappearance of the amino acid fluorescence emission (λ_{max} = 353 nm) with irradiation time was observed and taken as measurement of the extent of oxidation (Figure 8B). To check the involvement of a type II mechanism, a parallel experiment was performed in D_2O, where the lifetime of $^1O_2$ is longer than in water. As a matter of fact, the reaction rate was increased by a factor of 2 under these conditions, in agreement with Trp oxidation by $^1O_2$.

■ DISCUSSION

Nanosecond LFP of the statin drug RSV reveals a long-lived transient at λ_{max} ca. 550 nm, which was assigned to

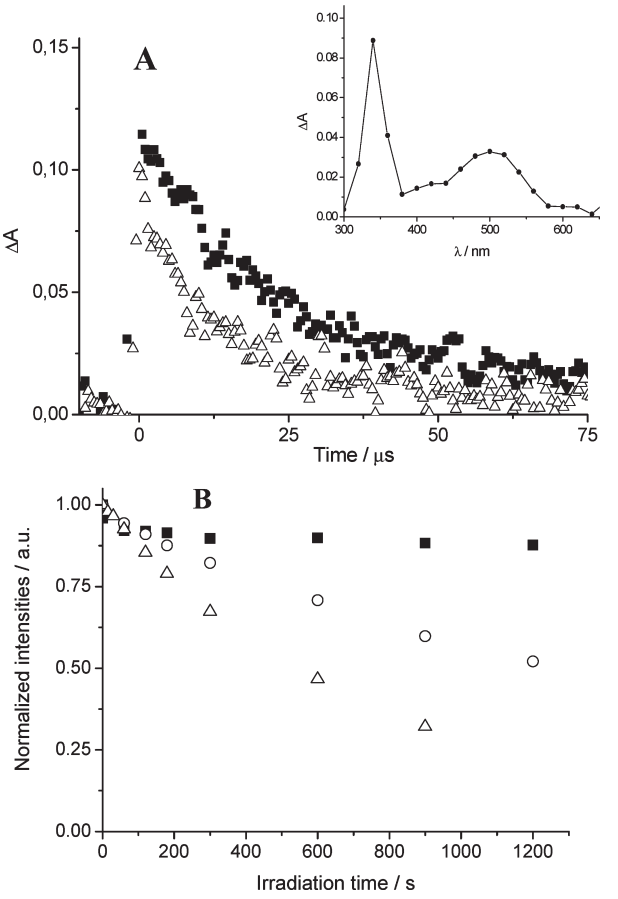

Figure 8. (A) LFP at 308 nm excitation of ppRSV (3.3×10^{-5} M) in deaerated PBS, transient decay monitored at 400 nm without (■) and with 5×10^{-6} M (Δ) Trp. Inset: Transient absorption spectrum of ppRSV in the presence of 1.8×10^{-5} M Trp registered 10.5 μs after the laser pulse. (B) Tryptophan photodegradation. Normalized emission at λ_{em} = 353 nm monitored as a function of irradiation time: Trp alone in PBS (■), and in the presence of equimolar amounts of Trp and ppRSV (2×10^{-5} M) in PBS (○) and in deuterium oxide as solvent (Δ).

8a,9-dihydrophenanthrene intermediate.[12] This result agrees well with RSV photochemistry corresponding to an electrocyclization process followed by a sigmatropic H transfer to form the primary photoproduct ppRSV.[8a] Moreover, the lack of triplet excited state detection and the low fluorescence emission indicate that RSV should be considered as an unlikely photosensitizer, as previously reported for ATV.[9] In the last case, we demonstrated that a photoproduct rather than the parent drug is the origin of the photosensitized damage to biomolecules.

With this background, the attention has been placed on ppRSV. It is noteworthy that its dihydrophenanthrene-like structure is the origin of interesting photophysical properties with respect to the phenanthrene-like photoproduct of ATV. Indeed, the photosensitizing capacity of this latter is mainly governed by singlet oxygen reactivity.[9]

As shown in Scheme 1, after promotion to its singlet excited state (eq 1, Scheme 1), ppRSV can undergo different deactivation pathways, including fluorescence (eq 2) and intersystem crossing (eq 3). Based on LFP experiments, an efficient triplet excited state formation (Φ_{ISC} = 0.8) has been established and consequently a high potential as a photosensitizer. Moreover, a high triplet state energy (E_T ca. 67 kcal mol^{-1}) state has been

Scheme 1

$$\text{ppRSV} \xrightarrow{+h\nu} {}^1\text{ppRSV} \quad (1)$$

$${}^1\text{ppRSV} \xrightarrow{-h\nu_f} \text{ppRSV} \quad (2)$$

$${}^1\text{ppRSV} \xrightarrow{ISC} {}^3\text{ppRSV} \quad (3)$$

$${}^3\text{ppRSV} \xrightarrow{-h\nu_p} \text{ppRSV} \quad (4)$$

$${}^3\text{ppRSV} + O_2 \longrightarrow \text{ppRSV} + {}^1O_2 \quad (5)$$

$${}^3\text{ppRSV} + \text{Thd} \longrightarrow \text{ppRSV} + {}^3\text{Thd} \quad (6)$$

$${}^3\text{ppRSV} + \text{Trp} \longrightarrow \text{ppRSV}^{\bullet-} + \text{Trp}^{\bullet+} \quad (7)$$

$$\text{Trp} + {}^1O_2 \longrightarrow \text{Trp oxidation products} \quad (8)$$

determined by phosphorescence emission studies. Indeed, triplet excited states have been demonstrated to be crucial species in photosensitizing reactions.[1,16] Thus, in connection with ppRSV photophysical properties, three processes have been considered to investigate the interaction between ^{3}ppRSV and biomolecules (eqs 5−8, Scheme 1).

Thymidine dimers (Thd<>Thd) are the most important lesions formed upon direct irradiation of DNA; however, they can also be obtained during photosensitization by a well established process based on a triplet−triplet energy transfer.[20] Few compounds are known to exhibit the appropriate properties to act as efficient sensitizers, the requirement being mainly based on the E_T value. Here, the ^{3}ppRSV triplet excited state lies close to that of benzophenone or carprofen, reported as Thd<>Thd photosensitizer.[20] As a matter of fact, quenching of ^{3}ppRSV in the presence of Thd is in agreement with a triplet−triplet energy transfer process (eq 6, Scheme 1). As expected from the isoenergetic triplet excited states of the donor and the acceptor, the reaction occurs with a low bimolecular rate constant of ca. 10^7 M^{-1} s^{-1}.

Furthermore, oxidations represent an essential part of biomolecule photodamages. Here, tryptophan has been used as a probe to determine type I and/or type II character of ppRSV mediated photooxidation. Steady-state photolysis has shown a higher degradation of this amino acid in D_2O, pointing toward a $^1O_2$ mediated oxidation. This result is in accordance with the obtained ppRSV singlet oxygen quantum yield Φ_Δ of 0.3 (eq 5, Scheme 1). In addition, a type I reactivity has also been evidenced by LFP. Indeed, ^{3}ppRSV quenching by Trp (eq 7, Scheme 1) leads to formation of the tryptophanyl radical arising from an electron transfer process. Therefore, oxygen and Trp compete for ^{3}ppRSV quenching. The predominance of type I or type II mechanism can be inferred from the absolute quenching rates ($k_q \times [Q]$) that consider not only the bimolecular rate constant k_q but also the concentration of the quencher [Q]. Thus, considering the maximum quencher concentration used in this work (i.e. 1.27×10^{-3} M and 20×10^{-6} M for oxygen and Trp, respectively), a type II/type I ratio of 14 is obtained. Although this value only reflects the competition between processes (5) and (7) of Scheme 1 and does not take into account the subsequent steps, it clearly indicates that Trp is mainly decomposed by a singlet oxygen process.

In summary, it has been demonstrated that RSV, a photolabile UV-absorbing statin, presents the appropriate properties of an efficient photosensitizer, acting through photoproduct formation. This may be the origin of undesired side effects like phototoxicity and/or photogenotoxicity.

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ABBREVIATIONS

ATV, atorvastatin; BP, benzophenone; LFP, laser flash photolysis; MeNP, 1-methylnaphthalene; NP, naphthalene; ppRSV, rosuvastatin photoproduct; RSV, rosuvastatin

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