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Solvent dependence of the photophysical properties of 2-Chlorothioxanthone, the principal photoproduct of chlorprothixene

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Abstract

2-Chlorothioxanthone (CTX) is used as photoinitiator for the reticulation of synthetic resins and for the preparation of pharmaceuticals. It was previously determined that CTX is the primary photoproduct of z-chlorprothixene (CPTX) when irradiated at 313 nm and is formed in an autocatalyzed reaction through an energy transfer mechanism [Piñero, L. E. *et al.* (2009) *Photochem. Photobiol.* **85**, 895–900]. In this work, the photophysical properties of CTX were measured in acetonitrile/water solutions to determine if their magnitude can affect the side-effects of CPTX. The results show that CTX has higher absorption coefficients in the visible region (400–420 nm) and higher triplet quantum yields than its parent compound. Similar to for TX, both properties strongly depend on the solvent polarity/hydroxylicity. The quantum yield of the triplet intermediate is very closed to the value of the phenothiazine triplets. The phenothiazines are the most phototoxic antidepressants. Therefore, given the appropriate microenvironment, the photosensitization side-effects of CPTX can be intensified on the production of CTX.

Introduction

The thioxanthenes belong to a class of compounds closely related to the phenothiazines (Fig. 1). The major difference is that the nitrogen at the 10-position in the phenothiazines is replaced by a carbon atom with a double bond to the side chain in the thioxanthenes. The phenothiazine and thioxanthene compounds are dopamine receptor antagonists and calmodulin inhibitors, are used clinically as neuroleptic and antiemetic agents, and have modest but broad antimicrobial activities (1). They are specifically used in the treatment of nervous, mental, and emotional conditions. Improvement in such conditions is thought to result from the effect of the medicine on nerve pathways in specific areas of the brain.

For the thioxanthenes derivatives (flupenthixol, clopenthixol and chlorprothixene (CPTX)), a 2-substituent introduces *Z/E* geometrical isomers at the exocyclic 9-olefin function. Since its introduction into human therapy, CPTX has been widely used as a major tranquilizer in psychiatry, anesthesiology, pediatrics and in general medical practice. The elimination of the drug from the blood occurs through biotransformation in the liver. Three of the main metabolites formed and excreted in urine have been isolated and identified, namely chlorprothixene-sulfoxide, N-demethyl-chlorprothixene-sulfoxide, and chlorprothixene-

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sulfoxide-N-oxide (2–3). Under UVA irradiation, CPTX also undergoes a rapid *Z/E*-photoisomerization. Furthermore, prolonged exposure to light with $\lambda < 350$ nm causes the alkylamino chain split off, producing the corresponding 2-chlorothioxanthone (CTX) (4–5). This process also strongly depends on the water content and has been found to be autocatalyzed by CTX (5). The protonation state of the terminal amino group, on the other hand, has no effect on the isomerization process, but inhibits the formation of CTX. These results indicate that the phototoxicity of zCPTX is somehow affected by the formation of CTX.

Although CPTX is prescribed as a pure *Z*-eutomer, the photoinduced isomerization to its *E*-isomer and the formation of photoproducts like CTX can alter its pharmacokinetics and intensify the phototoxic response to this drug. Most of the CPTX side effects are predictable, based on the relative strength of its interaction with and the different neurotransmitter receptors. It may produce, among others: sedation, urinary retention, dry mouth, blurred vision, sinus tachycardia, photo-induced eruptions, and phototoxicity (6). UVA *in vitro* photohemolysis test demonstrated that CPTX is one of the most phototoxic antipsychotics (7).

Phototoxicity is a common side-effect of all tricyclic antidepressants. The mechanism for this type of phototoxicity is completely unknown, although several mechanisms have been proposed, including - among others: (a) the formation of dimers and higher multimers (8); (b) the free radicals (9–10); (c) the photoaddition of the drug to ds-DNA (11); (d) the drug metabolites (12); and (e) the ground state complex with double stranded DNA (13). In previous publications (14–15), we measured the photophysical properties of many 2-substituted-10-alkylated phenothiazines in several solvents and found a very large effect on the triplet state for the 2-halogenated derivatives in phosphate buffer (PBS). The quantum yields and the lifetime of this intermediate drop to less than 5% of the corresponding value in organic solvents. The triplet state of halogenated tricyclic antidepressants is efficiently quenched by a proton transfer mechanism and the rate of this quenching correlates very well with the phototoxicity of the corresponding drugs. Therefore, we have postulated that this specie is somehow involved in the mechanism of the phototoxic side-effect of these drugs.

In this work, the photophysics of CTX is characterized in different acetonitrile/water mixtures to determine the dependence of its photophysical properties on the solvent and to find out if the formation of its triplet state can in fact affect the efficiency and side-effects of the parent drug.

MATERIALS AND METHODS

Chemicals

2-Chlorothioxanthen-9-one (CTX), thioxanthen-9-one (TX), quinine sulfate, and benzophenone (BP) were obtained from Sigma-Aldrich (St. Louis, USA) and used after recrystallization from absolute ethanol. Naphthalene (NP) was obtained from Fisher Scientific (Cayey, PR) and used after recrystallization from methanol. Acetonitrile (MeCN) was of high quality spectrophotometric grade (Fisher Scientific). Nitrogen and argon were from Air Products (Humacao, PR). Aqueous solutions were freshly prepared with nanopure water (Barnsted).

Absorption and Emission spectroscopy

All absorption spectra were taken with a HP 8453 UV-VIS photodiode array spectrophotometer. The steady-state emission and excitation spectra were taken either with a Photon Technology International LPS-220B spectrofluorometer (PTI; Birmingham, NJ) or a Spex Fluorolog Tau 3 spectrofluorometer (Horiba, NJ). The corresponding emission quantum yields were determined with the Spex Fluorolog Tau 3 spectrofluorometer. The

fluorescence quantum yields of CTX in acetonitrile were determined relative to thioxanthone (TX) in acetonitrile ($\phi = 0.0051$ at 340 nm) (16), according to Demas and Crosby (17). The excitation wavelength was 340 nm, the monochromator bandwidth was set to 2.0 nm and the diluted reference and sample were optically matched ($A < 0.08$). For the acetonitrile/water mixtures, the reference was either quinine sulfate in 0.10 M H_2SO_4 ($\phi_f = 0.53$ at 366 nm) (18) or CTX in acetonitrile. Corrections were made for differences in the instrument sensitivity as a function of wavelength, for differences in refractive index, and for intensity in the excitation. The 0–0 singlet energy was obtained from the intercept of the normalized emission and the excitation spectra. The set-up used for lifetimes measurements has been described elsewhere (19). They were measured with a TimeMaster fluorescence lifetime spectrometer (TM-2/2003) from PTI by means of the stroboscopic technique, which is a variation of the boxcar technique. A hydrogen/nitrogen flashlamp (1.8 ns pulse width) was used as excitation source. The kinetic traces were fitted by monoexponential decay functions using a re-convolution procedure taking into account the lamp pulse profile. The goodness of the fitting was judged by the chi square values and the random error of the residuals.

Laser Flash Photolysis

These experiments were carried out using the third harmonic of a Continuum Quantel pulsed Nd:YAG spectrum laser (Santa Clara, CA). The single pulses ($\lambda_{exc} = 355$ nm) have ~10 ns duration and an average energy of ~3 mJ/pulse. The laser flash photolysis apparatus was previously described (15). Samples were contained in 10–10 mm Suprasil quartz cells and were deaerated for at least 15 minutes with argon or nitrogen prior to the experiments. Concentration for the CTX was 0.050 mM, corresponding to an absorbance of 0.12 at 355 nm. The appropriate concentrations of the quencher were obtained by adding microliter volumes of a concentrated stock solution. The quenching rate constants were determined by adding increasing amounts of quenchers (from 0 to 0.10 mM), while keeping the analytical drug concentration constant.

Triplet Absorption Coefficient (ϵ_T) and Quantum Yield (ϕ_T)

The triplet absorption coefficients were measured using the energy-transfer method (20). Briefly, a 0.045 mM solution of CTX in acetonitrile was excited at 355 nm in the absence and in the presence of naphthalene (NP). Under the condition that all the laser light is absorbed by CTX and ~95% of the energy is transferred (21), the ϵ_T value for $^3CTX^*$ is determined according to the following equation, using the differences in the triplet absorbances (ΔOD) and $\epsilon_{TNP} = (1.1 \pm 0.2) \times 10^4$ (Mcm) $^{-1}$ for $^3NP^*$ at 412 nm (22–23):

$$\epsilon_T^{CTX} = 0.95 \epsilon_T^{NP} \left(\frac{\Delta OD^{CTX}}{\Delta OD^{NP}} \right) \quad (1)$$

The intersystem crossing quantum yields were obtained by the comparative actinometry method using benzophenone triplet in acetonitrile ($^3BP^*$ with $\phi_T = 1.00$ and $\epsilon_T = (6.5 \pm 0.7) \times 10^3$ M $^{-1}$ cm $^{-1}$ at 520 nm) (12) or $^3TX^*$ in MeCN ($\phi_T = 0.66$, $\epsilon_T = (2.2 \pm 0.3) \times 10^4$ M $^{-1}$ cm $^{-1}$ at 635 nm) (24) as reference. Optically matched samples of the standard and CTX were irradiated at 355 nm in nitrogen-saturated solvent. The respective triplet state absorbance of $^3CTX^*$ (630 nm) and $^3BP^*$ (520 nm) or $^3TX^*$ (625 nm) were measured for different laser intensities. A linear dependence of ΔOD with laser intensity was obtained. The ϕ_T values for CTX were calculated according to the following equation:

$$\varphi_T^{CTX} = \varphi_T^{STD} \left(\frac{\alpha_T^{CTX} \varepsilon_T^{STD}}{\alpha_T^{STD} \varepsilon_T^{CTX}} \right) \quad (2)$$

where ε_T is the triplet molar absorption coefficient, *STD* stands for either $^3\text{BP}^*$ or $^3\text{TX}^*$, and α_T is the triplet optical density per mJ, *i.e.* it is the slope of a plot of triplet transient OD's against laser energy. The intrinsic decay rate constant of the triplet and the self quenching rate constant (k_Q , with $Q = \text{CTX}$) were obtained from Stern-Volmer analysis, according to Eq. (3). The reported quenching triplet lifetimes correspond to the reciprocal of the k_0 values, *i.e.* they are equal to the lifetime in the absence of self quenching processes.

$$k_{obs} = k_0 + k_Q [Q] \quad (3)$$

Density Functional Calculations

DFT-calculations were performed to obtain relative energies for the ground and singlet excited states of CTX in either water or acetonitrile. The geometry pre-optimizations were performed *in vacuo* with the PM3 semiempirical method using the Polak-Ribiere conjugated gradient protocol (1×10^{-5} convergence limit, 0.01 kcal/Å³*mol RMS-limit). The final optimizations were performed with DFT [B3LYP/6-31G(d) OPT SCRF=(PCM, Solvent=acetonitrile or water)] using Gaussian 03 (25) at the High Performance Computing Facility (UPR Rio Piedras). All conformational, thermodynamical and spectroscopic parameters were obtained with a DFT single point calculation.

RESULTS AND DISCUSSION

Ground and singlet excited state properties

The absorption spectrum of CTX in acetonitrile shows maxima at 259, 292, 305, and 386 nm (Fig. 2; Table 1). The band with $\lambda = 259$ nm experiences a small bathochromic shift and, except for the 1:1 mixture, there is also an hyperchromic shift with increasing water content, indicating a π - π^* character. The other bands ($\lambda > 300$ nm) are not affected very much by the solvent composition. The 305 nm band shows only a hypsochromic effect and disappears in the MeCN/water (1:4) mixture. The 386 nm one shifts bathochromic by only 3 nm with water content. The DFT theoretical calculations indicate that this band is produced by a combination of the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions from both the chlorine and the sulfur (Fig. 2, Inset). For comparison purposes, the spectrum of TX was measured in MeCN. It was found to be blue-shifted by 6 nm, but no effect is observed for the extinction coefficient (Table 1). These findings are in agreement with the reports of Neumann and coworkers (26). These authors found that substitutions at the TX aromatic system introduce only small changes in the absorption maximum ($\Delta\lambda = \pm 10$ nm) and the molar absorption coefficient ($\Delta\log\epsilon = \pm 0.2$). Furthermore, changes in solvent show no significant peak shifts or changes in the absorption intensities.

The room-temperature CTX fluorescence spectrum consists of a single broad band, which maximum is solvent dependent (Fig. 3; Table 1), as previously reported for TX (16). The Stokes shift increases significantly with increasing the solvent polarity. This indicates that the S₁-State of CTX ($^1\text{CTX}^*$) is very polar. Density functional (DFT) calculations predict a change in the dipole moment of almost 2.0 D between the ground and the S₁-state. For singlet states with n - π^* character in nonpolar solvents, increasing the solvent hydrogen-bonding ability is expected to induce an initial blue shift in the wavelength maximum. The

absence of such a blue shift suggests that the $^1\text{CTX}^*$ -state is a $\pi\text{-}\pi^*$ state in all water/MeCN mixtures, similar to the transitions found for TX in hydroxylic solvents (16).

Fluorescence lifetimes were measured for different MeCN/H₂O ratios to understand the effect of the microenvironment on the singlet excited state dynamics. An excited singlet energy of ~ 70 kcal/mol was obtained for CTX from the crossing point of normalized excitation and emission spectra (Fig. 3, Table 1). This value is in excellent agreement with the corresponding singlet energies for TX in MeCN and MeCN/H₂O (4:1) measured under identical conditions, which are 73 and 71 Kcal/mol, respectively. The Stokes shifts of TX in these solvents are $(1.8 \pm 0.2) \times 10^4$ and $(2.8 \pm 0.2) \times 10^4 \text{ cm}^{-1}$, respectively. These values also coincide very well with the corresponding values of CTX (Table 1). The fluorescence decay of the singlet state of CTX ($^1\text{CTX}^*$) has a monoexponential behavior (Fig. 4), as confirmed by the χ^2 values ($0.88 \leq \chi^2 \leq 1.11$). Similar to the observations made for TX (27), the dissolved oxygen has a small influence on the lifetime of $^1\text{CTX}^*$, they are slightly higher in the absence of oxygen. The obtained quantum yields also show the same solvent polarity/hydroxylic dependency found for TX (Fig. 5, Table 1). For TX and its derivatives, the lifetime becomes very short in non-hydroxylic solvents like MeCN. For CTX, the emission decays yield smaller fluorescence lifetimes with decreasing water content. In pure MeCN, the quantum yield is too small for the signal to be detected, resulting in fluorescence lifetime parameters with higher uncertainty. The CTX emission quantum yield also increases with solvent polarity and should have a limiting value of 0.51 in water, extrapolated assuming a lineal correlation ($r^2 = 0.9671$). Furthermore, electron donor groups in the 2-position of the thioxanthone ring have been found to induce high fluorescence quantum yields, red-shifts in the emission spectra and long singlet lifetimes (25). This is evident in the case of CTX, if the TX values [$\phi = 0.26$ and $\tau = 4.9$ ns in MeCN:H₂O (3:2)] (27) are compared with the corresponding values for CTX [$\phi = 0.31$ and $\tau = 5.4$ ns in MeCN:H₂O (1:1)].

This solvent effect on the singlet state photophysical properties of thioxantone derivatives was first attributed to the separation of the $n\text{-}\pi^*$ and $\pi\text{-}\pi^*$ excited states induced by the higher electron density of the aromatic system. According to this proximity effect theory, the dipole moment of the S_1 ($\pi\pi^*$) state is even larger than that of the S_2 ($n\pi^*$) or S_0 states (28). For polar solvents, the solvent relaxation around the electronically excited singlet induces a larger decrease in the energy of S_1 ($\pi\pi^*$) relative to S_2 ($n\pi^*$), increasing the S_2 ($n\pi^*$) - S_1 ($\pi\pi^*$) electronic energy gap and diminishing the relative importance of the proximity effect. Therefore, in nopolarsolvents, the singlet lifetime and the quantum yield values are expected to be small due to a small S_2 ($n\pi^*$) - S_1 ($\pi\pi^*$) gap (29). Recently this large solvent effect on the excited singlet was interpreted in terms of several solvatochromic parameters (27).

Modern quantum photochemistry postulates, on the other hand, that the decrease of the internal conversion rates from the excited to the ground state and the increase of the fluorescence quantum yield in polar solvents should be attributed to the fact that the intersections of the S_2 ($n\pi^*$) and S_1 ($\pi\pi^*$) states are less accessible in such media (30). Based on this pathways for the population of the first excited triplet state T₁: (a) a direct and fast $S_2 \rightarrow T_1$ process; and (b) an indirect and slow $S_1 \rightarrow T_2 \rightarrow T_1$ process. The slow rise time of the T₁ state was actually found to be equal to the decay time of the S_1 state and is also strongly solvent-dependent. Although the exact energies of the S_2 - and T_2 -states of TX are not precisely known, quantum theoretical calculations predict the following values (kcal/mol): $S_1 = 78.2$, $S_2 = 80.0$, $T_1 = 74.0$, and $T_2 = 78.9$ (30). The theoretical values for the first excited states correlate fairly well with the experimental ones (kcal/mol): $S_1 = 71.4$, and $T_1 = 64.5$ kcal/mol (32). Similar behavior and similar energy values were predicted for the deactivation of the excited singlet of BP (33). In strongly polar and hydroxylic solvents, $T_2(^3n\pi^*)$ is higher in energy than $S_1(^1\pi\pi^*)$, as illustrated by the energy values given for TX.

This results in a slower intersystem crossing rate (one of the population mechanisms disappears) and a higher fluorescence quantum yield. Moreover, according to the El Sayed's rules, the rate of intersystem crossing from the singlet state to the triplet manifold is relatively large if the radiationless transition involves a change of molecular orbital type. Therefore, the rate of the process $S_2(^1n-\pi^*) \rightarrow T_2(^3n-\pi^*)$ should have no contribution to the deactivation of the excited singlet (31–32). Similarly, it has been reported that the deactivation of the excited singlet of BP also follows a two step mechanism, $S_1(^1n-\pi^*) \rightarrow T_2(^3\pi-\pi^*) \rightarrow T_1(^3n-\pi^*)$ (34), only the character of the states differ from those of TX (32). The character exhibited by the excited state of BP is actually a function of the polarity/acidity of the solvent (35).

Laser flash photolysis studies

The typical transient absorption spectrum for the triplet excited state of CTX ($^3\text{CTX}^*$) generated upon 355 nm laser excitation of deoxygenated acetonitrile solutions is shown in Fig. 6. It has bands at 310 and 630 nm and depletion in the 350 – 400 nm region. The triplet absorption maximum of CTX was found to shift toward shorter wavelengths with increasing solvent polarity, similar to the band of TX (36). Both bands decay by first-order kinetics with solvent dependent lifetime values (Inset Fig. 6). In MeCN/water solutions, the spectra have the same maxima and the similar monoexponential kinetics (data not shown). However, the triplet lifetime was found to be solvent dependent. The shortest-lived triplet is found for acetonitrile, with $\tau = 77 \mu\text{s}$ (Table 1). It is important to notice that this value was calculated using the Stern-Volmer analysis. Thus, as previously indicated, it corresponds to the intrinsic $^3\text{CTX}^*$ lifetime in the absence of any self-quenching effect. This is relevant for *in vivo* situations, where low concentrations of CTX are present. A much smaller value of $6.7 \mu\text{s}$ has been reported for TX in acetonitrile (26), although in this case, self-quenching processes were not completely eliminated. For CTX, the triplet lifetime behaves similar to that of the singlet state with increasing water content (Fig. 5). In MeCN/water (1:1), for example, the lifetime increases by a factor of 2.17 compared to the value in pure acetonitrile.

Solvents and compounds with heavy atoms increase the rate of intersystem crossing. Such molecules enhance the efficiency of both the $S_1 \rightarrow T_1$ and $T_1 \rightarrow S_0$ transitions. Moreover, secondary isotope effects could affect the excited state lifetimes through vibrational coupling. Therefore, the yields of photophysical processes or photochemical reactions originating from the singlet excited state are adversely affected, while the efficiency of photoconversion from the triplet state is usually increased. Experiments with MeCN/D₂O (1:1) were performed to verify if the lifetimes and quantum yields of the excited triplet $^3\text{CTX}^*$ are sensitive to heavy atoms. Similar to the case of TX (37), the fluorescence quantum yield and the lifetime of $^1\text{CTX}^*$ in MeCN/D₂O (1:1) were not affected by the heavy atom, within the accuracy of the experiment. The corresponding triplet lifetime, on the other hand, is slightly higher than in the water containing solutions. This behavior is explained in terms of the exciplex formed between the protic solvent and both $^1\text{CTX}^*$ and $^3\text{CTX}^*$ (37,38,39). The fact that the singlet excited state is not affected by solvent deuteration indicates that the singlet exciplex does not deactivate by internal conversion (37). The extinction coefficient and quantum yield values of $^3\text{CTX}^*$ in the 1:4 solutions are not available, because CTX is not very soluble in this solvent mixture. For water, an extrapolated triplet lifetime of $258 \mu\text{s}$ is obtained (Fig. 4, $r^2 = 0.9761$).

The quantum yields of $^3\text{CTX}^*$ are very high and decrease with the solvent polarity (Fig. 4, Table 1). In acetonitrile this value is 0.91 (relative to BP), but in MeCN/water (1:1) it is only 0.25 (relative to TX). These values are obtained under the assumption that the triplet extinction coefficient does not change with the water content (Eq. 2, Fig. 7). The extrapolation for an aqueous $^3\text{CTX}^*$ solution predicts a lifetime of $258 \mu\text{s}$, but this transient should form with the smallest quantum yield (ϕ_T 0.07, assuming an exponential dependence,

$r^2 = 0.9919$). The triplet quantum yields reported for TX are neither equal to unity, as sometimes assumed for carbonyl compounds (40) and decrease with solvent polarity (0.85 in n-hexane and 0.56 in methanol) (24). The quantum yields of $^3\text{CTX}^*$ decreases with water content, because water has a higher hydrogen bonding ability with the carbonyl group of CTX (24,39). One of the major reasons accounting for the lack of reproducibility in the triplet quantum yield values is that their determination requires accurate values for the molar absorption coefficients of the transient states, an experimental constraint associated to the conventional actinometric methods. The self-quenching constants of $^3\text{CTX}^*$ are in the range of $(0.56 - 1.10) \times 10^8 \text{ M}^{-1}\text{s}^{-1}$, slightly smaller than the corresponding values of other thioxanthone derivatives, $(2.0 - 4.0) \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ (41). The deuterium isotope effects on this constant ($k_{\text{D}_2\text{O}}/k_{\text{H}_2\text{O}} = 1.79$) is typical for triplet state processes, indicating a higher vibronic interaction between the solvent and CTX.

CONCLUSIONS

Z-Chlorprothixene shares all side effects with the chlorinated tricyclic antidepressants, including the phototoxicity. Phototoxic responses after administration of photosensitive pharmaceuticals have been recognized as undesirable side effects, and predicting potential hazardous side effects is gaining importance as new drugs are introduced to the market. The photochemistry of zCPTX is the same in water and water-containing acetonitrile (5). The formation of CTX is not due to a reaction between $^3\text{CPTX}^*$ and dissolved oxygen. A mechanism involving water traces in the solvent seems to be more plausible. The photolysis of CPTX-HCl never yielded CTX, even after very long irradiation times. Based on these results, the formation of the CTX was proposed to be involved in the phototoxic side-effects of this drug because: (a) it is part of the damaging metabolic products of zCPTX; (b) thioxanthenes photoreact easily from the singlet and the triplet state; and (c) CTX auto-sensitizes its own formation, increasing the formation of $^3\text{CPTX}^*$ and – thus – the toxic potency of zCPTX (5). Wavelengths within the UV-A (320–400 nm) and the visible range are more likely to cause drug-induced photosensitivity reactions. The absorption maxima of CPTX and CTX are 324 and 386 nm, respectively. This 62 nm red-shift increases the amount of light absorbed and, therefore, increases the probability of induced phototoxicity.

Besides, the carbonyl group in the thioxanthone is expected to increase the photoreactivity, because it behaves as an electrophilic radical in the triplet excited state, it is an excellent hydrogen-abstracting group (39), or can transfer energy to an acceptor (5). In the case of $^3\text{CTX}^*$, the corresponding lifetime is high enough to promote the energy transfer process. The aryl chloride in CTX is also liable to homolytic and/or to heterocyclic dechlorination, as in the case of other chlorinated derivatives (15,42). The study of the photodegradation of a CTX was not part of the scope of this work, but it cannot be ruled out since this compound absorbs significantly ambient light ($\lambda > 330 \text{ nm}$). It is important to notice that most information about photoreactions available in the literature refers to the conditions where such processes are most easily observed, *i.e.* in dilute solutions using organic solvents. Nevertheless, what really matters for having high drug photostability are relative concentrated aqueous solutions or the solid state. Under dilute conditions, if the drug is not protected from the sun light, its photoreactivity may dramatically affect its stability. Therefore, CTX can be present in the solution prior to the drug administration. Based on the findings in this work, it can be concluded that the formation of CTX in pre-irradiated CPTX solutions is an important factor affecting the drug photosensitive/phototoxic potential. This photoproduct may increase the damaging capacity of the parent drug *in vivo* because it forms in an autocatalytic mechanism and its triplet is long lived in water.

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Molecule	Abbr.	R ₁	R ₂
Promazine	PZ	N(CH ₂) ₂ N(CH ₂) ₂	H
Chlorpromazine	CPZ	N(CH ₂) ₂ N(CH ₂) ₂	Cl
<i>cis</i> -Chlorprothixene	<i>c</i> CPTX	C-(CH ₂) ₂ N(CH ₂) ₂	Cl
2-Chlorothioxantone	CTX	C=O	Cl

Figure 1.
Structures of the derivatives used in this work.

Figure 2. CTX ground-state molar absorption spectrum in: (●) MeCN, (▼) MeCN/Water (4:1), (■) MeCN/Water (2:1), (◆) MeCN/Water (1:1). Inset: Molecular orbitals involved in the 386 nm transition.

Figure 3.

Normalized emission and excitation spectra of CTX in different acetonitrile/water mixtures:

(■) Excitation spectrum in MeCN; Emission spectra in (●) MeCN, (●) MeCN:H₂O (4:1), (▼) MeCN:H₂O (2:1), (▲) MeCN:H₂O (1:1), and (■) MeCN:H₂O (1:4).

Figure 4. Fluorescence decay curve for CTX in a MeCN/Water (2:1) solution (blue), the mono-exponential fitting (red, $\chi^2=1.109$), the IRF (black) and the corresponding residuals (bottom).

Figure 5. Dependence on the water content in MeCN/Water solutions of the fluorescence quantum yield (■), the triplet quantum yield (▲), and the triplet lifetime (●, right scale). The values for 100% water were extrapolated.

Figure 6.

Transient absorption spectra of a 0.050 mM CTX solution in N₂-saturated MeCN after a 355 nm laser pulse (Energy < 3 mJ): (●) 0.83 μs , (○) 1.22 μs , (▼) 2.02 μs , and (△) 4.77 μs . Inset: Decay curves for the transients at 320 nm (●) and 630 nm (○).

Figure 7.

Dependence of the absorption on the laser energy for CTX in MeCN ($\circ$, $r^2 = 0.9885$), TX in MeCN ($\bullet$, $r^2 = 1.0000$), CTX in MeCH/Water (4:1) ($\blacktriangledown$, $r^2 = 0.9749$), and CTX in MeCN/Water (1:1) (Δ , $r^2 = 0.9802$).

Table 1

Photophysical properties of 2-chlorothioxanthene-9-one in different MeCN/water mixtures. All values are an average of, at least, three independent measurements.

MeCN:Water	1:0 ^{d)}	4:1 ^{b)}	2:1	1:1	1:1 (D ₂ O)
Absorbance					
$\lambda_{\text{max}} \pm 1$ (nm) [log $\epsilon \pm 0.1$]	259 [4.6], 292 [3.7], 305 [3.7], 386 [3.8]	258 [4.6], 290 [3.6], 303 [3.6], 385 [3.7]	260 [4.7], 293 [3.7], 305 [3.6], 389 [3.8]	261 [4.5], 294 [3.6], 305 [3.5], 388 [3.7]	261 [4.8], 293 [3.9], 305 [3.8], 389 [4.0]
Singlet State					
$\lambda_{\text{max}} \pm 2$ (nm)	415	431	433	438	436
$E_s \pm 0.4$ (kcal/mol)	71.5	69.9	69.6	69.4	69.4
$E_S \pm 3$ (kJ/mol)	299	292	291	290	290
Stokes shift ± 0.2 (cm ⁻¹ /10 ⁴)	1.8	2.8	2.6	2.9	2.8
ϕ_F	0.01 ^{c)}	0.1 ^{d)}	0.2 ^{e)}	0.3 ^{d)}	0.3 ^{f)}
τ_s (ns)	< 1	3.85 (Air)	3.47 (Air)	4.78 (Air)	4.42 (Air)
		4.00 (Ar)	3.63 (N2)	5.40 (N2)	4.65 (N2)
$k_F^0 \pm 0.04$ (s ⁻¹ /10 ⁷)		3.11	5.19	6.48	6.56
Triplet State					
λ^{T-T} (nm)	310, 630	310, 610	330, 610	310, 610	310, 600
$\epsilon \times 10^{-4}$ (M ⁻¹ cm ⁻¹)	2.5 $\pm$ 0.3	i)		i)	
$\tau_T \pm 9$ (μ s)	77	125	138	167	201
ϕ_T	0.91 ^{h)}	0.57	0.43	0.25 ^{i,j)}	
$k_{SQ}/10^8$ (M ⁻¹ s ⁻¹)	1.1 $\pm$ 0.1	0.64 $\pm$ 0.03	0.59 $\pm$ 0.08	0.56 $\pm$	1.0 $\pm$ 0.2

^{a)}The corresponding spectrum of TX has the following maxima (nm) and log (ϵ): 255 [4.6], 286 [3.6], 299 [3.6, 380 [3.8]. The singlet state has the following values (26): λ (nm) = 407, τ_s (ns) = 0.13, and ϕ_F = 0.006. The triplet state has the following values (43): λ (nm) = 625, τ_T (ns) = 6.7, and ϕ_T = 0.66

^{b)}The solubility of CTX in MeCN/water (1:4) is very low. The corresponding values are: (1) Absorption: λ (nm) = 202, 262, 292, 394; (2) Emission: λ (nm) = 443; E (kcal/mol) = 68.5; Stokes Shift (cm⁻¹) = 2807; ϕ_F = 0.39^{c,f)}; τ_s (ns): 7.62 and 7.68 under air and nitrogen, respectively. k_F^0 (s⁻¹)/10⁷ = 5.11

^{c)}The excitation wavelength was 340 nm and TX in MeCN was used as reference. The maximum error in all quantum yields is 15%.

^{d)}The reference was CTX in MeCN.

^{e)}The reference was CTX in 4:1 MeCN:H₂O (ϕ_F = 0.12).

- f)* The reference was CTX in 1:1 MeCN:H₂O ($\phi_f = 0.31$).
- g)* The reference was quinine sulfate in 0.10 M H₂SO₄.
- h)* The reference was BP in MeCN.
- i)* The reference was CTX in MeCN, assuming that the extinction coefficient is independent of the water content.
- j)* The reference was TX in MeCN.