Substitution and Solvent Effects on the Photophysical Properties of Several Series of 10-Alkylated Phenothiazine Derivatives

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Received: December 1, 2004; In Final Form: February 15, 2005

The photophysical properties of several 2-substituted, 10-alkylated phenothiazines were measured in several solvents to investigate the relevance of the molecular structure in their photophysics and consequent photochemistry. Because the interaction modes of each drug and its corresponding species strongly depend on the variety of microenvironments in the cells, the properties of each one of these species must also be determined separately to understand fully the mechanism of action of the drug and the mechanism of its side effects. Information on the chemical interactions of the different species at the cellular level can be inferred from the corresponding electronic properties. In this work, we present absorption, steady-state, and time-resolved emission, laser flash photolysis, and quantum theoretical results for the ground state, the first excited singlet and triplet states, and the cation radical of promazine hydrochloride (PZ), 2-chlorpromazine hydrochloride (CPZ), 2-trifuoromethylpromazine hydrochloride (TFMPZ), 2-trifluoromethylperazine dihydrochloride (TFMP), 2-thiomethylpromazine (TMPZ), and thioridazine hydrochloride (TR). The corresponding nonalkylated phenothiazines are included as references. The photophysical properties of this drug family depend more on the solvent and the 2-substituents than on the dialkylaminopropyl chain. The largest effect was found for the triplet state of the 2-halogenated derivatives in phosphate buffer (PBS). Both the quantum yield and the lifetime of this intermediate drop to less than 5% of the corresponding value in organic solvents. The triplet state of halogenated promazines is efficiently quenched by a proton-transfer mechanism, and the rate of this quenching correlates very well with the phototoxicity of the promazine drugs. Therefore, we postulate that this species is directly related to the phototoxic side effect of neuroleptic drugs.

Introduction

Promazines, piperidines, and perazines are derivatives of phenothiazine (Figure 1) and belong to the most commonly used families of tricyclic antidepressant (TCA)* drugs in the treatment of mental illness. The structural requirements for the neuroleptic activity of these drugs include a substitution at the 2-position of the phenothiazine group and an alkylamino chain at the 10-position.[1,2] This substitution must be in the 2-position and must possess electron-withdrawing properties. 1-Chlorpromazine (1-CPZ), for example, does not exhibit neuroleptic activity but is a very good antidepressant.[3] It is believed that the enhancing effect of the 2-substituent on the neuroleptic activity might be due to a direct interaction with the receptor at a site corresponding to one of the dopamine oxygens.[4] Nevertheless, because the neuroleptic promazines are fairly flexible at the alkylamino chain, they certainly have several dopamine-active conformations in solution.[5] It is also essential for the antipsychotic activity of the phenothiazines that the alkylamino chain has three carbons between both nitrogens. The amino group may be either at the end of the alkyl chain, as in the promazines, or incorporated into a alkylcycloamino ring system, as in the perazines. Derivatives with chains consisting of only two carbon atoms, such as promethazine, are very powerful antihistaminic, antiallergic, and sedative drugs but have no antipsychotic activity.[6,7]

Changes in the structure of phenothiazine drugs not only change their neuroleptic activity but also change the spectrum and intensity of the side effects. Halogenated promazine derivatives have been found to be more neuroactive but also induce a more pronounced phototoxicity than other analogues. Therefore, both structural modifications have also been correlated to the magnitude of this side effect.[8] For instance, 2-chlorpromazine (CPZ) is still the most potent drug of the phenothiazines and also has the most remarkable side effects. These include, among others, itching, rash, hypertrophic papillae of the tongue, angioneurotic edema, erythema, allergic purpura, exfoliative dermatitis, and photosensitivity.[9−11] Phototoxic and photoallergic reactions have been reported to occur in the eyes and skin of psychotic patients receiving CPZ treatment. Significant pigmentation has been observed in eye lenses and cornea.[12] Other phenothiazine derivatives also enhance light sensitivity, but it is still not known why CPZ differs so much from the rest of the group. Several hypotheses have been proposed to explain this phenomenon, including photodehalogenation,[13] the direct involvement of the excited states of the drugs[14] or its cation radical,[15−17] and the involvement of secondary toxic photoproducts.[18−20] The involvement of the high-energy cation radical in the in vivo photosenzitation of

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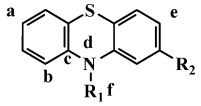
10.1021/jp044530j CCC: $30.25
Published on Web 03/26/2005



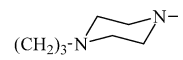
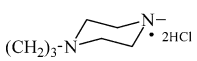
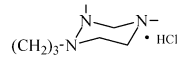
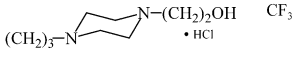
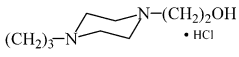
Phenothiazine Derivative		R_1	R_2
Abbrev.	Name		
PH	Phenothiazine	H	H
CPH	2-Chlorphenothiazine	H	Cl
TFMPH	2-Trifluoromethylphenothiazine	H	CF_3
PZ	Promazine hydrochloride	$(CH_2)_3-N(CH_3)_2 \bullet HCl$	H
CPZ	2-Chlorpromazine hydrochloride	$(CH_2)_3-N(CH_3)_2 \bullet HCl$	Cl
TFMPZ	2-Trifluoromethylpromazine hydrochloride	$(CH_2)_3-N(CH_3)_2 \bullet HCl$	CF_3
TMPZ	2-Thiomethylpromazine	$(CH_2)_3-N(CH_3)_2$	SCH_3
PCP	Prochlorperazine		Cl
TFMP	2-Trifluoromethylperazine dihydrochloride		CF_3
TR	Thioridazine hydrochloride		SCH_3
FP	Fluphenazine hydrochloride		CF_3
PP	Perphenazine hydrochloride		Cl

Figure 1. Structure of phenothiazine and its derivatives.

promazines has been questioned[21,22] because it is formed by a two-photon process and the intensity of radiation required for this multiphotonic process is not available under normal conditions. Nevertheless, recent reports still found some phenothiazine derivatives to photoionize monophotonically and associate the cation radical with the phototoxic effects of the corresponding drugs.[23]

The spectrum and the magnitude of the side effects of the phenothiazines are close related to their chemical structure.[24,25] Therefore, there must also be a correlation between the molecular structure and the photophysical properties of the corresponding transients.[14] In this work, we study the photophysics of the first excited singlet and triplet states and the cation radical for three sets of phenothiazine derivatives, which differ either in the 2-substitution or the alkyl chain. The 2-substituents include strong electron-withdrawing (CF_3) and electron-donating (SCH_3) groups. The 10-substitution includes the amino group at the end of the chain and as part of a ring system. This combination of substituents (Figure 1) allows a better discrimination of the specific contributions to the photophysical properties of these drugs. Non-alkylated 2-substituted phenothiazines are included for comparison purposes.

Materials and Methods

Chemicals. Monobasic (Na_2HPO_4) and dibasic (NaH_2PO_4) sodium phosphate were purchased from Sigma and used to prepare 10 mM phosphate buffer solutions (PBS). Promazine hydrochloride (PZ, Sparine), chlorpromazine hydrochloride (CPZ, Thorazine), prochlorperazine dimaleate (PCP, Compazine), thioridazine hydrochloride (TR, Mellaril) and 2-trifluoromethylperazine dihydrochloride (TFMP, Stelazine) were obtained from RBI-Sigma (St. Louis, MO). 2-Thiomethylpromazine (TMPZ) and 2-trifluoromethylpromazine hydrochloride (TFMPZ, Vesprin) were a gift from the NIH-National Cancer Institute (Drug Synthesis & Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment). All drugs were used as received, except PCP. For this substance, the free base was employed for all measurements because the maleate counterion interferes with the photoprocesses of the molecule.[26] This free base was obtained by dissolving the drug in a basic aqueous solution and extracting with ether. All solvents were HPLC grade and were obtained from Fisher Scientific (Cayey, Puerto Rico).

Absorption and Emission Spectroscopy. Absorption spectra were taken with an HP 8453 UV−vis photodiode array spectrophotometer. Luminescence was measured with a Spex Fluorolog Tau 3.11 spectrofluorimeter (Spex Industries, NJ). To avoid concentration gradients due to photobleaching, all solutions were stirred before and during the absorption and emission measurements. The quantum yields of the fluorescence (ϕ_F) were measured relative to quinine sulfate in 1 N H_2SO_4 ($\phi_f = 0.55$),[27] according to the method of Demas and Crosby.[28] The excitation wavelength was set to 320−350 nm, reference and samples were optically matched ($A < 0.1$), and the monochromator slits were set to 2.5 nm. Corrections were made for differences in the instrument sensitivity as a function of wavelength and for differences in refractive indexes. The fluorescence lifetimes (τ_f) were measured by exciting the molecules with 350 nm light because at this wavelength the lamp has a relatively high intensity. This wavelength also corresponds to the red edge of the absorption spectra of the promazines n $\rightarrow \pi^*$ transition. These singlet lifetimes were measured using the frequency domain method implemented by the Spex. The Spex Fluorolog implements the lifetimes fitting algorithms of the Global Unlimited version 3.0 software for fluorescence/anisotropy decay.[29] The triplet energy (E_T) is obtained from the phosphorescence spectra taken at 77 K in ethanol. The TCA solution was poured in a quartz tube of 4 mm inside diameter and inserted into an optical Dewar flask containing liquid N_2 inside the phosphoroscope accessory of the SLM 4800 spectrofluorimeter. The SLM 4800 was recently upgraded to photon-counting detection by ISS (Urbana, IL).

Nanosecond Laser Flash Spectroscopy. Transient absorptions intermediates were generated by laser flash photolysis using the 355 nm harmonic output of a Nd:YAG Surelite II laser (Continuum, Santa Clara, CA), as previously described.[30] Transient species were monitored at right angles to the laser beam using a 300 W xenon arc lamp (Oriel Corp.). The probe beam was discriminated with an Acton Research monochromator (model 305) and detected with a Hamamatsu R928 five-stage dynode wired photomultiplier tube. Output from the photomultiplier was digitized with a 400 MHz bandwidth oscilloscope (model 9310, Lecroy Corp.). A flow-through system was used to ensure a fresh sample for each laser pulse. The whole spectrokinetic system was controlled with custom software developed under LabView 6.1 (National Instruments, Austin, TX). Kinetics decay analyses were carried out using Levenberg−Marquardt nonlinear fitting programs in LabView.

The triplet molar extinction coefficients of all compounds (ϵ_T) were determined using the energy-transfer method[31] with merocyanine 540 (MC540) as the energy acceptor. The TCA solutions were excited with 355 nm light in the absence or presence of 7.5×10^{-4} M MC540 because at this wavelength MC540 shows negligible absorption. The absorption changes produced by the drug triplet (OD_T^X) were measured at the drug triplet absorption maxima (~460 nm) in the absence of MC540. With MC540 present, the rate constants of the energy transfer were measured from either the decay of the drug triplet or the growth of the optical density of the MC540 triplet ($\lambda_{max} = 670$ nm, $\epsilon = 71\,000\ M^{-1}\ cm^{-1}$).[32] The maximum value of the

unknown triplet extinction (ϵ_T^X) is then calculated according to the following equation:

$$\epsilon_T^X = \epsilon_T^R \left(\frac{OD_T^X}{OD_T^R \text{ max}} \right) \quad (1)$$

where OD_T^i = maximum observed optical density of the drug or MC540 triplet at the corresponding λ_{max}.

The triplet quantum yields (ϕ_T) were determined with the comparative method as described by Bensasson et al.[33] using benzophenone (BP) in acetonitrile as the actinometer. The benzophenone triplet has a maximum at 515 nm with $\epsilon = 7460$ M^{-1} cm^{-1} [34] and $\phi_T = 1.0$.[35] If the same monophotonic laser-induced excitation is used for optically matched thin solutions of drug X (ϕ_T^X, unknown) and standard R = BP (ϕ_T^R, known), then

$$\phi_T^X = \phi_T^R \left(\frac{OD_T^X}{OD_T^R} \right) \left(\frac{\epsilon_T^R}{\epsilon_T^X} \right) \quad (2)$$

where, for species *i*, OD_T^i = maximum optical density of the triplet−triplet transition and ϵ_T^i = molar extinction coefficient of this transition. The triplet quantum yield is determined under conditions where the triplet optical density follows a linear relationship with the laser intensity, as previously described.[36] The triplet optical density for the reference and sample can be substituted by the slope of the linear relation between the OD and the laser intensity. In this case, we have found that the phenothiazines show a linear laser intensity dependence up to ~3 mJ. In summary, all triplet properties were measured following the procedures given in García et al.[14] because they take into consideration the laser power dependence.

PM3/CI Theoretical Calculations. The geometry optimization using a combination of molecular mechanics (MM+), molecular dynamics, and quantum dynamic calculations for closed shells (PM3/RHF/CI) and open shells (PM3/UHF) was performed with HyperChem 7.0 (HyperCube Inc., FL) and Spartan'02 (Wavefunction, Inc; CA). MM+ was used to preoptimize all closed-shell systems. To minimize the computation time and enforce Franck−Condon-type excitations, we used the optimized ground-state structure as the starting configuration for the first singlet excited state (S_1), which in turn was the starting structure for T_1. The optimized T_1 structure was finally used as the starting configuration for all other open-shell species. For the optimization of S_1, the PM3/RHF/half-electron method was applied. All optimizations for the ground state were performed at least three times using different starting structures. At the semiempirical level, the optimizations were done with the Polak−Ribiere conjugated gradient protocol (1×10^{-5} convergence limit, 0.01 kcal/Å·mol RMS limit). The lowest-energy conformations were found with the conformational search program in HyperChem and the conformer distribution option in Spartan. To ensure that the counterion remains at the standard distance in all protonated compounds, we restrained the $H^+ \cdots Cl^-$ separation to 1.32 Å with the default force constant (7 kcal/mol·Å^2). For systems with a perazine cycle and two counterions, the $H^+ \cdots X^-$ distance was restrained to the corresponding calculated value and a higher force constant (>15 kcal/mol·Å^2).

All molecular parameters were obtained with PM3/RHF/CI single-point calculations starting with the most stable PM3-optimized conformation and using three occupied and three virtual orbitals. The gas-phase ionization potential (IP_g) was taken either as the negative of the HOMO energy (Koopman's theorem) or calculated from the formation enthalpies of the molecule and the corresponding cation ($IP_{g[X]} = \Delta H_{f[X^+]} - \Delta H_{f[X]}$). The second method ("adiabatic" process) was used for further calculations of the ionization potential in solution. The spin density was obtained from the difference between the α- and β-electron populations.

Results and Discussion

Ground-State Conformations Determined by Quantum Theoretical Calculations. According to PM3 theoretical calculations, protonation of the alkylamino terminal is a thermally favored process. For PZ, for example, the difference in the formation enthalpy between the free base (ΔH_f = 54.3 kcal/mol) and the protonated derivative (ΔH_f = 25.6 kcal/mol, Table 1) is 28.7 kcal/mol. For all other compounds in Table 1, the protonation process decreases the enthalpy of formation by 25−35 kcal/mol while making the system more soluble in polar solvents ($\Delta\mu$ > 3 D). For TFMP, this difference is almost doubled (~55 kcal/mol) because it is protonated twice. Besides, 2-trifluoromethyl-substituted derivatives are thermally more stable than 2-thiomethyl- and 2-chloro-substituted derivatives by more than 150 kcal/mol. Table 1 shows only the two most stable conformations for molecules with the *N*,*N*-dimethylamino propyl chain, but they have at least nine low-energy conformations (differing by more than 0.3 kcal/mol). Derivatives with the perazine group, however, have no more than six or seven stable equivalent conformations. The smaller number of conformations can be explained in terms of the steric effect of the perazine group resulting in a lower number of degrees of freedom for rotation. Nevertheless, all conformations are thermally accessible, and the total energy difference is less than 5 kcal/mol.

The magnitude of the tricyclic ring folding in the 2-substituted phenothiazine parent compounds depends on the nature of the substituents and the physical state. The folding angle is measured between the two planes of the benzo rings in phenothiazine. For PH, Bell and co-workers measured an angle of 153.3° for its monoclinic crystal structure.[37] We calculated an angle of 135.2° in vacuum. In solution, the actual value for this folding should be in the range of 135−153°. The same behavior is observed for CPH and TFMPH (footnotes of Table 1). The 10-alkylation forces the phenothiazine system to bend further by 8−23°. For instance, both the crystallographic values and our theoretical calculations indicate that the dihedral angle of reference compound CPH and of CPZ differs by 7−12°. Our theoretical value for the bending of CPZ is in better agreement with the experimental folding value than the DFT 157.8° given by Phillip et al.[38] In general, the theoretical prediction of the torsion angle for alkylated promazines using the PM3/RHF/CI approach is impressively good. The difference found for the nonalkylated compounds is probably due to the stacking of the simple parent molecules in the crystallization process, which forces the molecules to flatten. This is why the less the steric hindrance of the substitution, the more accurate the theoretical values. This hypothesis is corroborated with the following series of simple 10-substituted phenothiazines (experimental values from ref 5): PH (153 exp., 135.2 theory), 10-methyl PH (143.7 exp., 128.2 theory), 10-ethyl PH (135.0 exp., 127.5 theory), and 10-isopropyl PH (146.8 exp., 142.6 theory).

The magnitude of the folding angle along the S−N axis depends, to a large extent, on the nature and position of the substituent. This bending and the high polarity of the protonated molecule do not allow promazines to intercalate into the DNA

TABLE 1: Ground- and Excited-State Properties of the Most Stable Conformations of the Phenothiazine Derivatives

drug	state	ΔH_f (kcal/mol) protonated	ΔH_f (kcal/mol) free base	ring torsion angle (degrees) PM3/RHF[a]	ring torsion angle (degrees) exptl[b]	chain torsion angle (degrees)[c]	μ (D)
PZ	S_0 (1)	25.6	54.3	136.5	140[d]	−61.3	5.0
	S_0 (2)	25.9		136.6		−61.2	5.0
	S_1	87.5	114.0	107.9		−13.9	5.3
	T_1	65.3	92.9	138.7		−9.5	6.6
	$PZ^{\bullet+}$	194.3	221.3	178.1	172.7[e]	−3.6	7.0
CPZ	S_0 (1)	19.0	47.7	138.9	137.8[f]	−60.7	4.3
	S_0 (2)	19.2		135.5		−59.5	4.3
	S_1	79.21	106.5	137.1		−3.5	4.2
	T_1	58.0	91.8	138.7		−8.6	5.6
	$CPZ^{\bullet+}$	189.1	227.4	149.4	169.9	−3.4	5.4
TFMPZ	S_0 (1)	−131.4	−103.6	139.6	136 or 145 [g]	−61.2	7.3
	S_0 (2)	−131.0		140.2		−65.7	6.6
	S_1	−73.4	−46.5	161.4		−8.9	2.9
	T_1	−92.1	−56.9	143.9		−33.8	7.5
	$TFMPZ^{\bullet+}$	43.8	70.4	179.9		−7.7	11.7
TMPZ	S_0 (1)	23.6	53.8	135.5	141.1	−63.0	6.5
	S_0 (2)	24.2		145.5		−58.5	6.5
	S_1	85.3	113.0	152.1		−0.7	7.0
	T_1	71.2	99.3	136.3		−5.1	7.0
	$TMPZ^{\bullet+}$	192.4	220.7	179.2		−0.6	7.2
PCP	S_0 (1)		50.2	137.7	136.1	−63.9	1.3
	S_0 (2)		52.2	137.6		−77.4	1.2
	S_1		109.0	159.7		−2.9	2.4
	T_1		88.0	139.8		−8.6	3.4
	$PCP^{\bullet+}$		218.9	174.0		−3.2	8.8
TFMP	S_0 (1)	−156.9	−101.0	138.6	141.1	−66.3	3.4
	S_0 (2)	−154.7		137.5		−56.6	3.7
	S_1	−98.9	−43.9	159.2		−4.6	4.6
	T_1	−111.0	−53.7	143.1		−33.8	4.6
	$TFMP^{\bullet+}$	20.5	73.3	149.3		−13.1	11.3
TR	S_0 (1)	14.9	44.4	134.7	134.4 or 145.8	−45.7	5.6
	S_0 (2)	15.1		126.0		−45.0	5.9
	S_1	75.2	103.5	120.1		−11.5	6.6
	T_1	60.1	90.1	138.3		−33.6	6.5
	$TR^{\bullet+}$	181.4	210.0	167.9		−16.7	6.0

[a] Measured for the **acde** torsion angle of the PM3/RHF optimized structure. (See Figure 1.) [b] Crystallography experimental values from ref 5 and references therein. [c] Measured for the **bcdf** torsion angle of the PM3/RHF optimized structure. (See Figure 1.) [d] Crystallographic and PM3/RHF values for the corresponding phenothiazine (PH) are 153 and 135.2, respectively. [e] Crystallographic and PM3/RHF values for the corresponding phenothiazine cation radical (PH) are 175.8 and 179.1, respectively. [f] Crystallographic and PM3/RHF values for the corresponding phenothiazine (CPH) are 153 and 135.1, respectively. [g] Crystallographic and PM3/RHF values for the corresponding phenothiazine (TFMPH) are 171 and 135.8, respectively.

bases under psychological conditions, as demonstrated by Kochevar and co-workers using fluorescence quenching measurements.[39] The different substituents considered in this work do affect this folding angle, but not to a great extent so as to explain the difference in the side effects of these compounds. Therefore, there should be no contribution from this parameter to the promazines' phototoxicity because these molecules do differ a lot in the potency of this side effect.

Singlet and Triplet Excited State Conformations Determined by Quantum Theoretical Calculations. On excitation, the S_1-transient species of the substituted promazines are predicted to flatten, in good agreement with previous experimental and theoretical results.[40] In general, the ring torsion angle for the ground-state molecules is not affected by the protonation state (angle difference is ∼5°), in contrast to that of the first excited singlet. For this state, the free-base molecule is predicted to be more planar than the protonated one by more than 20°. The S_1-protonated form has an angle of 140−160°, whereas the free base is practically planar (∼178°). The exception to this rule is TR, which has a smaller angle for both the protonated form and the free base (120.1 and 143.6°, respectively). This is obviously due to the steric effect of the alkylamino chain.

The triplet excited state has approximately the same torsion angle as the ground-state molecules, but interestingly, the cation radical of all promazines is almost planar. This is in excellent agreement with the ESR,[41] Raman,[38,40] and crystallographic experimental values (Table 1). Nevertheless, this should not make a great contribution to the extent of the intercalation because the cation radical formation quantum yields are low, even at high light intensity.[14] Besides, if the cation radical forms while the promazine is in its free base form, it is yet to be measured if its lifetime is of the same magnitude as the time required for intercalation. (See below.)

Absorption Spectra. In general, the experimental absorption spectra of neutral phenothiazines present two main absorption bands in the 250−265 and 300−320 nm wavelength ranges (Table 2 and Figure 2). The first band is attributed to a $\pi \rightarrow \pi^*$ transition, and the other belongs mainly to an $n \rightarrow \pi^*$ transition because of the presence of the sulfur lone-electron pairs in the phenothiazine ring.[42] Besides, a blue shift of the 300−320 nm band is observed in polar solvents, which further confirms the $n \rightarrow \pi^*$ nature of this band. The same behavior is observed for all derivatives in this study (Table 2). Quantum theoretical results indicate that these transitions strongly depend on the

TABLE 2: Absorption Properties and Quantum Theoretical Parameters of Phenothiazine and Its Derivatives

phenothiazine derivative	λ_{max} (nm) [$\epsilon \times 10^{-4}$ (M^{-1} cm^{-1})]			λ_{max} (nm) [f] PM3/RHF/CI
	acetonitrile	methanol	PBS (pH 7.4)	
PH	253 [4.98 ± 0.06], 318 [0.48 ± 0.01]	254 [5.1 ± 0.3], 318 [0.48 ± 0.01]	[a]	208 [0.08], 253 [0.32], 262 [1.08], 323 [0.11]
PZ	258 [3.40 ± 0.09], 305 [0.42 ± 0.01]	255 [3.3 ± 0.2], 307 [0.42 ± 0.03]	252 [2.04 ± 0.03], 302 [0.272 ± 0.001]	255 [0.23], 265 [0.88], 306 [0.05], 334 [0.15]
CPH	256 [7.5 ± 0.3], 323 [0.70 ± 0.01]	256 [7.4 ± 0.3], 323 [0.70 ± 0.02]	[a]	206 [0.46], 216 [0.16], 262 [1.12], 321 [0.23]
CPZ	258 [3.46 ± 0.02], 312 [0.44 ± 0.01]	258 [3.66 ± 0.01], 311 [0.46 ± 0.01]	255 [2.7 ± 0.1], 307 [0.35 ± 0.02]	217 [0.26], 263 [0.86], 312 [0.14], 331 [0.20]
TFMPH	258 [4.02 ± 0.07], 322 [0.41 ± 0.01]	259 [4.09 ± 0.02], 324 [0.43 ± 0.01]	[a]	210 [0.07], 238 [0.04], 255 [0.26], 268 [1.02], 333 [0.16]
TFMPZ	258 [3.27 ± 0.06], 310 [0.40 ± 0.01]	259 [3.31 ± 0.03], 310 [0.37 ± 0.01]	256 [2.45 ± 0.02], 306 [0.32 ± 0.01]	252 [0.12], 260 [0.66], 300 [0.09], 320 [0.23]
TMPZ	264 [3.86 ± 0.02], 315 [0.49 ± 0.01]	264 [3.32 ± 0.02], 314 [0.39 ± 0.01]	262 [3.44 ± 0.02], 312 [0.42 ± 0.03]	253 [0.15], 272 [0.59], 311 [0.18], 313 [0.16]
PCP	259 [3.67 ± 0.01], 314 [0.46 ± 0.01]	258 [3.48 ± 0.02], 313 [0.45 ± 0.03]	255 [2.99 ± 0.07], 309 [0.38 ± 0.001]	238 [0.06], 248 [0.71], 299 [0.16], 320 [0.22]
TFMP	259 [3.31 ± 0.02], 310 [0.41 ± 0.01]	260 [3.8 ± 0.3], 310 [0.46 ± 0.01]	257 [2.97 ± 0.02], 308 [0.37 ± 0.01]	251 [0.15], 261 [0.60], 296 [0.09], 323 [0.22]
TR	264 [3.36 ± 0.04], 316 [0.44 ± 0.01]	263 [3.99 ± 0.01], 314 [0.54 ± 0.01]	263 [3.11 ± 0.03], 312 [0.43 ± 0.02]	254 [0.39], 277 [0.62], 314 [0.28], 326 [0.20]

[a] Insoluble.

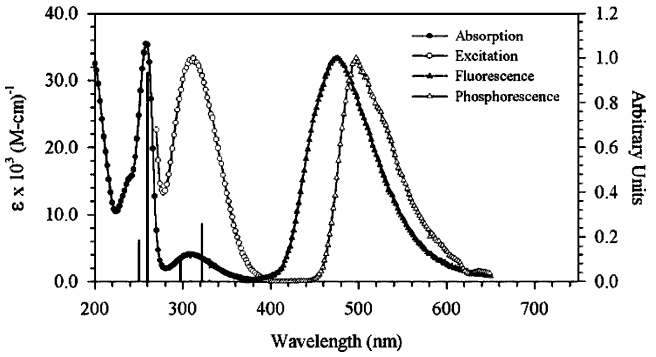


Figure 2. Absorption and emission spectra of trifluoromethyperazine (TFMP, 0.5 mM) in acetonitrile. The absorption spectrum shows the vertical theoretical oscillator values (Table 2). The excitation spectrum was measured at 470 nm. The fluorescence emission spectrum was obtained with $\lambda_{exc} = 350$ nm. The phosphorescence spectrum was obtained in ethanol glass ($\lambda_{exc} = 310$ nm).

substituent at the phenothiazine 2-position. Figure 3 shows the relative energy of the involved molecular orbitals for TFMP and TR. It shows that, for TR, only phenothiazine unoccupied MOs are involved in the UV transitions of the molecules, whereas an MO of the CF_3 group in TFMP participates in the n → π^* transition (296 nm). This explains the similarity in the absorption properties of promazines and perazines listed in Table 2 and the effect of the 2-substitution on the n → π^* band. All derivatives with an SCH_3 group have HOMO orbitals with higher energies than those with chlorine or CF_3 (i.e., these systems have lower ionization potentials; see below). Besides, SCH_3 systems also have a higher number of electronic transitions than the other derivatives. This is due to the contribution of the 2-sulfur n-electrons to the frontier molecular orbitals (i.e. all occupied molecular orbital of the SCH_3 derivatives have contributions from this substituent). These thiomethyl derivatives also have larger oscillator strengths for both bands, whereas no effect is observed for the chain substituents, which is due once again to the coupling of the transitions to the SCH_3 group.

These results clearly demonstrate that, in general, the 2-substituent affects the absorption and thermodynamic properties of all phenothiazine species to a greater extent than the nitrogen substitution at the 10-dimethylaminopropyl group. This is further support by the values published by Elisei and co-workers for perphenazine (PP) and fluphenazine hydrochloride (FP).[23] These two promazine derivatives are similar to PCP and TFMP, respectively, except that they have an extra *para*-OH group at the perazine end. Because this substituent does not affect the chromophore of the drugs, the absorption properties they measured for both derivatives correspond within the experimental errors to the values of PCP and TFMP given in Table 2. The same substituent-property correlation had been previously observed between the corresponding neuroactivity and phototoxicity.[14]

Excited Singlet State and Fluorescence Spectra. The emission spectra of all 10-alkylated phenothiazines consist of a broad band with a maximum between 440 and 470 nm in all solvents used in this work (Table 3 and Figure 2). These derivatives also present a Stokes shift larger than 10^4 cm^{-1}, which is a considerable magnitude. The emission maxima are more solvent-dependent than the corresponding absorption maximum. (See previous section.) For 2-CF_3 derivatives, for instance, the emission maximum is red shifted by ~25 nm relative to the others and appears around 470 nm. The maximum of 2-SCH_3 analogues is between 450 and 455 nm. In all systems, the emission is further shifted toward the red with increasing solvent polarity. This behavior is opposite to the effects observed for the absorption spectra and is, once again, characteristic of the π^* nature of the S_1 state. This red shift is obviously explained in terms of the involved molecular orbitals, which are located on the phenothiazine moiety. Therefore, there is absolutely no contribution of the *n*-alkyl chain to the emission properties of the phenothiazines. This is supported by the fact that the emission maxima of the phenothiazines are exactly the same as those of the corresponding 10-alkylated derivative:[43] PH (442 nm), CPH (450 nm), and TFMPH (471 nm). The

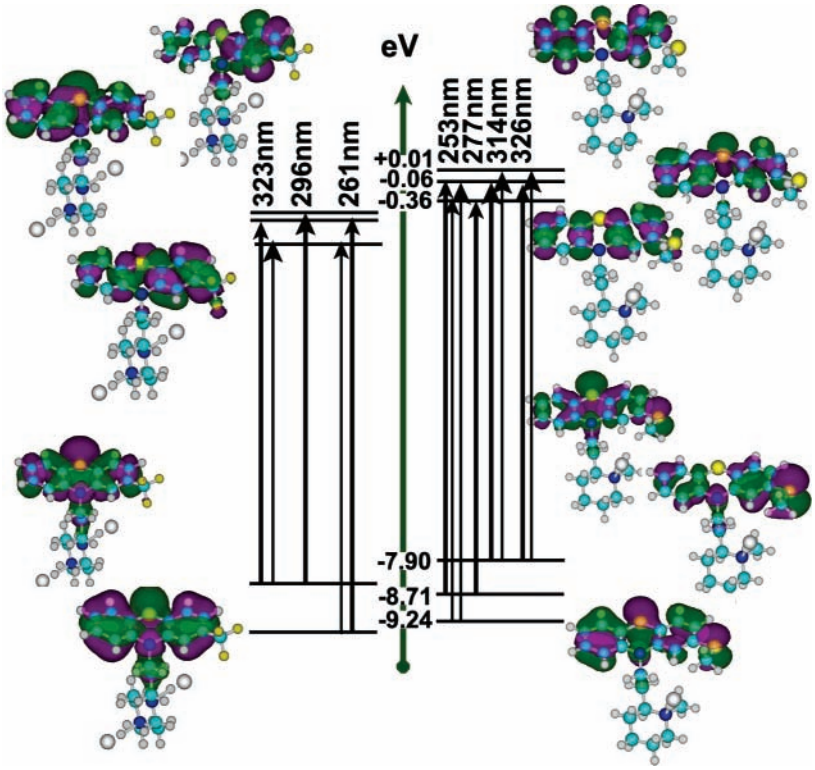


Figure 3. Molecular orbital transitions of trifluoromethylperazine dihydrochloride (TFMP, left) and thioridazine hydrochloride (TR, right).

TABLE 3: Emission Properties of the Phenothiazine Derivatives

phenothiazine derivative	λ^{max} (nm), Stokes shift (cm^{-1}), $\phi_f \times 10^{-3}$ and τ_f (ns)				
	acetonitrile			PBS (pH 7.4)	
	this work	ref 23	methanol	this work	ref 23
PZ	444		444	452	
	8924		10 050	10 449	
	20.0		4.5	14.3	
	1.85		1.75[b]	2.04[b]	
CPZ	451		449	453[a]	
	9372		9474	9772	
	0.89		0.95	3.6	
	0.92		0.89[b]	0.35[b]	
TFMPZ	470		463	475	
	10 982		10 659	11 627	
	1.1		1.0	1.4	
	2.66		2.47	3.17	
TMPZ	460		455	475	
	10 007		9869	11 102	
	3.0		3.0	3.0	
	1.16		1.15	1.17	
PCP	449	458[c]	449	457[a]	467[c]
	8688		9175	9174	
	8.4	1.00	46.6	3.3	7.0
	0.91	0.81	0.89		
TFMP	470	475[d]	467	482	470[d]
	10 981		10 844	11 720	
	1.0	20	1.0	1.4	20
	2.68	2.7	2.52	3.01	3.3
TR	455	460	453	466	475
	9668		9772	10 592	
	5.0	7.0	4.1	6.0	11.0
	1.03	1.1	0.96	1.47	1.7

[a] Measured in PBS (pH 6.9). [b] Values from Garcia et al.[14] [c] Corresponding values for perphenazine. [d] Corresponding values for fluphenazine hydrochloride.

phenothiazines, in general, have transitions with charge-transfer character (i.e., the absorption at wavelengths greater than 270 nm is mainly due to n $\rightarrow \pi^*$ transitions originating from the electronic density of the middle-ring sulfur and the 2-SCH_3, in the case of TMPZ and TR; see Figure 3). In the excited state, the electron density is transferred from the heteroatoms to the benzene rings, which have greater π character. This charge transfer is correlated with a decrease in the torsion angle, which induces an increase in the dipole moment of the excited state relative to that of the ground state (Table 1). With the increase in the dipole moment on excitation, polar solvents stabilize the S_1 state and decrease its energy relative to that of the ground state in the same solvent. This energy decrease or solvatochromic effect is manifested in the relatively high Stokes shift.

As previously mentioned, the substituents considered in this work do not change the ring torsion angle in the ground state to a great extent (Table 2), but for the first excited singlet these torsion angles are distributed from 107.9 to 161.4°. This parameter is, once again, dependent on the 2-substituent. Photoionization studies indicate that an increase in the dihedral angle gives better π-orbital overlap between the ring carbons and nitrogen atoms as well as the ring carbons and sulfur atoms.[44] The resulting planarity coincides with the nitrogen nonbonding electrons' participation in the benzene π system. Therefore, the mentioned charge separation can definitely occur in the excited state for the most planar derivatives, such as TFMPZ.

Fluorescence Quantum Yield (ϕ_f). The fluorescence quantum yields are given in Table 3, including values reported for similar promazines.[23] Compared to the parent promazine, chlorinated derivatives have small quantum yields, especially in methanol. For the 2-CF_3 phenothiazines, the ϕ_f is practically constant in all three solvents. The same behavior was observed for fluphenazine hydrochloride,[23] although it is not clear why this compound has a fluorescence quantum yield 20 times larger than that of the similar TFMP. The 2-SCH_3 analogues show ϕ_f values 3 to 6 times larger than those of the corresponding CF_3 substituted analogues, but they are still lower than the value for promazine. All promazines have ϕ_f values on the order of

$10^{-2}-10^{-3}$. Therefore, other deactivation mechanisms for the S_1 state must be competing favorably with the fluorescence process for these molecules. The 10-alkylamino chain was found to be very flexible in solution, giving several stable conformations to all molecules. Because all of them are thermally accessible, the interconversion between conformations should diminish the values of the quantum yield. Besides, the phenothiazine ring flattens on excitation, and this also can affect the S_1 state. The fluorescence quantum yields for the nonalkylated phenothiazines are not available for comparison, but no effect is observed between the side chain of the promazines and the perazines. In both derivatives, the alkyl chains are the same size and have the same effects on the stability of the conformations. In terms of the 2-substitution, however, the methylthio group (SCH_3) is responsible for the increase in ϕ_f relative to the values for the CF_3 and Cl groups. The higher ϕ_f for SCH_3 can be explained in terms of a higher planarity of these derivatives and the corresponding better orbital interaction of the substituent with the tricyclic ring. All of this information leads to the conclusion that fluorescence is still not the major energy-dissipation pathway for 10-alkylated phenothiazines because there is only a 0.1 to 0.6% energy dissipation attributed to this process. This low yield is expected to correspond to a high efficiency in the thermal deactivation due to the alkyl chain (internal conversion) and/or the intersystem crossing (ISC). Nanosecond laser flash photolysis experiments were performed to determine if these drugs have, in fact, high triplet quantum yields (see below) or the energy of the S_1 excited state is otherwise dissipated.

In summary, the emission properties of promazines are also very sensitive to the solvent and the 2-substituent but not to the alkylamino chain. The data of ref 23 strongly supports this finding. Although we found smaller values for the quantum yields, all other parameters match within the experimental error.

Fluorescence Lifetime (τ_f). All obtained fluorescence decay curves were best described by a monoexponential decay function. This photophysical parameter shows the same substituent and solvent dependency observed for the quantum yield. The 10-alkyl chain has no effect on the lifetime values. This is seen in the fact that, within the experimental error, all phenothiazines have the same fluorescence lifetime as the corresponding promazine:[43] PH ($\tau_f \leq 1$ ns), CPH ($\tau_f \leq 1$ ns), and TFMPH ($\tau_f = 2.5$ ns). Moreover, our τ_f values are exactly the same values of the corresponding analogues reported by Elisei and co-workers.[23] The fluorescence lifetimes for the 2-CF_3 derivatives are similar and follow the same trend in different solvents. A τ_f value of 2.4−2.7 ns is obtained for both trifluoromethyl derivatives in acetonitrile and methanol, whereas a value of ∼3.1 ns is observed in PBS. This small increase in the fluorescence lifetime in polar solvents such as water is, once again, indicative of the solvatochromic effect of the polar S_1 state. The observed lifetimes are within the range of values reported for promazine in PBS ($\tau_f = 1.75-2.04$ ns).[45] The τ_f values of the 2-chloro derivatives, CPZ and PCP, however, show a larger difference between PBS and methanol, with values of 350 and 890 ps, respectively.[14] The main problem for PCP (and perphenazine[23]) in PBS is the solubility, which precludes the measurement of τ_f. The small lifetime values (<1.0 ns) of these chlorinated derivatives are due to the presence of the chlorine atom, which can enhance the spin−orbit coupling in the $S_1 \rightarrow S_0$ nonradiative or ISC processes.[46] The fluorescence lifetime of the 2-SCH_3-substituted phenothiazines are ∼50% lower than the values of the corresponding CF_3 derivatives and promazine. This can also be explained by the presence of a sulfur atom at the 2-position. Analogous to the chlorine atom effect in CPZ, this atom can enhance spin−orbit coupling through a heavy-atom effect. Further experiments showed that, in fact, the ISC is one of the main decay pathways (vide infra).

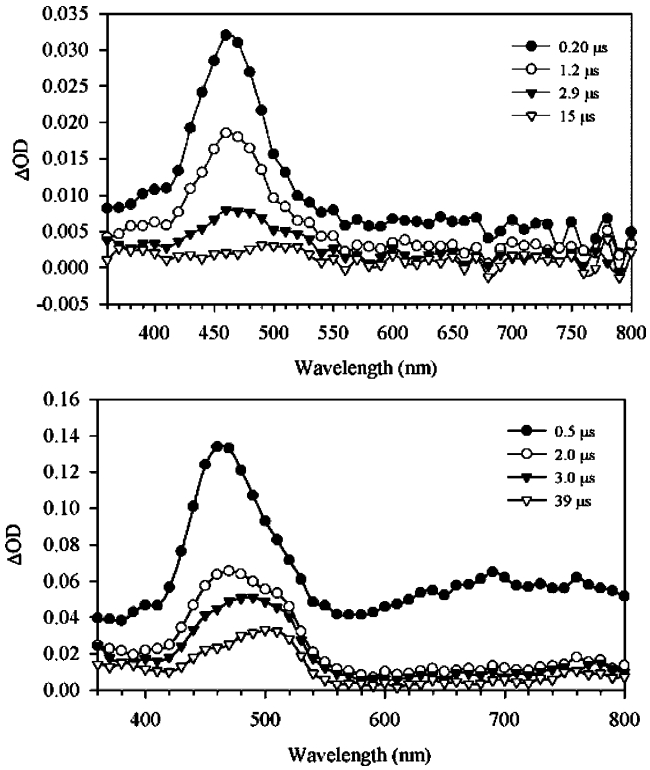


Figure 4. Transient absorption spectra of the 355 nm laser flash photolysis of TFMPZ in nitrogen-saturated PBS 7.4 at low pulse energy ($E < 3$ mJ, top) and at high pulse energy ($E > 10$ mJ, bottom).

Laser Flash Photolysis. In general, the transient absorption spectrum of nitrogen-saturated solutions of 10-alkylated phenothiazines at high laser intensities consists of an intense band with maxima between 460 and 480 nm and near 530 nm and another very broad one extending into the red region of the spectrum. For TFMPZ, for example, transient absorption is observed with maxima at 470 nm, 520 nm, and a band extending from 630 to 800 nm (Figure 4). The 470 nm band disappears under aerobic conditions and therefore is assigned to the triplet state (Table 4). In fact, this species is quenched by triplet-state quenchers oxygen ($k > 10^9$ M^{-1} s^{-1}) and 1,3-cyclohexadiene $\{k \approx (3-7) \times 10^9\}$.[43,47] Similar behavior was observed for the other drugs in both acetonitrile and methanol ($\lambda_{max} = 470 \pm 10$ nm). Furthermore, it was observed that the triplet-state decay rate constants increase with ground-state concentration, indicating a self-quenching process as previously reported by Barra et al.[43] From a plot of k_{obs} versus ground-state concentration (data not shown), the self-quenching rate constants were obtained for several derivatives in all solvents. For acetonitrile, methanol, and PBS, we report the following results: TFMPZ 4.0×10^7, 4.5×10^7, and 2.4×10^8 M^{-1} s^{-1}, respectively; TFMP 1.7×10^8, 6.3×10^7, and 2.7×10^8 M^{-1} s^{-1}, respectively. The values for the rest of the derivatives are also on the order of 10^7-10^8 M^{-1} s^{-1}, in excellent agreement with those previously reported for the nonsubstituted phenothiazines.[43] The highest values are observed in PBS for all drugs. Promazine derivatives are known to exhibit surfactant behavior because the heterocyclic rings impart hydrophobic character and the protonated aminopropyl group is polar. This is evident in the large dipolar moments given in Table 1. Thus, the high self-quenching rate constant observed in PBS must be due to the high hydrophobic

TABLE 4: Properties of the Triplet State of the Phenothiazine Derivatives

phenothiazine derivative	λ^{max} (nm), $\epsilon_T \times 10^{-4}$ (M^{-1} cm^{-1}), ϕ_T, τ_T (μs)				
	acetonitrile			PBS (pH 7.4)	
	this work	ref 23	methanol	this work	ref 23
PZ[a]	460		460	460	
	4.33[b]		2.65		
	0.57		0.41	0.58	
	62[b]		61	61	
CPZ[a]	460		460	460	
	6[b,c]		1.95		
	0.73		0.90		
	0.91[b]		2.2[b]		
TFMPZ	470		460	460	
	2.84		2.2	2.52	
	0.68		0.67	0.39	
	49		91[d]	3.4	
TMPZ	470		470	475	
			1.3		
	0.84		0.89	0.90	
			59	33	
PCP	470	480[e]	470		490[f]
	4.2		3.5		
	0.40	0.58	0.35		
	0.80	0.66	55.0		2.9
TFMP	470	450[g]	470	470	480[g]
	3.23	1.15	2.38	2.8	3.3
	0.39	1.0	0.50	0.22	
	66	0.95	111	2.13	
TR	470	470	470	480	480
	1.8	1.1	1.54	1.27	3.3
	0.70	1.0	0.74	0.67	
	100	4.2	143	35[h]	

[a] Values from Garcia et. al.,[14] unless otherwise indicated. [b] Values from this work. [c] The required absorption of MC540 was 0.1, which introduces an error of ~30%. [d] 63 ns for an air-saturated solution. [e] Corresponding values for perphenazine. [f] Corresponding values for perphenazine in water. [g] Corresponding values for fluphenazine hydrochloride. [h] 650 ns for an air-saturated solution.

interactions between the promazine molecules. At high drug concentrations, molecules in the excited triplet state are in close contact with molecules in the ground state, resulting in the triplet excited state deactivation. However, the triplet ground state quenching mechanism should not be relevant for the in vivo studies because the drug concentration used and the corresponding triplet concentration formed are normally smaller than the apparent critical concentration required for self-quenching (0.1−0.8 mM).

Triplet-State Extinction Coefficient (ϵ_T), Quantum Yield (ϕ_T), and Lifetime (τ_T). The triplet-state molar absorption coefficients were determined using merocyanine-540 (MC540) as the acceptor in deareated methanol and acetonitrile. Because MC540 is not very soluble in PBS and it forms some type of complex with PZ and CPZ, it was assumed that ϵ_T has the same value for this solvent as in methanol. Figure 5 shows, for example, the transient absorption profiles detected for the 355 nm excitation of TFMP in the absence or presence of 0.35 mM MC-540. From the respective maximum triplet absorbances, values on the order of $(1.5-6) \times 10^4$ M^{-1} cm^{-1} were calculated using eq 1. These values are within the range of those reported for CPZ (19 500 $M^{-1}cm^{-1}$) and PZ (26 500 $M^{-1}cm^{-1}$) in methanol[14] and those reported for similar compounds.[23] The bimolecular quenching constant of MC-540 and the promazines triplet state is close to 6.5×10^9 M^{-1} s^{-1} under our experimental conditions, a third of the diffusion limit rate constant in all cases, resulting in a high probability of energy transfer.

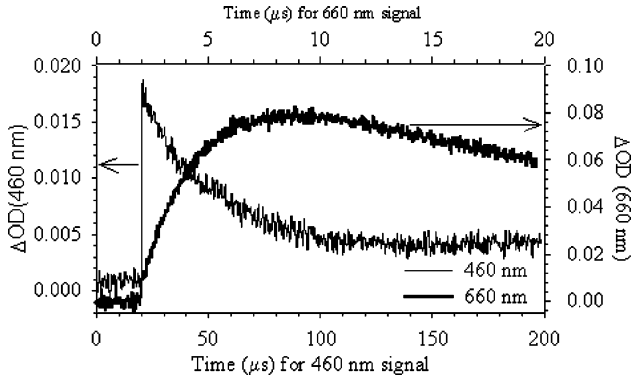


Figure 5. Laser-induced (355 nm) transient absorption decay of TFMP in acetonitrile under nitrogen at 460 nm (TFMP, 0.25 mM) and 660 nm (TFMP, 0.20 mM in the presence of MC-540, 0.35 mM).

The intersystem crossing quantum yields were determined using benzophenone, and the lowest practical laser pulse energies were employed to minimize nonlinear effects (e.g., photoionization). From the linear dependence of the benzophenone (BP) triplet−triplet absorption, used as an actinometer, and of promazine as a function of the laser energy at the excitation wavelength of 355 nm (data not shown), the ϕ_T values were calculated using eq 2 and are given in Table 4. These values are in the range of 0.2−0.9 and show some solvent dependence. For the chlorinated derivatives, for instance, the values are not given in PBS because their triplet states are rapidly quenched in aqueous solution. This behavior had been reported by Garcia et al.[14] and explains why Elisei and colleagues could not determine the triplet quantum yield of perphenazine in water.[23] As a matter of fact, besides this special case of chlorinated promazines, smaller quantum yields are observed for TFMPZ and TFMP in PBS, which are also halogenated at the phenothiazine 2-position. All triplets form in very good yields in methanol, especially for those derivatives having a methylthio substituent ($\phi_T > 0.7$). The CPZ triplet also forms with an impressive quantum yield of 0.90 in methanol, whereas PCP has a value of only 0.35. This large difference must be due to the bulky perazine group in PCP because the same relation is observed for the following pairs: TFMPZ (0.68)/TFMP (0.39) and TMPZ (0.89)/TR (0.74). With the limitations of the small data set that we have screened, it can be concluded that perazines have smaller triplet quantum yields than the corresponding promazines. This general statement about the N-side chain substitution on the triplet quantum yield is supported by the fact that for phenothiazine $\phi_T = 0.96$.[43] This indicates, once again, that the chain may induce the deactivation of the singlet state, thus diminishing the intersystem crossing efficiency.

The triplet lifetimes is a very sensitive property. Curiously, the 10-substitution has the least effect, noted by the fact that there is not a large variation in the values of τ_T for the phenothiazines, promazines, and perazines in a given solvent. For the chlorinated derivatives, for example, the values in acetonitrile (μs) are CPH/CPZ/PCP/PP = 0.80:0.91:0.80:0.66. The small values reported by Elisei et al.[23] for FP (0.95 vs 66 μs for TFMP) and TR (4.2 vs 100 μs measured in this work) in acetonitrile might be due to triplet−triplet annihilation processes induced by high laser intensities and/or high ground-state concentrations. The 2-substitutions induce a larger variation in the triplet lifetime values, as noted for the promazines in methanol (μs): PZ/CPZ/TFMPZ/TMPZ = 61:2.2:91:59. The same trend is observed for the perazines (μs): PCP/TFMP/TR = 55:111:143. The largest effect on τ_T, though, is induced by

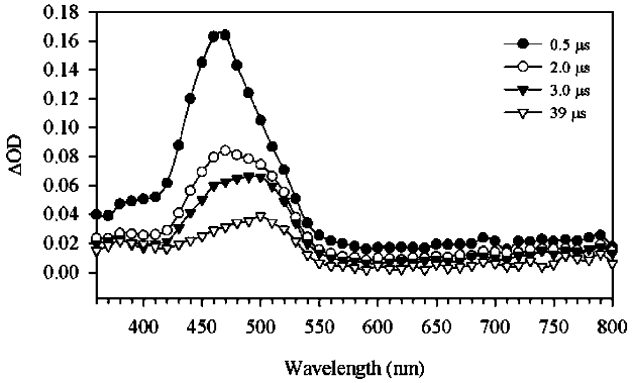


Figure 6. Transient absorption spectra following the high pulse energy (E > 10 mJ) 355 nm laser flash photolysis of TFMPZ (0.5 mM) in an N_2O-saturated PBS 7.4 solution containing 0.10 M *tert*-butyl alcohol.

the solvent. Except for PZ, the lifetime values are drastically reduced when switching from acetonitrile or methanol to water. For TFMP, for example, it decreases from 111 μs in methanol to only 2.13 μs in PBS. This effect is even more drastic when combined with the previous 2-substituent one. For the chlorinated derivatives, it is not even possible to see the triplets in water, as mentioned before. Considering all of these findings, we consider that a very fast proton-transfer mechanism is responsible for this triplet state quenching, as we had previously proposed.[14]

Cation Radical. For laser pulse energies higher than 10 mJ, a more complex transient spectrum is commonly observed for all derivatives. (See Figure 4.) Moreover, in nitrogen-saturated acetonitrile solutions, the triplet lifetimes change with the laser intensity. The lifetime for TFMP, for example, is 49 μs for E = 5 mJ and only 8 μs for E = 20 mJ (not corrected for self-quenching). This decrease in the lifetime value is mainly due to triplet−triplet annihilation reactions, as indicated in the previous section. In addition to the triplet absorption at 470 nm, an intense residual absorption at 520 nm and a broad absorption extending to the red are also observed in PBS. The broad absorption is due to the solvated electron, identified by its characteristic spectrum, and the fact that it is not observed in the presence of electron scavengers such as N_2O. In the presence of N_2O and *tert*-butyl alcohol, the only species that are not quenched are the triplet state and the radical cation. The excitation of aerated solutions results in the quenching of both the triplet state and the solvated electron ($k_q = 5.9 \times 10^9$ M^{-1} s^{-1}).[48] Nevertheless, the absorption of a stable intermediate is still observed under air-saturated conditions at 520 nm. By comparing these spectra with those observed in solutions containing N_2O and *tert*-butyl alcohol (Figure 6), this intermediate was assigned to the radical cation, as previously characterized for PZ and CPZ in one-electron oxidation experiments using pulse radiolysis techniques.[14]

In acetonitrile under high pulse energies, the only bands observed correspond to the triplet and the radical cation absorptions. The electron rapidly reacts with the solvent to form the dimer anion $(CH_3CN)_2^-$, which does not absorb in the wavelength range of λ > 350 nm.[49] Electron photoejection photonicity studies were performed for TFMPZ and TFMP in aqueous solution by measuring the initial solvated electron absorbance at 720 nm as a function of laser intensity (data not shown). A quadratic dependence between ΔOD and the laser energy was found for both molecules, which corresponds to biphotonic processes. García et al. previously reported the photoejection mechanism for PZ and CPZ.[14] They showed that the excited triplet is not the intermediate state responsible for the electron photoejection in the nanosecond laser flash photolysis. Using picosecond laser absorption spectroscopy, these authors also demonstrated that the singlet excited state absorbs the second photon producing the solvated electron, in agreement with several previous reports.[21,22] In a similar way, we postulate that a two-photon mechanism should apply for the photoionization of TFMPZ and TFMP. Evidence for this postulate is the fact that under air-equilibrated conditions the transient absorption spectra for both molecules show maxima at 460 nm (T−T band) 100 ns after the laser pulse and at 520 nm. It is known that under air-saturated conditions the triplet state is expected to be quenched within the laser pulse because of the diffusional rate constant for the reaction between this state and oxygen. Therefore, a decrease in the T−T signal is observed along with a corresponding decrease in the lifetime. For TFMPZ, for example, the triplet lifetime decreases from 91μs in N_2-saturated methanol to only 63 ns in air-saturated solution (Table 4). Nevertheless, the cation radical signal at 520 nm does not decrease in the same proportion. Therefore, under those conditions where the triplet is being quenched, the second photon must be absorbed by the singlet excited state, although a recent report still postulate a monophotonic process.[23]

TABLE 5: Ionization Potentials of the Phenothiazine Derivatives in Water (ϵ = 78.5 D), Methanol (ϵ = 32.6 D), and Acetonitrile (ϵ = 37.5 D)

molecule	vol. (Å^3)	IP_{gas} (eV)			IP_+ (eV)			$IP_{solution}$ (eV)[d]		
		a	*b*	*c*	H_2O	CH_3OH	CH_3CN	H_2O	CH_3OH	CH_3CN
PH	589	8.0	7.4	7.3	1.37	1.34	1.35	4.73	5.06	4.55
PZ	938.2	8.1	7.3	7.2	1.17	1.15	1.15	4.83	5.15	4.65
CPH	631.7	8.0	7.5		1.34	1.31	1.32	4.86	5.19	4.68
CPZ	979.9	8.2	7.4	7.2	1.15	1.13	1.14	4.95	5.27	4.76
TFMPH	667.3	8.3	7.7		1.31	1.29	1.29	5.09	5.41	4.91
TFMPZ	1024	8.5	7.6	7.3	1.14	1.12	1.12	5.16	5.48	4.98
TFMP	1195	8.6	6.8	7.3	1.08	1.06	1.06	4.42	4.74	4.24
PCP	1066	8.2	7.3	7.3	1.12	1.10	1.11	4.88	5.20	4.69
TMPZ	1051	8.1	6.8		1.13	1.11	1.11	4.37	4.69	4.19
TR	1092	7.9	7.2	7.0	1.11	1.09	1.10	4.79	5.11	4.60

[a] Obtained from Koopman's theorem. [b] Calculated with the formation enthalpy of the molecule and the corresponding cation. [c] Photoelectron spectra experimental values from ref 5. [d] Calculated with the "adiabatic" gas ionization potential.

From a thermodynamic point of view, the absorption of a single 355 nm photon does not provide enough energy to induce the photoionization of these molecules. Their ionization potentials in solution ($IP_{solution}$) were calculated using the following equation,[50] and the results are given in Table 5.

$$IP_{solution} = I_g + P_+ + V_0 \qquad (3)$$

In this equation, IP_g is the ionization potential of the molecule in the gas phase, P_+ is the adiabatic electronic polarization energy of the medium by the positive ion, and V_0 is the ground-state energy of the "quasi-free" electron in the liquid relative to the electron in vacuum. The IP_g values were determined from the difference in formation enthalpies, as described in the Experimental Section. The value of P_+ was obtained using Born's equation: $P_+ = -e'(\epsilon - 1)/2r^+\epsilon$, where $e' = e^2/4\pi\epsilon_0$, ϵ is the solvent optical dielectric constant, and r^+ is the cation radius (calculated from the molecular volume, which is obtained using the grid method implemented in Hyperchem). V_0 has the following values: −1.5 eV for acetonitrile,[51,52] −1.3 eV for water,[53] and −1.0 eV for methanol. All other calculated values are summarized in Table 5, including several experimental ionization potentials for the gas phase.[5,44] The excellent agreement between these values and our calculated adiabatic IPs is used to validate the correctness of the method. Using this

approach, we estimated $IP_{solution}$ values larger than 4.2 eV for all drugs in all solvents. Because this $IP_{solution}$ value is larger than the energy provided by a 355 nm laser photon (3.49 eV), the absorption of two photons (6.98 eV) is required to exceed the ionization threshold. Moreover, under in vivo conditions the contribution of the monophotonic processes should be very small, as indicated by the transient distribution for low-energy irradiations.

The experimental IP values given in Table 5 are the values obtained from the photoelectron spectra reported by Domelsmith et al.[44] They found that the first ionization potential is N-centered and the second ionization potential is S-centered for all of the compounds. Only small variations in both the experimental and theoretical ionization potentials are found among the compounds studied in this work. Nevertheless, phenothiazines should be easier to photoionize than promazines (by ∼0.1 eV), the 2-Cl substitution has almost no effect on this parameter, and the 2-CF_3 induces the largest increase in the IPs (∼ 0.3 eV). In terms of the solvent dependence, all compounds should be easier to oxidize in acetonitrile and then in water. All factors considered, these small differences in the ionization potential of promazine derivatives cannot account for the differences in their phototoxicity. This suggests that there is no direct relationship between the ionization potential and the neuroleptic activity for these compounds. This is in complete agreement with the exclusion of the cation radical in the phototoxicity of CPZ.[22,54]

Conclusions

Phenothiazine compounds possess many pharmacological activities that depend on the nature and position of the substituent. CPZ, TFMPZ, and triethylperazine are antipsychotic agents;[55] promethazine is an antiallergic agent;[7] perphenazine (PP) is a potent tranquilizer; and other similar derivatives have antiparkisonian activities.[56] In this regard, the effects of the 2-substituent and the 10-alkylamino chain on the antipsychotic potency of promazines have been carefully investigated, and it was concluded that changes in these two factors cause variations in the potency covering at least 3 orders of magnitude.[57,58] The 2-susbtitution has also been found to affect the distribution of the promazine drugs in vivo by altering the number of possible conformations in the molecule and/or affecting the dopamine-type receptor site.[4]

The mechanism for the phototoxicity of the promazine drugs is completely unknown, although several proposals have been made to account for the exaggerated phototoxicity of CPZ.[59,60] Kochevar et al.[8] attributed the response to the formation of dimers and higher multimers of the drug produced by preirradiation of CPZ. Other mechanisms consider that the biochemical damage is produced by the free radicals[61,62] or the photoaddition of CPZ to ds-DNA.[18] On the basis of the observation that the photobinding of CPZ in vivo can be induced even with long-wave UVA light, Ljunggren and Möller[19] suggested that these adverse photobiological effects can be caused by CPZ metabolites. Therefore, attention was once focused on chlorpromazine sulfoxide, the major metabolite of CPZ.[20] The ground-state CPZ−DNA complexes had also been considered to participate in the phototoxicity mechanism. The involvement of this covalent binding of CPZ to DNA in the phototoxic side effect has been questioned many times because there is no complex formation under physiological conditions. Moreover, in a fluorescence quenching study, Kochevar et al.[39] showed that ground-state complex formation at acidic pH values does not promote the photoaddition of CPZ to DNA. However, dimers and polymers of CPZ cannot form in a concentration high enough to be toxic because the in vivo therapeutic concentration of CPZ is only 0.03−3.0 μM.[63] Rosenthal et al.[13] found no toxic effect on *E. coli* attributed to CPZ sensitization, and Schoonderwoerd et al.[45] ruled out the metabolic product of CPZ to be responsible for the drug phototoxicity. They based their conclusions on the bioavailability of CPZ and its oxidation product (CPZSO) in the skin and on the absorption spectrum in the spectral region of the UVA lamps used for the experiments. They also concluded that the sulfoxidation of CPZ results in less photobinding. In vitro studies indicate that the cation radical of CPZ inhibits enzyme systems,[17] interferes with membrane functions,[16] binds to DNA and proteins,[15] and induces DNA damage.[64] Nevertheless, the involvement of $CPZ^{\bullet+}$ in the in vivo photosensitization of CPZ has been questioned[21,22] because the intensity of radiation needed for the two-photon formation of $CPZ^{\bullet+}$ is not available under normal conditions. In complete agreement with all these data, we found that the 2-substituent and the nature of the 10-alkylamino chain do not affect the photophysical properties of the molecular ground state, the first excited state, and its cation radical. Although there does not appear to be a direct correlation between the dihedral angle and the neuroleptic potency, a nonplanar shape seems to be necessary for neuroleptic activity. Moreover, Keiser et al. postulated that a nonplanar conformation of the side chain is a prerequisite for antidepressant activity.[3] A nonplanar conformation of the phenothiazine was found only for the first excited singlet state and the cation radical. Both of them have been ruled out of participating in the phototoxicity of the promazines because the first one has a very short lifetime and the other requires very high light intensity to form. Besides, the small variation in the first ionization potentials of the promazines does not correlate with the large differences in their phototoxicity. As a matter of fact, the IP values do not correlate with their antipsychotic activity either.[44]

The S_1 state of most promazines converts very efficiently to the triplet state, which in turn has been proposed to be the precursor in the biphotonic formation of the cation radical.[21] Using a two-laser/two-wavelength setup, it was demonstrated that, in fact, the cation radical is not formed from the triplet state but from the singlet.[14] All of these mechanisms consider the triplet state only as a short-lived intermediate in the formation of whatever is producing the undesirable side effects of the drugs. However, the "more direct" participation of the triplet state in this process has not been considered. Several reports confirmed that the triplet state, the cation radical, and the solvated electron are the most important transients formed upon irradiation of the drugs. The relative yields of $CPZ^{\bullet+}$ and $^3CPZ^*$ do not depend on the nature of the solvent, as proposed by Navaratnam et al.,[65−66] but on the pH of the solution. The triplet lifetime of halogenated promazines drastically decreases with the proton concentration of the solution. We interpreted this finding in terms of a proton-transfer quenching of the triplet state. Other parameters such as the ionic strength and the excitation wavelength have no effect on the distribution of the transients and their corresponding lifetime. Chlorine and thiomethyl, which are weakly electron-withdrawing substituents, give neuroleptic drugs of similar moderate activity and high phototoxicity (CPZ and TR, respectively).[67] A potent electron withdrawer, trifluoromethyl, gives a drug of high potency and less phototoxicity (TFMP).[67] The relative neuroleptic potency of some phenothiazines follows the order[68] PZ < TR < CPZ < TFMPZ < TFMP < PP < FP. The phototoxicity index of

Felmeister and Schaubman[69] indicates the following order: PZ $\ll$ TFMPZ < TFMP < CPZ $\approx$ PCP.

The effectiveness of the quenching of ^{3}Drug* in water ($\tau_{PZ} > \tau_{TFMPZ} > \tau_{TFMP} > \tau_{CPZ} \approx \tau_{PCP}$) has an excellent correlation with this phototoxicity index (i.e., the more effective the quenching, the smaller the triplet lifetime and the more phototoxic the drug). This indicates that this species might be directly involved in the phototoxicity mechanism of promazines. This is energetically possible because the formation of the triplet is a monophotonic process, which is more relevant to the in vivo conditions.

In summary, the detailed mechanism of phototoxic and photoallergic side effects of neuroleptic drugs is by no means resolved. Our finding of the proton-transfer quenching of the triplet state of 2-halogenated promazines makes this short-lived transient a very interesting species to investigate in terms of its interaction with the different components of the membrane and the consequent phototoxic side effect. More specific experiments are underway to better elucidate and quantify the proton-transfer quenching of the TCAs triplet states.

Acknowledgment. This work has been supported by NIH-MBRS grant S06GM08216 to UPR-Humacao. R.O. also appreciates the financial support from the GAAN fellowship program of the U.S. Department of Education and the Puerto Rico Industrial Development Company.

Abbreviations. BP, benzophenone; CPH, 2-chlorphenothiazine; CPZ, chlorpromazine hydrochloride; DFT, density functional theory; f, oscillator strength; FP, fluphenazine hydrochloride; IP, ionization potential; MC540, merocyanine 540; PCP, prochlorperazine; PBS, phosphate buffer solution; PH, phenothiazine; PP, perphenazine; PZ, promazine hydrochloride; TCA, tricyclic antidepressant drugs; TFMP, 2-trifluoromethylperazine dihydrochloride; TFMPH, 2-trifluoromethylphenothiazine; TFMPZ, 2-trifluoromethylpromazine hydrochloride; TMPZ, 2-thiomethylpromazine; TR, thioridazine hydrochloride.

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