

# PCCP

Accepted Manuscript



This article can be cited before page numbers have been issued, to do this please use: F. M. Cabrerizo, F. A. O. Rasse-Suriani, M. P. Denofrio, J. G. Yaňuk, M. M. Gonzalez, E. Wolcan, M. Seifermann and R. Erra-Balsells, *Phys. Chem. Chem. Phys.*, 2015, DOI: 10.1039/C5CP05866J.



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

## CHEMICAL AND PHOTOCHEMICAL PROPERTIES OF CHLOROHARMINE DERIVATIVES IN AQUEOUS SOLUTIONS

*Federico A. O. Rasse-Suriani<sup>a,‡</sup>, M. Paula Denofrio<sup>a,‡</sup>, Juan G. Yañuk<sup>a</sup>, M. Micaela Gonzalez<sup>a</sup>, Ezequiel Wolcan<sup>b</sup>, Marco Seifermann<sup>c</sup>, Rosa Erra-Balsells<sup>d,\*</sup> and Franco M. Cabrerizo<sup>a,\*</sup>*

<sup>a</sup> Instituto de Investigaciones Biotecnológicas - Instituto Tecnológico de Chascomús (IIB-INTECH), Universidad Nacional de San Martín (UNSAM) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Intendente Marino Km 8.2, CC 164 (B7130IWA), Chascomús, Argentina. E-mail: [fcabrerizo@intech.gov.ar](mailto:fcabrerizo@intech.gov.ar)

<sup>b</sup> Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA, UNLP, CCT La Plata-CONICET), Diag. 113 y 64, Sucursal 4, C.C. 16, (B1906ZAA) La Plata, Argentina.

<sup>c</sup> Institute of Pharmacy and Biochemistry, University of Mainz, Staudingerweg 5, Mainz, Germany.

<sup>d</sup> CIHIDECAR - CONICET, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, 3p, Ciudad Universitaria, (1428) Buenos Aires, Argentina. E-mail: [erra@qo.fcen.uba.ar](mailto:erra@qo.fcen.uba.ar)

<sup>‡</sup> These authors contributed equally.

\* To whom correspondence should be addressed ([fcabrerizo@intech.gov.ar](mailto:fcabrerizo@intech.gov.ar) and [erra@qo.fcen.uba.ar](mailto:erra@qo.fcen.uba.ar))

**ABSTRACT**

Thermal and photochemical stability ( $\Phi_R$ ), room temperature UV-vis absorption and fluorescence spectra, fluorescence quantum yields ( $\Phi_F$ ) and lifetimes ( $\tau_F$ ), quantum yields of hydrogen peroxide ( $\Phi_{H_2O_2}$ ) and singlet oxygen ( $\Phi_\Delta$ ) production, and triplet lifetimes ( $\tau_T$ ) have been obtained for the neutral and protonated forms of 6-chloroharminine, 8-chloroharminine and 6,8-dichloroharminine, in aqueous media. When it was possible, the effect of pH and oxygen concentration was evaluated. The nature of electronic transitions of protonated and neutral species of the three investigated chloroharminines were established by Time-Dependent Density Functional Theory (TD-DFT) calculations. The impact of all the foregoing observations on the biological role of the studied compounds is discussed.

## INTRODUCTION

$\beta$ -Carbolines ( $\beta$ Cs) are a group of alkaloids present in a wide range of species that are different from a phylogenetic point of view such as mammals,<sup>1-5</sup> plants,<sup>6-8</sup> arthropods,<sup>9</sup> insects,<sup>10</sup> etc. Although the great majority of  $\beta$ Cs described do not contain halogen atoms in their structure, recently, more than forty halogenated- $\beta$ Cs have been isolated from sea invertebrates, algae, cyanobacteria and other organisms.<sup>11-13</sup>

*Ascidia Eudistoma olivaceum* (from the Caribbean Sea) contains some halogenated full-aromatic  $\beta$ Cs such as Eudistomin N (6-bromo-9*H*-pyrido[3,4-*b*]indole or 6-bromo-norharmane) and Eudistomin O (7-bromo-9*H*-pyrido[3,4-*b*]indole or 7-bromo-norharmane),<sup>14-16</sup> among others. *Ascidia Eudistoma fragum* (from coastal zone of New Caledonia) contains other non-full aromatic bromine derivatives ((1*R*)-6-bromo-2-methyl-1-[(2*S*)-1-methyl-2-pyrrolidinyl]-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole, or woodinine).<sup>17, 18</sup> Hydroid *Aglaophenia pluma* produces 6-bromo-1-ethyl-9*H*-pyrido[3,4-*b*]indole (or 6-bromo-1-ethyl-norharmane), 6-bromo-1-methyl-9*H*-pyrido[3,4-*b*]indole (or 6-bromo-1-methyl-norharmane or 6-bromo-harmane), 6,8-dibromo-1-ethyl-9*H*-pyrido[3,4-*b*]indole (or 6,8-dibromo-1-ethyl-norharmane), among others.<sup>19</sup> Chlorinated  $\beta$ C derivatives were also found in various species. Chloro-nostocarboline (or 6-chloro-2-methyl-norharmanium) was found in *Nostocales* (cyanobacteria spread in Europe, Southwest Asia, Australia and New Zealand) and other phototrophic organisms.<sup>11</sup> Bauerine alkaloids A (or 7-chloro-9-methyl-2*H*-pyrido[3,4-*b*]indole), B (or 7,8-dichloro-9-methyl-2*H*-pyrido[3,4-*b*]indole) and C (or 7,8-dichloro-1-hydroxy-9-methyl-2*H*-pyrido[3,4-*b*]indole) were isolated from *Dichotrix baueriana* (a terrestrial blue-green alga).<sup>20</sup>

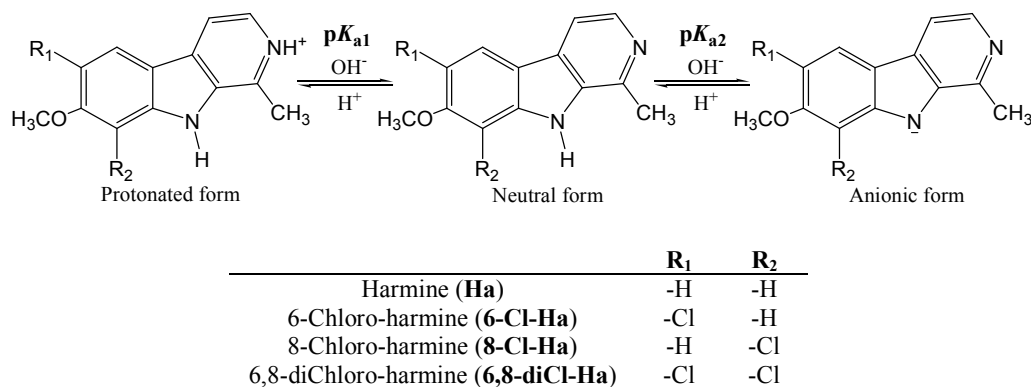
These halogenated- $\beta$ Cs have been described as bioactive compounds. *In vitro*, they have shown antimicrobial activity against parasites, viruses, yeasts and bacteria.<sup>11, 15, 16, 21</sup> Some halogenated- $\beta$ Cs have a high cytotoxic activity against various cancer cell-lines<sup>22</sup> and some chlorinated-derivatives were suggested as potent and selective synthetic kinase inhibitors.<sup>23</sup> Despite their abundance, the main biological role of these alkaloids as well as many fundamental aspects of the mechanisms involved still remain poorly understood; as consequence the potential use of synthetically modified (halogenated)  $\beta$ Cs still is not clearly known. Moreover, taking into account that non-halogenated  $\beta$ Cs are photoactive compounds<sup>24-30</sup> and having in mind their abundance in nature (including phototrophic organisms),<sup>11</sup> the photochemical and photobiological properties of halogenated- $\beta$ Cs deserve to be explored in detail.

Tarzi *et. al.*<sup>31-33</sup> described the effect of chlorine and bromine atoms as substituents on the UV-vis absorption and fluorescence emission spectra of norharmane, harmane and harmine in organic solvents (ethanol and acetonitrile). However, studies in water environment, which are

relevant from the biological point of view, were not attempted. Very recently, we have described the spectroscopic and fluorescence behaviour of three protonated chloroharmines (*i.e.*, in acidic aqueous solutions and in acetonitrile/TFA) upon one- and two-photon excitation.<sup>34</sup> Briefly, solvent-dependent behaviour on the one- and two-photon absorption spectra of protonated chloroharmines was observed. As it was expected, chlorine atoms introduce quite strong changes on the electronic distribution (on both ground and excited states) of the  $\beta$ C ring. However, having in mind the well-known dependence of the physicochemical and photochemical properties of other related  $\beta$ Cs on the pH and oxygen concentration,<sup>35-38</sup> the effect of these environmental conditions still need to be addressed for chloroharmines. Moreover, no information is available in the literature regarding the chemical (thermal) and photochemical stability of chlorinated- $\beta$ Cs.

In the following sections we describe different aspects related to the physicochemical and photochemical properties of three chloro-substituted  $\beta$ Cs obtained from 7-methoxy-1-methyl-9H-pyrido[3,4-*b*]indole (harmine, Ha): 6-chloroharmine (6-Cl-Ha), 8-chloroharmine (8-Cl-Ha) and 6,8-dichloroharmine (6,8-diCl-Ha), in aqueous media (Scheme 1). These compounds might serve as representative examples of other naturally occurring  $\beta$ Cs. On the other hand, studies on synthetic chloro- $\beta$ Cs are also relevant from the biotechnological point of view (*i.e.*, development of novel antimicrobial and/or photosensitizing agents).

The study started by outlining the pH effect on the spectroscopy and also on the chemical and photochemical stability of the three investigated compounds. Reactive oxygen species produced upon one-photon excitation are monitored and quantified and the role of oxygen and its highly reactive species on the photochemistry of chloroharmines is investigated. The impact of all the foregoing observations on the biological role of the studied compounds is discussed.



**Scheme 1.** Structures of chloroharmines studied and acid-base equilibria present in aqueous solution, in the pH range 2-13.

## EXPERIMENTAL

*$\beta$ -Carbolines.* 7-Methoxy-1-methyl-9H-pyrido[3,4-*b*]indole (harmine, Ha) (> 98%) from Sigma-

Aldrich was used without further purification. The method used to synthesize and purify chloroharmines derivatives has been published elsewhere.<sup>39</sup>

*pH adjustment.* The pH of the solutions was adjusted by adding drops of aqueous NaOH or HCl solutions (concentration ranged from 0.1 M to 2.0 M) with a micropipette. When solutions free of chloride ion were needed, HClO<sub>4</sub> was used for acidification. The ionic strength was approximately 10<sup>-3</sup> M in all the experiments. In experiments using D<sub>2</sub>O as solvent, D<sub>2</sub>O (> 99.9%; Sigma), DCl (99.5%; Aldrich) in D<sub>2</sub>O, and NaOD (Aldrich) in D<sub>2</sub>O were used. The pD of the solution was adjusted as it was described elsewhere,<sup>33</sup> the final isotope purity was greater than 96%.

*Dissolved oxygen concentration.* Chloroharmines aqueous solutions were equilibrated (saturated) to three different ambient oxygen partial pressure conditions by bubbling nitrogen (or argon), air, and oxygen through the solution, for approximately 20 min, leading to a dissolved oxygen concentration ([O<sub>2</sub>]) of 0, 0.28 and 1.33 mM, respectively.

*Elapsed irradiation.* Aqueous βC solutions were irradiated at 350 nm or 365 nm, in 1 cm quartz cells at room temperature with a Rayonet RPR lamp (bandwidth ~15 nm, Southern N.E. Ultraviolet Co.). Experiments were performed in the presence and absence of air. Oxygen-free solutions were obtained by bubbling with Ar or N<sub>2</sub> gas for 20 min.

*UV/vis Analysis and pK<sub>a</sub> measurement.* Electronic UV-vis absorption spectra were recorded on Shimadzu PC2101 and Perkin-Elmer λ-25 spectrophotometers. Measurements were made using 1 cm path length quartz cuvettes, at room temperature. pK<sub>a</sub> values were determined from absorption changes following the procedure described elsewhere.<sup>40</sup>

*High-Performance Liquid Chromatography (HPLC).* A Waters 1525 binary Pump controller with a Waters 2475 multi-λ fluorescence detector was used to monitor and quantify the photochemical reactions. Aqueous solutions of commercial standards were employed for the calibration curves. Stationary phase: GracePrevail C18 (250 × 4.6 mm, 5 μm). Mobile phase: 50/50 (v/v) mixture of formic acid aqueous solution (0.08% (v/v), pH 3.2) and MeOH. Flow rate: 1 mL / min.

*Determinations of quantum yield of chloroharmines consumption.* Quantum yields of reactant disappearance ( $\Phi_R$ ) were obtained using the following equation:

$$\Phi_R = - \frac{(d[R]/dt)_0}{q_{n,p,\lambda}} \quad (1)$$

where  $(d[R]/dt)_0$  is the initial rate of reactant consumption and  $q_{n,p,\lambda}$  is the number of photons of the excitation wavelength  $\lambda$ , per time interval (spectral photon flux, amount basis) absorbed by the reactant per volume, V. For determining the consumption rate, experiments were carried out using

solutions with an initial reactant concentration of  $\sim 150 \mu\text{M}$  (except for Ha at pH 10.0 where  $\sim 30 \mu\text{M}$  was used). Under these conditions, the time evolution of the reactant concentration followed a zero-order rate law over a period of time within which the change of  $q_{n,p,\lambda}$  was negligible. In our experiments, this condition was fulfilled at irradiation times less than 120 min. The initial rates were obtained from the slope of the corresponding plots of concentration vs. irradiation time within this time window.

Aberchrome 540 (Aberchromics Ltd.) was used as actinometer for the measurements of the incident photon flux at the excitation wavelength (*i.e.*,  $q_{n,p,\lambda}^0$  according to Braslavsky *et al.*).<sup>41</sup> The general approach used is described in detail elsewhere.<sup>35, 42</sup> Values of the photon flux absorbed ( $q_{n,p,\lambda}$ ),<sup>41</sup> were calculated from  $q_{n,p,\lambda}^0$  according to the Lambert-Beer law:

$$q_{n,p,\lambda} = q_{n,p,\lambda}^0 (1 - 10^{-A}) / V \quad (2)$$

where  $A$  is the absorbance of the sensitizer at the excitation wavelength.

*Detection and quantification of H<sub>2</sub>O<sub>2</sub>.* For the determination of H<sub>2</sub>O<sub>2</sub>, a Cholesterol Kit (Wiener Laboratories S.A.I.C.) was used. H<sub>2</sub>O<sub>2</sub> was quantified after reaction with 4-aminophenazone and phenol.<sup>35, 43</sup> Briefly, 600  $\mu\text{l}$  of irradiated solution (UVA) was added to 1.6 ml of reagent. The absorbance at 505 nm of the resulting mixture was measured after 30 min at room temperature, using the reagent as a blank. Aqueous H<sub>2</sub>O<sub>2</sub> solutions prepared from commercial standards were employed for obtaining the corresponding calibration curves.

*Detection and quantification of chloride ion.* A chloride ion-selective electrode Weiss Research RCL3001 was used for monitoring the chloride ion release during both thermal and photochemical reactions studied. The electrode was used following the specifications of the fabricant. Several NaCl solutions (0 ppm - 100 ppm of Cl<sup>-</sup>), prepared from a chloride standard solution (1000 ppm, WE-2815, Weiss Res. Inc.), were used for electrode calibration. The ionic strength of samples and standard solutions was adjusted by adding 0.1 M of NaNO<sub>3</sub> (from a 5 M stock solution, WE-2510, Weiss Res. Inc.).

*Fluorescence emission.* Steady-state fluorescence measurements were performed using a Fluoromax4 (HORIBA Jobin Yvon), whereas a single-photon-counting equipment FL3 TCSPC-SP (HORIBA Jobin Yvon) spectrofluorometer was used for time-resolved measurements. Corrected fluorescence spectra were recorded in a 1 cm path length quartz cell at room temperature.

Fluorescence quantum yields ( $\Phi_F$ ) were determined from the corrected fluorescence spectra, integrated over the entire emission profile and were calculated as the average of  $\Phi_F$  values obtained using different excitation wavelengths over the entire range of the lowest-energy absorption band.

The standard used was quinine sulfate at pH 4 ( $\Phi_F = 0.52 \pm 0.02$ ).<sup>44</sup> To avoid inner filter effects, the absorbance of the solutions at the excitation wavelength was kept below 0.10.

*Nanosecond Time-Resolved Experiments.* (i) Quantum yields of photosensitized singlet oxygen production,  $\Phi_\Delta$ , were obtained using a pulsed Nd:YAG laser as the excitation source ( $\lambda = 355$  nm), looking at the 1270 nm  $^1\text{O}_2$  phosphorescence with a cooled germanium detector.<sup>36</sup> Experiments were performed by comparing the magnitude of the integrated time-resolved phosphorescence signal produced upon one-photon irradiation of the molecule under study to that obtained upon one-photon irradiation of a standard sensitizer (perinaphthenone-2-sulfonic acid (PNS) in deuterated water with  $\Phi_\Delta = 0.97 \pm 0.05$ ).<sup>45,46</sup> (ii) Triplet state kinetics were studied in a pump-probe experiment using instruments and an approach that have likewise been previously described.<sup>37</sup> Typically, chloroharmine acidic aqueous solutions (200  $\mu\text{M}$ ) were irradiated at 355 nm. The absorption of the resultant triplet state was probed using the output of a steady-state 200W xenon lamp that had been passed through a water filter to remove the IR-components. The light from the xenon lamp transmitted through the sample was dispersed by a grating onto a PMT (Hamamatsu R928). A filter was inserted to remove residual scattered laser light. The resulting time-resolved signal was digitized and monitored with an oscilloscope (Tektronix TDS5032B). Changes in the amount of transmitted light were monitored at 450 nm where the protonated species of chloroharmine triplet state absorbance is strong.

*Computational methods.* The electronic structure of the chloroharmine derivatives were determined using tools of Density Functional Theory (DFT)<sup>47-49</sup> as implemented in Gaussian 09 package.<sup>50</sup> Their vacuum geometries were optimized at the B3LYP/TZVP level of theory. Vibrational frequencies were computed at the same level of theory to confirm that these structures were minima on the energy surfaces. The vertical transition energies were calculated at the optimized ground-state geometry by Time-Dependent Density Functional Theory (TD-DFT)<sup>51-53</sup> using B3LYP hybrid functional and aug-cc-pVDZ basis set including solvent effects (water) through the Polarizable Continuum Model<sup>54-56</sup> to produce a number of 110 singlet-to-singlet transitions. Percentage compositions of different molecular fragments to molecular orbitals (MOs) from output files generated from Gaussian 09 were calculated using the AOMix program.<sup>57,58</sup>

Absorption spectra were simulated with Gaussian distributions with a full-width at half-maximum (fwhm) set to 3000  $\text{cm}^{-1}$  with the aid of GaussSum 2.2.5 program. Convolved spectra are obtained by summing Gaussian functions centred at each calculated wavelength with the maxima related to the value of the oscillator strengths (which are also plotted) using equation 3.<sup>59</sup>



$$\varepsilon(\tilde{\nu}) = \frac{2.175 \times 10^8 \text{ L mol}^{-1} \text{ cm}^{-2}}{\Delta_{1/2} \tilde{\nu}} (f_{\text{osc}}) \exp \left[ -2.772 \left( \frac{\tilde{\nu} - \tilde{\nu}_{i \rightarrow f}}{\Delta_{1/2} \tilde{\nu}} \right)^2 \right] \quad (3)$$

where,  $\Delta_{1/2} \tilde{\nu}$  is the parametrical value of the fwhm of the band (in  $\text{cm}^{-1}$ ),  $f_{\text{osc}}$  is the oscillator strength and  $\tilde{\nu}_{i \rightarrow f}$  is the frequency (in  $\text{cm}^{-1}$ ) corresponding to the wavelength of the calculated electronic transition.

## RESULTS

### Determination of $\text{p}K_{\text{a}}$ values

In the pH range 2-13,  $\beta\text{Cs}$  show two acid-base equilibria (Scheme 1).<sup>35, 38</sup> We report herein the corresponding  $\text{p}K_{\text{a}}$  values (Table 1) for the three chloroharmane derivatives, assessed using UV-vis spectroscopy. Spectra and titration curves are shown as Electronic Supplementary Information (Figures ESI.1 to ESI.3). It is worth mentioning that 6-Cl-Ha showed a reversible acid-base equilibrium in the pH range 2-10.5 whereas an irreversible chemical reaction was observed under higher alkaline conditions, *i.e.*,  $\text{pH} > 11.2$  (see red line in Figure ESI.1a). Irreversible reactions were also observed for 8-Cl-Ha and 6,8-diCl-Ha when were subject to alkaline conditions, even at  $\text{pH} \sim 8$ , (*vide infra*). Thus,  $\text{p}K_{\text{a}1}$  values were found to be  $6.3 \pm 0.2$ ,  $6.4 \pm 0.2$  and  $5.4 \pm 0.4$ , for 6-Cl-Ha, 8-Cl-Ha and 6,8-diCl-Ha, respectively. On the other hand,  $\text{p}K_{\text{a}2}$  was only determined for 6-Cl-Ha ( $10.5 \pm 0.3$ ) due to the quite fast chemical reaction followed by neutral and/or anionic species of 8-Cl-Ha and 6,8-diCl-Ha under alkaline conditions (*vide infra*).

The latter data evidences that chlorine atom increases the acidity of the  $\beta\text{C}$  moiety: *i.e.*, chloroharmines showed  $\text{p}K_{\text{a}}$  values lower than Ha (the unsubstituted  $\beta\text{C}$  ring) and other related  $\beta\text{Cs}$ .<sup>35, 60</sup> Note that  $\text{p}K_{\text{a}1}$  values obtained herein are lower than those reported in the literature (8.3 and 8.5 for 6-Cl-Ha and 8-Cl-Ha, respectively).<sup>32</sup> However, the chlorine-atom effect on other  $\beta\text{Cs}$  described (norharmane and harmane) showed the decrease of their basic character, matching these results with the chlorine-atom-effect on proton affinity (PA) predicted by calculation for ground-state chloro- $\beta\text{Cs}$ , including chloroharmines.<sup>32</sup> These facts support the trend of  $\text{p}K_{\text{a}}$  values measured for chloroharmines in the present work.

### Thermal stability of acidic and alkaline solutions

The chemical stability of acidic and alkaline aqueous solutions of three chloroharmines (Scheme 1) was studied using UV-vis spectroscopy for monitoring. Briefly, all the investigated compounds remained stable after 50 days storage in acidic ( $2 < \text{pH} < 5$ ) solution in the dark;

whereas alkaline ( $\text{pH} > 7.4$ ) solutions of the three chloroharmines, kept in the dark, showed irreversible chemical reactions (Figures ESI.1a-3a and ESI.4). Previously, it has been shown that both protonated and neutral chloroharmines were stable when stored in ethanolic solutions.<sup>32</sup> These results show the strong structure and solvent-dependent chemical properties of  $\beta\text{Cs}$ .

The rate of the irreversible thermal reaction observed strongly depends on the chloroharmines derivative structure ( $6,8\text{-diCl-Ha} > 8\text{-Cl-Ha} \gg 6\text{-Cl-Ha}$ ). In particular, more than 50% of the two former  $\beta\text{Cs}$  were consumed during the first 10 min of reaction; whereas hardly any significant UV-vis change was observed when 6-Cl-Ha alkaline ( $\text{pH} 8$ ) solutions were incubated for more than 30 min, at room temperature (data not shown). The short distance between the chlorine atom and the indolic-NH group in 8-chloro-substituted  $\beta\text{Cs}$ , would account for the formation of a hydrogen-bridge-like-interaction.<sup>32</sup> That would be a reasonable explanation for the higher rate of reaction (probably hydrolysis-like reaction) observed when 8-Cl-Ha and 6,8-diCl-Ha are kept in alkaline aqueous solutions.

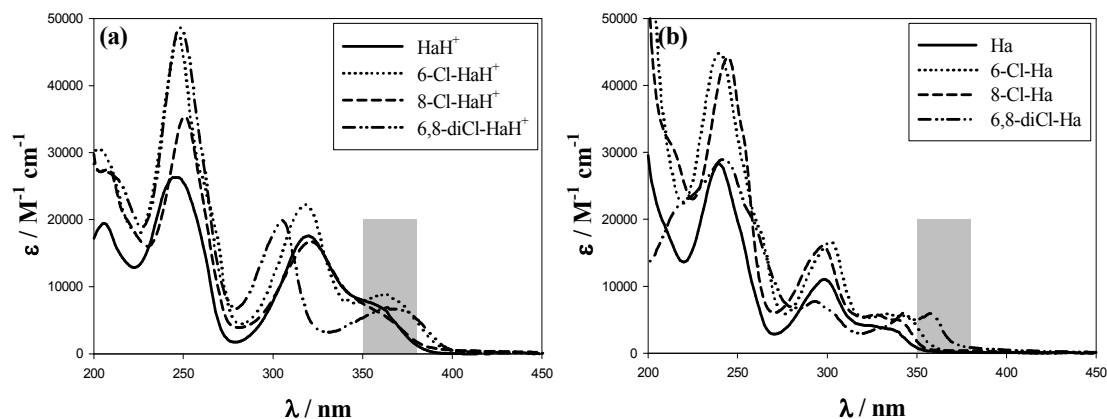
According to the UV-vis spectra (Figures ESI.1a-3a and ESI.4), the nature of the product/s of the thermal reaction is  $\beta\text{C}$ -like because the main absorption band, centred at  $\sim 360$  nm, remains present in all cases. The latter fact is in agreement with a nucleophilic substitution of the chlorine atom by a hydroxyl group and/or a  $\beta\text{C}$  dimerization-like reaction (*vide infra*). This hypothesis should be further investigated.

### UV-vis absorption spectra

We conducted experiments with Cl- $\beta\text{Cs}$  solutions at two different pH-ranges (3.0 - 4.0 and 8.0 - 9.0) where protonated and neutral form, respectively, are the dominant species (Scheme 1). The corresponding experimental UV-vis absorption spectra are shown in Figure 1. For comparative purposes, the corresponding absorption spectra of Ha were also included.

Data reported herein show both similarities and remarkable differences with those reported for chloroharmines in ethanolic solutions.<sup>32</sup> Briefly, (i) in the range 200 - 500 nm, all compounds show three absorption bands. Table 1 collects relevant data for the electronic transitions  $S_0 \rightarrow S_1$  and  $S_0 \rightarrow S_2$ , located in the 320 - 400 region and in the 275 - 320 region, respectively. (ii) The relative spectral behaviour observed for the protonated and neutral species of each compound was similar to that observed in organic protic solvents (*i.e.*, ethanol). The protonated species show bathochromic shifts and hyperchromicity in their absorption bands with respect to the corresponding neutral species. (iii) With respect to Ha, protonated chloroharmines show a clear bathochromic shift of the lowest absorption band ( $S_0 \rightarrow S_1$ ) and a hypsochromic shift of the  $S_0 \rightarrow S_2$  transition band (with the

exception of 8-Cl-HaH<sup>+</sup>). (iv) The chlorine atom on neutral species induces a clear effect only on the lowest energy absorption band: the S<sub>0</sub>→S<sub>1</sub> transition of the three chloroharmines investigated are red-shifted with respect to Ha, whereas the maximum of absorption of the other electronic transitions showed no significant differences.<sup>61</sup> (v) On the contrary to what was observed in organic protic solvents, the presence of a chlorine atom at position C-6 of the protonated βC ring prompts a better resolution of the absorption bands (*i.e.*, transitions S<sub>0</sub>→S<sub>1</sub> and S<sub>0</sub>→S<sub>2</sub>, in 6-Cl-Ha and 6,8-diCl-Ha).



**Figure 1.** UV-vis absorption spectra of protonated (a) and neutral (b) species of the investigated Cl-βCs recorded in aqueous solutions. Grey bar depicts the irradiation range (*i.e.*, 365 ± 15 nm) in photostability experiments conducted.

### Computational analysis

TD-DFT calculations were performed on different geometry optimized structures corresponding to the existing species in aqueous solutions to better understand the substituent and protonation effects on the photophysical properties of the chloroharmines derivatives. In the following section, TD-DFT results corresponding to the two lowest energy bands of the chloroharmines derivatives will be discussed in detail, as the electronic transitions in this near UV-vis energy region are the ones responsible of the observed fluorescence and the photochemical properties. The calculated TD-DFT results are summarized and compared with experimental data for 6-Cl-Ha, 8-Cl-Ha, 6,8-diCl-Ha and their corresponding protonated analogues (Table 1, entries 4, 6 and 7). It is observed that the main spectral features are predicted to a great accuracy, both in position and relative intensities, by TD-DFT calculations. The main molecular orbitals (MOs) involved in the S<sub>0</sub>→S<sub>1</sub> and S<sub>0</sub>→S<sub>2</sub> electronic transitions are the highest occupied MO (HOMO, H), the lowest unoccupied MO (LUMO, L) and the second highest occupied MO (H-1). The second lowest unoccupied MO (L+1) makes only minor contributions to those electronic transitions. For all the compounds, with the exception of 8-Cl-HaH<sup>+</sup>, the first excited state, which corresponds to a S<sub>0</sub>→S<sub>1</sub> electronic transition, is predominantly a H→L transition.<sup>62</sup> The second excited state, S<sub>0</sub>→S<sub>2</sub>,

corresponds to a H-1→L transition. 8-Cl-HaH<sup>+</sup> is an exception, since the S<sub>0</sub>→S<sub>1</sub> electronic transition corresponds to a H-1→L transition and S<sub>0</sub>→S<sub>2</sub> electronic transition is of H→L nature.

Figures 2 and 3 show spatial plots of those MOs, which provide interesting insights to the electronic transitions of protonated and neutral chloroharmines, respectively. We give below a description of H-1, H and L MOs of chloroharmine derivatives based on Figures 2 and 3:

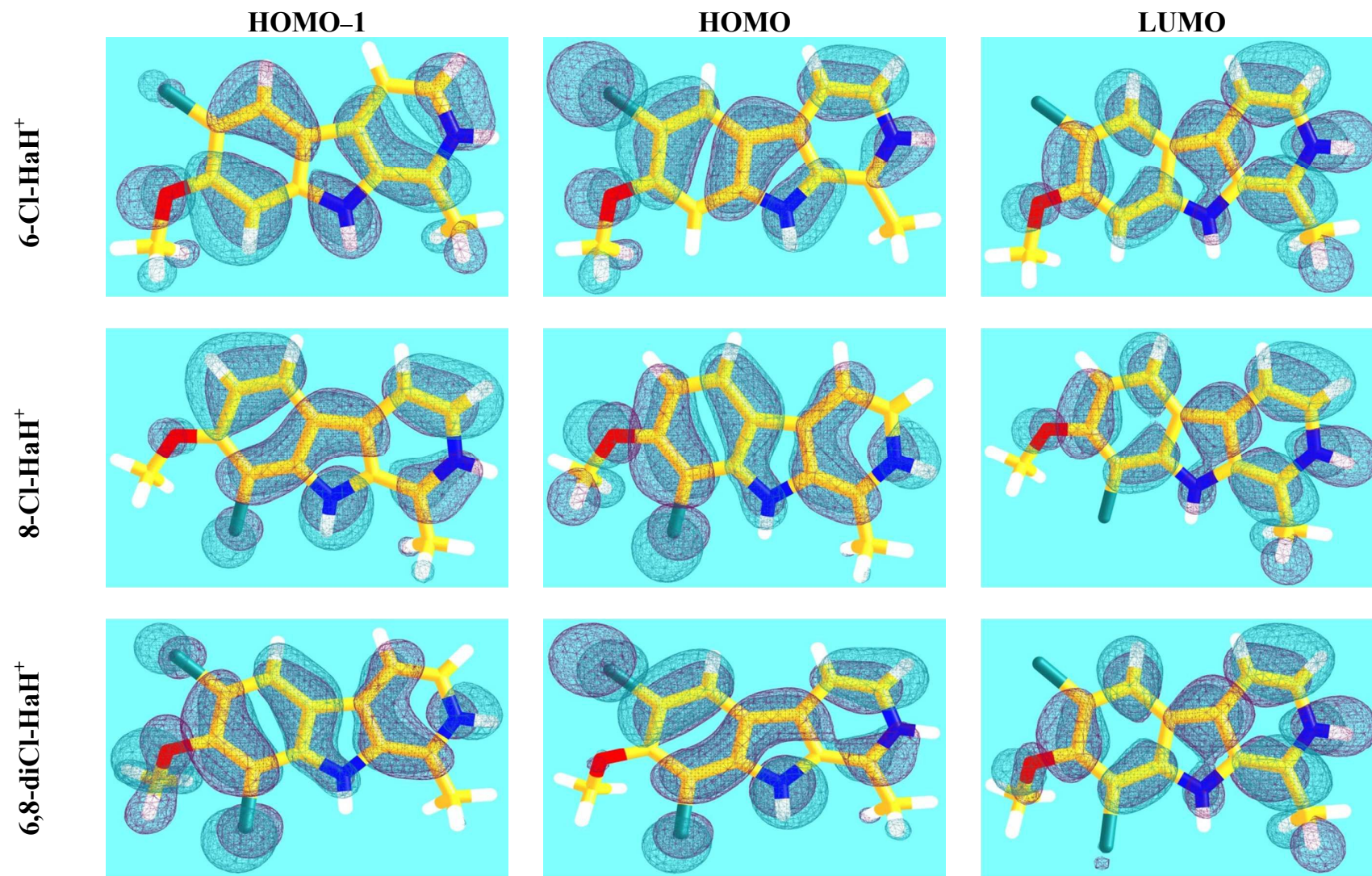
*6-Cl-Ha and 8-Cl-Ha.* H has a significant amount of electron density on the chlorine atom as well as on the pyrrolic NH. The electron density over the chlorine atom and pyrrolic NH is lower in H-1 than in H. However, the electron density residing in O-CH<sub>3</sub> substituent is higher in H-1 than in H. On the other hand, L orbital has very little electron density on both chlorine atom and pyrrolic NH (at an isocontour value of 0.02 no electron density is observed on Cl of 6-Cl-Ha), while the electron density over O-CH<sub>3</sub> substituent is of comparable amount for L and H. Furthermore, the amount of electron density over pyridinic N is higher in L than in H or H-1.<sup>62</sup>

*6,8-diCl-Ha.* H and H-1 have a significant amount of electron density on both chlorine atoms. While H has a significant amount of electron density over pyrrolic NH, H-1 has not. The electron density over O-CH<sub>3</sub> substituent is significant in H-1 while it is nil (at an isocontour value of 0.02) for H. Furthermore, the amount of electron density over pyridinic N is higher in L than in H, though in L it is comparable to that of H-1.<sup>63</sup> The amount of electron density over 8-Cl is lower in L than in both H and H-1 while the electron density over 6-Cl is nil in L. Similar conclusions can be made from Figure 2 based on the relative electron density contributions of Cl, pyrrolic NH, pyridine NH<sup>+</sup> and O-CH<sub>3</sub> to H-1, H and L MOs of 6-Cl-HaH<sup>+</sup>, 8-Cl-HaH<sup>+</sup> and 6,8-diCl-HaH<sup>+</sup>.

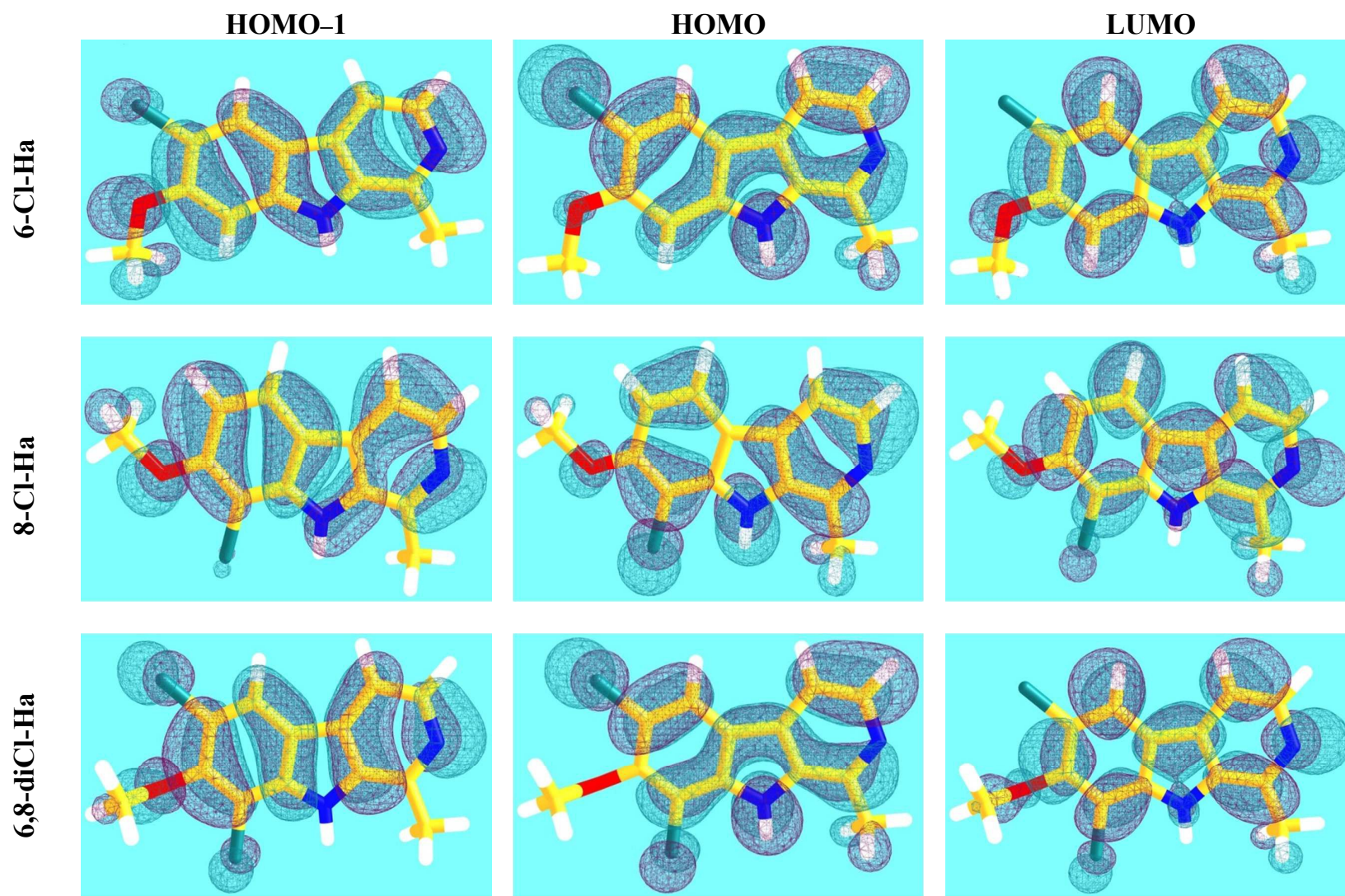
With the aid of AOMIX program, which takes advantage of Mulliken population analysis to get information on electronic structure of molecules, we calculated the orbital percentage composition of the relevant MOs based on the contributions of the different fragments in which the whole molecule can be split for analysis. The following fragments were defined: (i) pyridinic nitrogen (N or NH<sup>+</sup> in case of protonated chloroharmines), (ii) NH of pyrrolic ring, (iii) Cl substituent on 6 and/or 8 position, (iv) CH<sub>3</sub> substituent, (v) O-CH<sub>3</sub> substituent (vi) phenyl ring (without Cl and O-CH<sub>3</sub> substituents) and (vii) pyridine ring (excluding N). Table ESI.1 (see Supplementary Information) lists the orbital percentage composition of the relevant MOs of chloroharmine derivatives based on the fragments defined above. Inspection to Table ESI.1 gives a more precise description of electronic transitions than the one that can be inferred from visual inspection of MOs plots as it helps, in addition, to understand electron density differences in MOs aromatic rings. In this regard, in 6-Cl-Ha, 8-Cl-Ha and 6,8-diCl-Ha there is a higher amount of electron density on the pyridine ring in L than in H. In 6-Cl-HaH<sup>+</sup>, 8-Cl-HaH<sup>+</sup> and 6,8-diCl-HaH<sup>+</sup>, however, the electron density over the phenyl ring is substantially higher in H than in L while the

electron density over the pyridine ring is substantially higher in L than in H. Based on both Figures 2 and 3 and Table ESI.1, in 6-Cl-Ha, 8-Cl-Ha and 6,8-diCl-Ha compounds H→L transition can be visualized as an electronic transition where electron density moves from NH of pyrrolic ring and Cl towards N of pyridine ring and phenyl ring. On the other hand, for 6-Cl-HaH<sup>+</sup> and 8-Cl-HaH<sup>+</sup> compounds H→L transition involves the transfer of electron density from NH of pyrrolic ring, Cl, O-CH<sub>3</sub> and phenyl ring towards NH<sup>+</sup> and the rest of pyridine ring. In 6,8-diCl-HaH<sup>+</sup> H→L transition involves the transfer of electron density from NH of pyrrolic ring, Cl and phenyl ring towards NH<sup>+</sup> and the rest of pyridine ring. Alternatively, for 6-Cl-Ha, 8-Cl-Ha and 6,8-diCl-Ha compounds H-1→L transition can mainly be viewed as an electronic transition where electron density is transferred from O-CH<sub>3</sub> and phenyl ring towards N and the rest of pyridine ring. For 6-Cl-HaH<sup>+</sup>, H-1→L is an electronic transition where the electron density is transferred from NH of pyrrolic ring, O-CH<sub>3</sub> and phenyl ring towards NH<sup>+</sup> and the rest of pyridine ring. In 8-Cl-HaH<sup>+</sup> H-1→L transition is an electronic transition where the electron density is transferred from NH of pyrrolic ring and phenyl ring towards NH<sup>+</sup> and the rest of pyridine ring. Finally, in 6,8-diCl-HaH<sup>+</sup> H-1→L transition is an electronic transition where the electron density is transferred from both Cl atoms, O-CH<sub>3</sub> and phenyl ring towards NH<sup>+</sup> and the rest of pyridine ring.

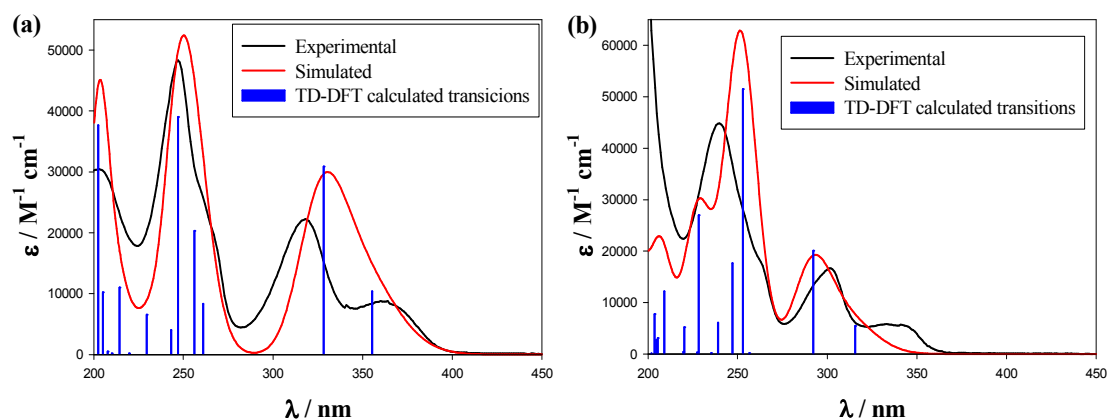
In previous work the electronic structures of protonated species of chloroharmines were determined using TD-DFT at the CAM-B3LYP/aug-cc-pVDZ/Polarizable Continuum Model level of theory. When comparing the absolute positions of the bands with the experiment it was found that the calculated electronic transitions were blue shifted by 0.5-0.6 eV relative to the experimental absorption bands.<sup>34</sup> In this paper we decided to study the electronic structure of neutral and protonated species of chloroharmines at the B3LYP/aug-cc-pVDZ/PCM level of theory. Figure 4 shows, as a representative example, data obtained for 6-Cl-Ha (results for other two compounds are presented as Supplementary Information in Figures ESI.5 and ESI.6). Calculated electronic spectra, obtained by using equation 3, are simulated from the theoretical results for comparison with experimental data. The comparison between calculated electronic transitions and experimental data improves substantially when using the B3LYP functional instead of CAM-B3LYP (Table 1, entries 3-6). Indeed, the main spectral features are predicted to a great accuracy, both in position and relative intensities,<sup>64</sup> by TD-DFT calculations at the B3LYP/aug-cc-pVDZ/PCM level of theory.



**Figure 2:** Frontier orbital diagram for 6-Cl-HaH<sup>+</sup>, 8-Cl-HaH<sup>+</sup> and 6,8-diCl-HaH<sup>+</sup> compounds (isocontour value = 0.02). H → L and H-1 → L are the main transitions involved in the low energy absorption bands of the compounds in H<sub>2</sub>O in the 300 - 350 nm wavelength region. The vertical transition energies were calculated at the optimized ground-state geometry by TD-DFT calculations (see text for details).



**Figure 3:** Frontier orbital diagram for 6-Cl-Ha, 8-Cl-Ha and 6,8-diCl-Ha compounds (isocontour value = 0.02). H $\rightarrow$  L and H-1 $\rightarrow$  L are the main transitions involved in the low energy absorption bands of the compounds in H<sub>2</sub>O in the 300 - 350 nm wavelength region. The vertical transition energies were calculated at the optimized ground-state geometry by TD-DFT calculations (see text for details).



**Figure 4:** Comparison of the UV-vis experimental absorption spectrum (black lines) with TD-DFT calculated electronic transitions (blue lines) and simulated spectra (red lines) for (a) 6-Cl-HaH<sup>+</sup> and (b) 6-Cl-Ha. The vertical transition energies were calculated at the optimized ground-state geometry by TD-DFT calculations (see text for details).

### Steady-state and time-resolved fluorescence emission

Fluorescence measurements were recorded in both acidic and alkaline aqueous solutions (Figure 5). Briefly, under acidic conditions (pH 4.0), a single emitting species was observed for the three chloroharmines investigated. Emission spectra showed one intense-broad band (centred at  $\sim 430$  nm) and time-resolved analysis yields mono-exponential decays over the entire emission range (with lifetimes  $\sim 2$  ns). The latter emitting species were assigned to protonated or cationic (C) chloroharmines. On the other hand, fluorescence spectra recorded under alkaline conditions (pH 9.0) showed two additional pH-dependent emission bands centred at  $\sim 380$  nm and  $\sim 470$  nm (shoulder), besides the emission band at  $\sim 430$  nm. Fluorescence decays recorded at each maximum wavelength of emission (treated as bi-exponential) showed the presence of three distinctive emission-wavelength-dependent species, with lifetimes of  $\sim 0.5$  ns,  $\sim 2$  ns and  $\sim 8$  ns. According to the information reported for other related  $\beta$ Cs and data recorded under acidic conditions, the three emitting species were assigned to neutral (N), protonated or cationic (C) and zwitterionic (Z)<sup>65</sup> species of  $\beta$ Cs, respectively.

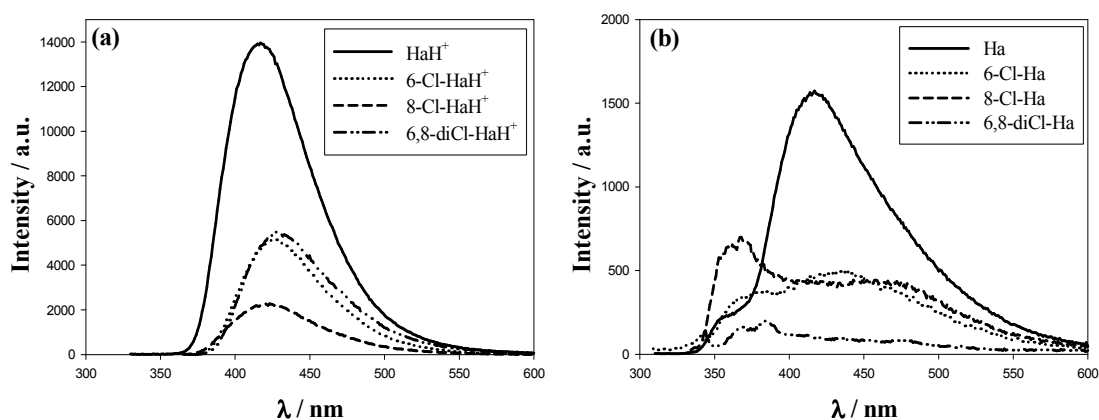
Both protonated and neutral species show a noticeable shift of the maximum of emission relative to Ha of  $\sim +15$  nm. This is consistent with the bathochromic shift observed on the UV-vis absorption spectra (*vide supra*). Besides, the presence of zwitterionic species upon excitation of alkaline (pH  $\leq 9.0$ ) chloroharmines represents another distinctive behaviour with respect to Ha. Note that, although the presence of Ha-zwitterion species in alkaline (pH  $> 11$ ) organic-protic solvents has been reported in the literature, it was not detected under our experimental conditions (*i.e.*, moderate-alkaline aqueous solution). This is further supported by time-resolved fluorescence data (*vide infra*).



The presence of the emission band at  $\sim 430$  nm evidences that photoexcited chloroharmines show the typical enhancement of the basicity of the  $\beta$ C pyridinic nitrogen.<sup>36</sup> When the electronic excited state becomes populated, even in water at pH 9.0, a fraction of the excited neutral species is protonated during the lifetime of its  $S_1$  state. However, in contrast to what was observed for Ha and other  $\beta$ Cs,<sup>27, 35, 36</sup> the relative intensity of both emitting bands of chloroharmines is quite similar (Figure 5b) suggesting that only a fraction ( $\leq 50\%$ ) of neutral excited chloroharmines follows protonation to yield the corresponding protonated excited chloroharmines cation, which then emits.

It is also important to note that the intensity of fluorescence observed upon excitation of the three compounds studied is significantly lower than that of Ha, showing a considerable difference in  $\Phi_F$  (Table 1). This suggests that chlorine atoms, attached to the  $\beta$ C moiety, enhance other deactivation pathways different than fluorescence emission. It is well known that heavy atoms attached directly to aromatic compounds can reduce significantly the  $\Phi_F$  (because of the so called intramolecular heavy atom effect).<sup>66, 67</sup> Typically, the spin-orbital coupling mechanism of the heavy atom enhances the rate of intersystem crossing to the triplet state ( $S_1 \rightarrow T_1$ ). However, our singlet oxygen data independently obtained as well as the data on chloroharmines photodegradation (*vide infra*) suggest that internal conversion ( $S_1 \rightarrow S_0$ ) might be the operative deactivation pathway.

The corresponding quantum yields of fluorescence ( $\Phi_F$ ) and other selected photophysical properties are collected in Table 1. Chloroharmines show the same trend to that observed for other  $\beta$ Cs: neutral species have  $\Phi_F$  values rather smaller than the corresponding protonated species. In the case of 6-Cl-Ha, it was also demonstrated that the data obtained are consistent with the corresponding low values of quantum yield of 6-Cl-Ha consumption ( $\Phi_R$ ) observed. To the best of our knowledge, emission spectra and  $\Phi_F$  values of both acid-base species of chloroharmines dissolved in aqueous solution have not previously been reported.



**Figure 5.** Fluorescence emission spectra of chloroharmines recorded in: (a) acidic (pH 4) and (b) alkaline (pH 9.0) aqueous solutions.

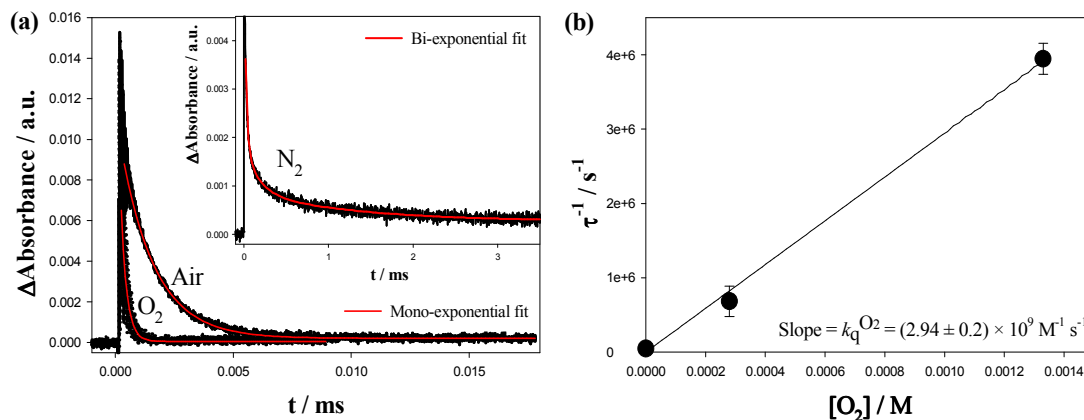
On the contrary to what had been previously described for other protonated  $\beta$ C derivatives, the typical oxygen-concentration-dependent decrease in  $\Phi_F$  was not observed in the case of the three chloroharmines studied. The data clearly showed that  $\Phi_F$  was independent, within the experimental error, on the increase of oxygen partial pressure (Table 1, entries 9-11). This fact could be the consequence of the relatively short fluorescence lifetime of protonated chloroharmines derivatives ( $\sim 2$  ns, see Table 1) compared with other  $\beta$ CsH<sup>+</sup> described ( $\sim 20$  ns).<sup>36,37</sup>

### Laser flash photolysis

Due to the extremely low solubility of chloroharmines in alkaline aqueous media, laser flash photolysis experiments were performed exclusively with the protonated species (acidic conditions). According to the literature, the excitation of oxygen-free solutions of related  $\beta$ Cs (such as norharmane, harmane, harmine, among others) generates a transient species that absorbs throughout the visible spectrum with triplet-lifetimes ranging from 30  $\mu$ s to 5 ms, depending on the pH and the solvent used.<sup>37,68-70</sup> In aqueous solutions, the transient absorption spectra of protonated  $\beta$ Cs (pH < 5) show two bands at  $\sim 450$  and 580 nm, whereas the spectra of neutral species (pH > 9) has only one band centred at  $\sim 510$  nm. Thus, for protonated-chloroharmines 450 nm was the wavelength used to monitor and record the kinetic traces obtained in a time-resolved triplet state absorption experiment (see kinetic traces in Figures 6 and ESI.7).

Oxygen-free solutions of chloroharmines, when subject to laser-pulse excitation, generate two transient species (Table 1, entry 18). On the basis of previous reports, the short-lived transient was assigned to the triplet ( $T_1$ ) state; whereas the long-lived transient might originate from the chloroharmines photo-degradation. It is noteworthy that the traces do not reach the baseline, as expected for an irreversible photochemical transformation. Moreover, UV-vis absorption spectra of irradiated solutions showed noticeable changes, indicative for the presence of an irreversible photochemical reaction (*vide infra*).

In the presence of oxygen (*i.e.*, in aerated and O<sub>2</sub>-saturated solutions), all the kinetic traces show mono-exponential decay, where only the short-lived transient (with lifetimes  $\sim 1.5$   $\mu$ s and  $\sim 0.25$   $\mu$ s, in air-equilibrated and O<sub>2</sub>-saturated solutions, respectively) can be observed, corresponding to  $T_1$ .<sup>71</sup> For all three compounds in acidic water, the fast transient was quenched by O<sub>2</sub> with a rate constant of  $\sim 2.5 \times 10^9$  M<sup>-1</sup> s<sup>-1</sup> (Table 1, entry 21).



**Figure 6.** (a) Kinetic traces obtained in a time-resolved triplet state absorption experiment of 6-Cl-HaH<sup>+</sup>, dissolved in H<sub>2</sub>O at pH 4. Data are shown for N<sub>2</sub>-saturated (*inset*), air-equilibrated and O<sub>2</sub>-saturated solutions. Superimposed on the data are double and single exponential fitting functions used in the analysis of experiments run in absence and presence of oxygen, respectively. (b) Plot of the first-order rate constant for the decay of the triplet state against the dissolved oxygen concentration.

**Table 1:** Photochemical and photophysical parameters determined upon one-photon excitation of chloroharmine derivatives in aqueous solution under acidic and alkaline conditions (pH 4.0 and 9.0, respectively). Data for harmine are listed for comparative porpoise.

Entry	Acid-base species	6-Cl-Ha		8-Cl-Ha		6,8-diCl-Ha		Ha	
		Protonated	Neutral	Protonated	Neutral	Protonated	Neutral	Protonated	Neutral
1	$pK_{a1}$	6.3 ± 0.2		6.4 ± 0.2		5.4 ± 0.4		7.5, <sup>a</sup> 8.0 or <sup>b</sup> 7.7	
2	$pK_{a2}$	10.5 ± 0.3		nd		nd		<sup>a</sup> 14.5 or <sup>b</sup> > 14	
3	$\lambda_{obs}^{Abs} / nm$	362 (S <sub>0</sub> →S <sub>1</sub> ) 317 (S <sub>0</sub> →S <sub>2</sub> )	342 (S <sub>0</sub> →S <sub>1</sub> ) 302 (S <sub>0</sub> →S <sub>2</sub> )	361 <sup>(sh)</sup> (S <sub>0</sub> →S <sub>1</sub> ) 321 (S <sub>0</sub> →S <sub>2</sub> )	340 (S <sub>0</sub> →S <sub>1</sub> ) 298 (S <sub>0</sub> →S <sub>2</sub> )	369 (S <sub>0</sub> →S <sub>1</sub> ) 305 (S <sub>0</sub> →S <sub>2</sub> )	357 (S <sub>0</sub> →S <sub>1</sub> ) 293 (S <sub>0</sub> →S <sub>2</sub> )	357 <sup>(sh)</sup> (S <sub>0</sub> →S <sub>1</sub> ) 320 (S <sub>0</sub> →S <sub>2</sub> )	332 (S <sub>0</sub> →S <sub>1</sub> ) 298 (S <sub>0</sub> →S <sub>2</sub> )
4	<sup>c</sup> $\lambda_{calc}^{Abs} / nm$	355.26 (S <sub>0</sub> →S <sub>1</sub> ) 328.20 (S <sub>0</sub> →S <sub>2</sub> )	315.61 (S <sub>0</sub> →S <sub>1</sub> ) 229.19 (S <sub>0</sub> →S <sub>2</sub> )	345.70 (S <sub>0</sub> →S <sub>1</sub> ) 336.40 (S <sub>0</sub> →S <sub>2</sub> )	313.30 (S <sub>0</sub> →S <sub>1</sub> ) 290.50 (S <sub>0</sub> →S <sub>2</sub> )	362.00 (S <sub>0</sub> →S <sub>1</sub> ) 329.70 (S <sub>0</sub> →S <sub>2</sub> )	325.80 (S <sub>0</sub> →S <sub>1</sub> ) 281.90 (S <sub>0</sub> →S <sub>2</sub> )	nd	
5	$\epsilon_{obs} \times 10^{-4} / M^{-1} cm^{-1}$	0.902 (S <sub>0</sub> →S <sub>1</sub> ) 2.406 (S <sub>0</sub> →S <sub>2</sub> )	0.566 (S <sub>0</sub> →S <sub>1</sub> ) 1.666 (S <sub>0</sub> →S <sub>2</sub> )	0.588 (S <sub>0</sub> →S <sub>1</sub> ) 1.672 (S <sub>0</sub> →S <sub>2</sub> )	0.414 (S <sub>0</sub> →S <sub>1</sub> ) 1.603 (S <sub>0</sub> →S <sub>2</sub> )	0.633 (S <sub>0</sub> →S <sub>1</sub> ) 1.890 (S <sub>0</sub> →S <sub>2</sub> )	0.558 (S <sub>0</sub> →S <sub>1</sub> ) 0.871 (S <sub>0</sub> →S <sub>2</sub> )	0.734 (S <sub>0</sub> →S <sub>1</sub> ) 1.757 (S <sub>0</sub> →S <sub>2</sub> )	0.371 (S <sub>0</sub> →S <sub>1</sub> ) 1.102 (S <sub>0</sub> →S <sub>2</sub> )
6	<sup>c</sup> $f_{osc} calc$	0.1300 (S <sub>0</sub> →S <sub>1</sub> ) 0.3857 (S <sub>0</sub> →S <sub>2</sub> )	0.0697 (S <sub>0</sub> →S <sub>1</sub> ) 0.2558 (S <sub>0</sub> →S <sub>2</sub> )	0.0605 (S <sub>0</sub> →S <sub>1</sub> ) 0.4407 (S <sub>0</sub> →S <sub>2</sub> )	0.0752 (S <sub>0</sub> →S <sub>1</sub> ) 0.2847 (S <sub>0</sub> →S <sub>2</sub> )	0.0795 (S <sub>0</sub> →S <sub>1</sub> ) 0.3839 (S <sub>0</sub> →S <sub>2</sub> )	0.0772 (S <sub>0</sub> →S <sub>1</sub> ) 0.1343 (S <sub>0</sub> →S <sub>2</sub> )	--	--
7	Major contributions to Electronic Transitions (% coefficients)	H→L (81%), H-1→L (15%) (S <sub>0</sub> →S <sub>1</sub> ) H-1→L (81%), H→L (13%) (S <sub>0</sub> →S <sub>2</sub> )	H→L (93%) (S <sub>0</sub> →S <sub>1</sub> ) H-1→L (86%), H→L+1 (11%) (S <sub>0</sub> →S <sub>2</sub> )	H-1→L (80%), H→L (17%) (S <sub>0</sub> →S <sub>1</sub> ) H→L (79%), H-1→L (15%) (S <sub>0</sub> →S <sub>2</sub> )	H→L (85%) (S <sub>0</sub> →S <sub>1</sub> ) H-1→L (76%), H→L+1 (11%) (S <sub>0</sub> →S <sub>2</sub> )	H→L (96%) (S <sub>0</sub> →S <sub>1</sub> ) H-1→L (94%) (S <sub>0</sub> →S <sub>2</sub> )	H→L (95%) (S <sub>0</sub> →S <sub>1</sub> ) H-1→L (72%) (S <sub>0</sub> →S <sub>2</sub> )	--	--
9	<sup>d</sup> $\Phi_R (air)$	7.8 × 10 <sup>-3</sup>	3.2 × 10 <sup>-3</sup>	5.7 × 10 <sup>-3</sup>	nd	4.8 × 10 <sup>-3</sup>	nd	3.7 × 10 <sup>-3</sup>	1.63 × 10 <sup>-3</sup>
10	<sup>d</sup> $\Phi_{H2O2} (air)$	1.79 × 10 <sup>-3</sup>	0.76 × 10 <sup>-3</sup>	0.77 × 10 <sup>-3</sup>	nd	1.9 × 10 <sup>-3</sup>	nd	0.84 × 10 <sup>-3</sup>	0.37 × 10 <sup>-3</sup>
11	$\lambda_{max}^{fluor} / nm$	428 ± 1 (C)	375 ± 1 (N) 426 ± 1 (C) 467 ± 5 (Z)	423 ± 2 (C)	356 ± 2 (N) 423 ± 5 (C) 460 ± 3 (Z)	429 ± 1 (C)	361 ± 2 (N) 429 ± 5 (C) 475 ± 3 (Z)	<sup>e</sup> 416 ± 2 (C)	<sup>e</sup> 358 ± 1 (N) <sup>e</sup> 415 ± 1 (C)
12	$\Phi_F (N_2)$	0.16 ± 0.01	0.12 ± 0.03	0.11 ± 0.02	0.11 ± 0.01	0.20 ± 0.02	0.017±0.001	0.47 ± 0.05	0.39 ± 0.05
13	$\Phi_F (air)$	0.16 ± 0.02	0.12 ± 0.05	0.11 ± 0.02	0.12 ± 0.01	0.20 ± 0.02	0.017±0.001	0.49 ± 0.05	0.38 ± 0.05
14	$\Phi_F (O_2)$	0.16 ± 0.02	0.10 ± 0.04	0.11 ± 0.02	0.10 ± 0.01	0.20 ± 0.02	0.017±0.001	0.47 ± 0.05	0.36 ± 0.05
15	<sup>f</sup> $\tau_F / ns$	2.2(at 426 nm, C)	0.4 (at 375 nm, N) 2.1 (at 426 nm, C) 8.5-12 (at 510 nm, Z)	2.23(at 423 nm, C)	0.59 (at 360 nm, N) 2.32 (at 423 nm, C) 8.59 (at 480 nm, Z)	2.80 (at 429 nm, C)	0.22 (at 370 nm, N) 2.20 (at 430 nm, C) 7.50 (at 485 nm, Z)	7.06 (at 416 nm, C)	0.44 (at 358 nm, N) and 6.96 (at 415 nm, C)
16	$\lambda_{em}^{phos} / nm$	<sup>g</sup> 472	<sup>g</sup> 473	<sup>g</sup> 481	<sup>g</sup> 476	<sup>g</sup> 473	<sup>g</sup> 434	<sup>i</sup> 437, <sup>h</sup> 460	<sup>i</sup> 396, <sup>h</sup> 405
17	$E_T / kJ mol^{-1}$	253.5	252.9	248.7	251.3	252.9	275.6	<sup>1</sup> 274.4, <sup>h</sup> 265.8	<sup>1</sup> 302.1, <sup>h</sup> 295.4
18	<sup>j</sup> $\tau_T / \mu s (N_2)$	22 <sup>k</sup> (and 150)	nd	48 <sup>k</sup> (and 80)	nd	11 <sup>k</sup> (and 48)	nd	27 and 160, <sup>1</sup> 530	--
19	<sup>j</sup> $\tau_T / \mu s (air)$	1.5	nd	1.5	nd	1.7	nd	1.6, <sup>1</sup> --	--
20	<sup>j</sup> $\tau_T / \mu s (O_2)$	0.25	nd	0.28	nd	0.31	nd	0.25, <sup>1</sup> --	--
21	<sup>j</sup> $k_q^{O_2} / M^{-1} s^{-1}$	2.94 × 10 <sup>9</sup>	nd	2.49 × 10 <sup>9</sup>	nd	2.41 × 10 <sup>9</sup>	nd	2.97 × 10 <sup>9</sup>	nd
22	$\Phi_A (air, in D_2O)$	0.19 ± 0.03	nd	0.22 ± 0.04	nd	0.05 ± 0.02	nd	<sup>e</sup> 0.22 ± 0.02	<sup>e</sup> 0.13 ± 0.01
23	$\Phi_A (O_2, in D_2O)$	0.19 ± 0.03	nd	0.26 ± 0.04	nd	0.08 ± 0.02	nd	<sup>e</sup> 0.24 ± 0.02	<sup>e</sup> 0.13 ± 0.01
24	<sup>1</sup> $k_t^A (in D_2O) / M^{-1} s^{-1}$	1.4 × 10 <sup>7</sup>	nd	4.3 × 10 <sup>7</sup>	nd	1.6 × 10 <sup>7</sup>	nd	3.6 × 10 <sup>6</sup>	3.5 × 10 <sup>7</sup>

nd = non-determined data due to the extremely low solubility and/or due to the thermal decomposition of alkaline solutions of the investigated compounds in the course of the experiment. sh = the band appears as a shoulder of the S<sub>1</sub>→S<sub>0</sub> transition. <sup>a</sup> Data obtained from Ref. [60]. <sup>b</sup> Data obtained from Ref. [72]. <sup>c</sup> Method: TD-DFT calculations (see text for details). <sup>d</sup> Error of ± 0.3 on the mantissa shown. <sup>e</sup> Data obtained from Ref. [36]. <sup>f</sup> Decays recorded under acidic and alkaline conditions were mono- and bi-exponential, respectively. <sup>g</sup> Method: phosphorescence emission at low-temperature. Note that  $\lambda_{em}^{phos}$  values reported in Ref. [31] are inaccurate <sup>h</sup> Data obtained from Ref. [31]. <sup>i</sup> Data obtained from Ref. [68]. Method: measured in N<sub>2</sub>-saturated acidic (pH 0) aqueous media by microsecond flash photolysis. <sup>j</sup> Method: laser flash photolysis. <sup>k</sup> Lifetime shown in parenthesis is assigned to a transient species that lead to the degradation product. <sup>1</sup>  $k_t^A$  is the rate constant of total quenching of <sup>1</sup>O<sub>2</sub> by βC.

## Stability of chloroharmine upon UVA steady-state excitation

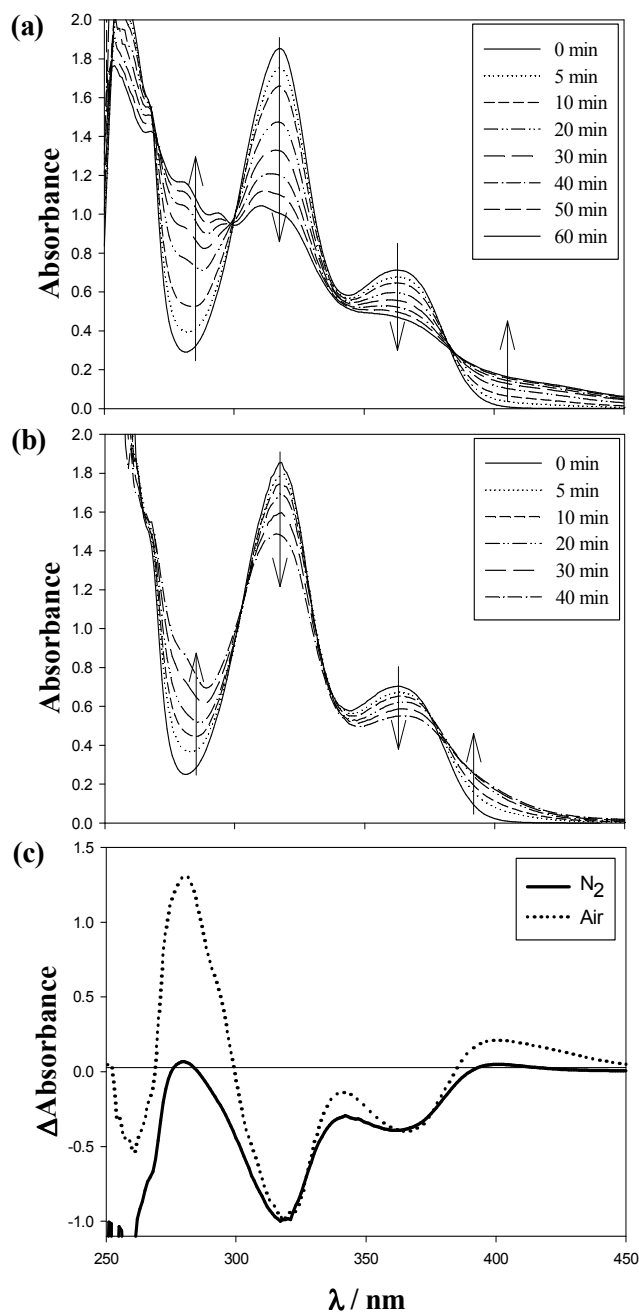
Air-equilibrated chloroharmine aqueous solutions were irradiated and reactions were monitored by UV-visible spectrophotometry and HPLC. Figure 7 shows, as a representative example, data obtained for 6-Cl-HaH<sup>+</sup>. Briefly, significant changes in the absorption spectrum were observed during UVA irradiation (Figure 7a). The decrease in the  $\beta$ C concentration, as a function of elapsed irradiation time, follows zero-order kinetics during the first 30 min of irradiation (Figure 8a and ESI.8). The three investigated chloroharmine showed quite small quantum yields values for the  $\beta$ C disappearance,  $\Phi_R$ , (Table 1, entry 9). Moreover, for those compounds that showed thermal stability under alkaline conditions (6-Cl-Ha and Ha)<sup>73</sup>, the expected dependence of  $\Phi_R$  on pH was observed, with the neutral species being more stable than the protonated ones.<sup>35, 36</sup> When comparing the observed  $\Phi_R$  values, it is evident that the presence of the chlorine atom makes 6-Cl-Ha less photo-stable than Ha (*vide infra*).

Experiments performed under anaerobic conditions (*i.e.*, N<sub>2</sub>-saturated) were used to further explore the role of dissolved molecular oxygen in the photochemical pathway/s of 6-Cl-HaH<sup>+</sup> consumption, as well as the presence of radical reactions. In contrast to what was observed for other  $\beta$ Cs<sup>35, 36</sup>, 6-Cl-HaH<sup>+</sup> showed to be unstable upon UVA excitation (Figure 7b), even in anaerobic conditions, with a  $\Phi_R$  value of  $7.9 \times 10^{-3}$ . The latter value was the same, within the experimental error, to that observed in the presence of oxygen. Nevertheless, photoinduced spectral changes indicate that the pattern of the studied reaction strongly depends on the dissolved-oxygen concentration (see comparative NED spectra in Figure 7c). The reactions in absence and presence of oxygen follow a quite different pattern, suggesting the presence of at least two distinctive types of reactions (*vide infra*).

When subject to UVA-irradiation, carbon-chlorine bonds usually follow homolytic-cleavage yielding Cl<sup>•</sup> and  $\beta$ C<sup>•</sup>. In aqueous environment, Cl<sup>•</sup> can take a hydrogen atom either from the solvent (water) or from organic molecules (6-Cl-HaH<sup>+</sup>) yielding, as consequence, H<sup>+</sup> and Cl<sup>-</sup> (reactions 5 and 7). To further explore this, we measured herein the evolution of chloride ion and proton concentrations during the irradiation of both anaerobic and aerobic 6-Cl-HaH<sup>+</sup> solutions. For these experiments, the initial pH of the solution was adjusted by adding drops of HClO<sub>4</sub>, in order to minimize the initial concentration of chloride in the solution. The profile and the rate of the photochemical reactions were independent of the inorganic acid used (see Figure ESI.9). In the studied example, chloride ions and protons are released while 6-Cl-HaH<sup>+</sup> is being irradiated (see entries 1 and 2 in Table 2 and Figure 8b). These results are consistent with the decrease in the calculated electron density distributions on chlorine atoms described for excited states (*vide supra*).

Note that: **(i)** Under both anaerobic and aerobic conditions about one chloride ion and two protons are released per each molecule of 6-Cl-HaH<sup>+</sup> consumed (see entry 2 in Table 2 and Figure 8b). **(ii)**  $\Phi_{\text{Cl}^-}$  observed in argon is slightly higher than that observed in the presence of oxygen, suggesting the presence of an additional deactivation process competing by the photo-excited chloroharmine. **(iii)** Curves of proton release as a function of irradiation time show a stationary-phase-like pattern (being more evident in anaerobic experiments). This fact suggests that protons are produced in a secondary reaction. **(iv)** Harmine was not detected as a photoproduct neither in anaerobic nor in aerobic conditions, suggesting that Ha<sup>•</sup> produced upon C-Cl cleavage in 6-Cl-Ha would not react with the solvent (via hydrogen abstraction) to yield Ha. Reaction 9 might represent a possible fate of this radical.

Results shown above are consistent with the reasonable expectation that, although the photo-initiated degradation of chloro- $\beta$ Cs depends on the presence of oxygen (reaction 12) (*vide infra*), a parallel and competitive radical-pathway might also play a key role in the mechanism of reaction: *i.e.*, through homolytic-cleavage of C-Cl bonds (reaction 4). These results are in agreement with laser flash photolysis results (*vide supra*).



**Figure 7.** Evolution of the UV-vis spectra of air-equilibrated (a) and N<sub>2</sub>-saturated (b) 6-Cl-HaH<sup>+</sup> ([ $\beta$ C]<sub>0</sub> = 80  $\mu$ M, pH 4.0) aqueous solutions as a function of irradiation time. (c) Normalized (at 317 nm) Experimental Difference (NED) spectra.

### Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) production under UVA steady-state irradiation

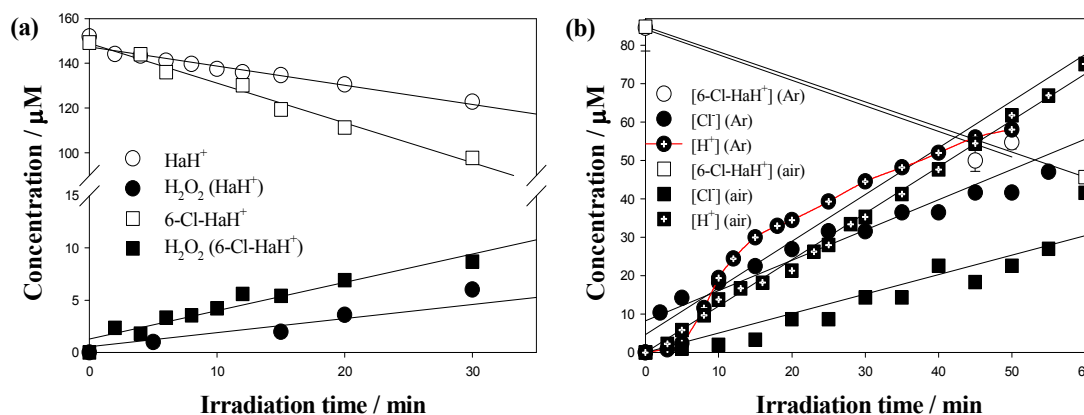
The production of H<sub>2</sub>O<sub>2</sub> was detected upon irradiation of air-equilibrated solutions of chloroharmines (Figure 8a and Figure ESI.8). Filled (black) symbols in Figure 8a represent the evolution of the concentration of H<sub>2</sub>O<sub>2</sub> as a function of elapsed irradiation time ([ $\beta$ C]<sub>0</sub> = 150  $\mu$ M, pH 4.0), whereas open symbols represents the evolution of the  $\beta$ Cs' concentration. Quantum yields

of  $\text{H}_2\text{O}_2$  formation ( $\Phi_{\text{H}_2\text{O}_2}$ ) listed in Table 1 (entry 10) show that although photoexcited chloroharmines have a very low efficiency of  $\text{H}_2\text{O}_2$  production,  $\Phi_{\text{H}_2\text{O}_2}$  depends noticeably on the position of the chlorine atom (being the 6-substituted derivatives 6-Cl-Ha and 6,8-diCl-Ha more efficient than 8-Cl-Ha) and also on the pH (being the neutral form less efficient than the protonated form).

The production of  $\text{H}_2\text{O}_2$  upon UVA irradiation of  $\beta\text{Cs}$  has been reported.<sup>35, 36</sup> In an earlier study of norharmane,<sup>35</sup> it was suggested that  $\text{H}_2\text{O}_2$  is formed by electron transfer from  $\text{S}_1$  to  $\text{O}_2$  yielding the superoxide anion ( $\text{O}_2^{\cdot-}$ ) and, as a result of the spontaneous disproportionation of  $\text{O}_2^{\cdot-}$ ,  $\text{H}_2\text{O}_2$  is produced. This may indeed be one source of  $\text{H}_2\text{O}_2$  that is applicable in our present work (reaction 14). This hypothesis is further supported by the increase in the  $\Phi_{\text{H}_2\text{O}_2}$  value observed when experiments were performed in the presence of SOD enzyme (Table 2, entry 4).

$\text{H}_2\text{O}_2$  might be also produced in the anaerobic pathway (*vide supra*) as a consequence of  $\text{HO}^{\cdot}$  self-annihilation (reaction 10). As it was expected, the latter hypothesis was confirmed by measuring the production of  $\text{H}_2\text{O}_2$  in irradiated argon-saturated 6-Cl-HaH<sup>+</sup> solutions. In particular, a  $\Phi_{\text{H}_2\text{O}_2}$  value of  $1.0 \times 10^{-3}$  was determined (Table 2, entry 1).

$\Phi_{\text{H}_2\text{O}_2}$  and  $\Phi_{\text{R}}$  values (Table 2) are the same order of magnitude suggesting that  $\text{H}_2\text{O}_2$  (or compounds related to its formation, *i.e.*,  $\text{O}_2^{\cdot-}$ ) may be involved, at least in part, in the photo-initiated degradation of chloroharmines. Comparison of  $\Phi$  values listed in Table 2 shows a ratio between 6-Cl-HaH<sup>+</sup> consumed and  $\text{H}_2\text{O}_2$  produced of  $\sim 4$  to 1 (*i.e.*, far from the 2 to 1 ration expected from the reactions described in the previous paragraph). The latter fact suggests the presence of additional reactive pathways of  $\text{O}_2^{\cdot-}$  and/or  $\text{HO}^{\cdot}$  consumption, that might be reactions 8, 9 and 15.



**Figure 8.** (a) Evolution of  $\beta\text{C}$  and  $\text{H}_2\text{O}_2$  concentration in air-equilibrated aqueous solutions of  $\text{HaH}^+$  (circles) and 6-Cl- $\text{HaH}^+$  (squares) as a function of irradiation time ( $[\beta\text{C}]_0 = 150 \mu\text{M}$ , pH 4.0). (b) Evolution of 6-Cl- $\text{HaH}^+$  and chloride ( $\text{Cl}^-$ ) concentration in Ar-saturated (circles) and air-equilibrated aqueous solutions (squares) as a function of irradiation time ( $[\text{6-Cl-HaH}^+] = 85 \mu\text{M}$ , pH 4.0).

### Quantum yield of chloroharmines sensitized $^1\text{O}_2$ production ( $\Phi_{\Delta}$ ) in aqueous solution



It has been proposed that  $\beta$ C alkaloids are excellent  $^1\text{O}_2$  photosensitizers.<sup>74</sup> Values of  $\Phi_\Delta$  for the neutral form of norharmane, harmane and harmine in aprotic organic solvents were found to be ranged from 0.31 to 0.40.<sup>69</sup> However, we have recently demonstrated that, in aqueous environment  $\beta$ Cs show quite small  $\Phi_\Delta$  values (ranged from 0.06 to 0.24, depending on the  $\beta$ Cs, the pH and also on the dissolved oxygen concentration).<sup>27, 35, 36</sup> Briefly, for a given  $\beta$ C derivative, the higher the pH, the lower the  $\Phi_\Delta$ ; whereas the higher the  $[\text{O}_2]$ , the higher the  $\Phi_\Delta$ .

We have determined herein the quantum yields for the photosensitized production of  $^1\text{O}_2$  and the rate constant of total quenching of  $^1\text{O}_2$  ( $k_t^\Delta$ ) by the three chloroharmines studied in acidic aqueous solution (alkaline solutions were unstable over the time interval of the measurements). The data, recorded as a function of ambient oxygen partial pressure (*i.e.*, exposure to air and oxygen), are listed in Table 1. Regarding this, some key points might be highlighted: (i) the efficiency of  $^1\text{O}_2$  production depends on the chemical structure of the  $\beta$ C derivative ( $\Phi_\Delta^{6\text{-Cl-Ha}} \approx \Phi_\Delta^{8\text{-Cl-Ha}} \gg \Phi_\Delta^{6,8\text{-diCl-Ha}}$ ). (ii) Within the experimental error, no change was observed in  $\Phi_\Delta$  of all investigated compounds with the increase in the oxygen concentration ( $\Phi_\Delta^{(\text{O}_2)} \approx \Phi_\Delta^{(\text{air})}$ ). This is in good connection with the oxygen-induced triplet state deactivation described above. Triplet-triplet energy transfer might be the operative pathway for  $^1\text{O}_2$  production. (iii) In the cases of 6-Cl-Ha and 8-Cl-Ha, the  $\Phi_\Delta$  values listed in Table 1 ( $\Phi_\Delta^{(\text{air})} = 0.19$  and  $0.22$ , respectively) are quite similar to the corresponding value reported for Ha ( $\Phi_\Delta^{(\text{air})} = 0.22$ ). Thus, no heavy-atom effect was observed due to the presence of chlorine atoms. (iv)  $\Phi_\Delta$  values obtained are entirely consistent with  $\Phi_F$  values recorded at the same oxygen concentration in independent experiments (the sum of  $\Phi_\Delta$  and  $\Phi_F$  does not exceed 1.0). (v) On the contrary to what was described for other  $\beta$ Cs, as an exception to the rule,<sup>36, 37</sup> the lack of a dependence of  $\Phi_F$  values on the dissolved oxygen concentration and the quite high  $\Phi_\Delta$  values observed for chloroharmines ( $\sim 0.2$ ) suggest that triplet-triplet energy transfer might be the operative pathway for  $^1\text{O}_2$  production reported herein. (vi)  $k_t^\Delta$  values observed for protonated chloroharmines were about one order of magnitude higher than that observed for the unsubstituted Ha (Table 1, entry 24). Although  $k_t^\Delta$  values for neutral chloroharmines could not be measured, a predictive modelling of the total deactivation rate constant of singlet oxygen by heterocyclic compounds suggest that neutral  $\beta$ Cs would have  $k_t^\Delta$  values one order of magnitude higher than protonated  $\beta$ Cs (*i.e.*,  $\sim 10^8$  and  $\sim 10^7$ , respectively).<sup>75</sup>

### Role of reactive oxygen species (ROS) in the photochemistry of 6-chloroharmane

The contribution of ROS produced by photoexcited 6-Cl-HaH<sup>+</sup> (*vide supra*) to its photo-degradation was investigated in four independent experiments. Briefly, quantum yields of 6-Cl-

HaH<sup>+</sup> consumption ( $\Phi_R$ ) were determined in the presence of propan-2-ol, SOD, catalase and sodium azide, to evaluate the role of HO<sup>•</sup>, O<sub>2</sub><sup>•-</sup>, H<sub>2</sub>O<sub>2</sub> and <sup>1</sup>O<sub>2</sub>, respectively (Figure ESI.10a).<sup>35</sup> Results, listed in Table 2, show that catalase has no significant effect on the efficiency of 6-Cl-HaH<sup>+</sup> consumption, whereas propan-2-ol, SOD and NaN<sub>3</sub> induce a small decrease (~ 5 - 25%) on the  $\Phi_R$  with respect to the experiment performed in the absence of these scavengers. Thus, Cl<sup>•</sup>, HO<sup>•</sup>, O<sub>2</sub><sup>•-</sup> and/or <sup>1</sup>O<sub>2</sub> might play a secondary role in the main mechanism of 6-Cl-HaH<sup>+</sup> photodegradation (*i.e.*, reactions 5, 8, 15 and 16).

The quantum yields of H<sub>2</sub>O<sub>2</sub> production ( $\Phi_{H_2O_2}$ ) were also measured in the presence of ROS scavengers in order to investigate which ROS (HO<sup>•</sup>, <sup>1</sup>O<sub>2</sub> and/or O<sub>2</sub><sup>•-</sup>) promotes the production of H<sub>2</sub>O<sub>2</sub> described above (Figure ESI.10b). Results, obtained in the presence of propan-2-ol, suggest that HO<sup>•</sup> is not the precursor of H<sub>2</sub>O<sub>2</sub> produced (Table 2, entry 3). On the other hand, although <sup>1</sup>O<sub>2</sub> might have a minor contribution through endoperoxide intermediates (Table 2, entry 5), the quite high increase on the  $\Phi_{H_2O_2}$  observed in the presence of SOD clearly indicates that O<sub>2</sub><sup>•-</sup> is the main H<sub>2</sub>O<sub>2</sub> precursor; chloroharmines follow the same trend to that described for other  $\beta$ CS.<sup>35, 36</sup>

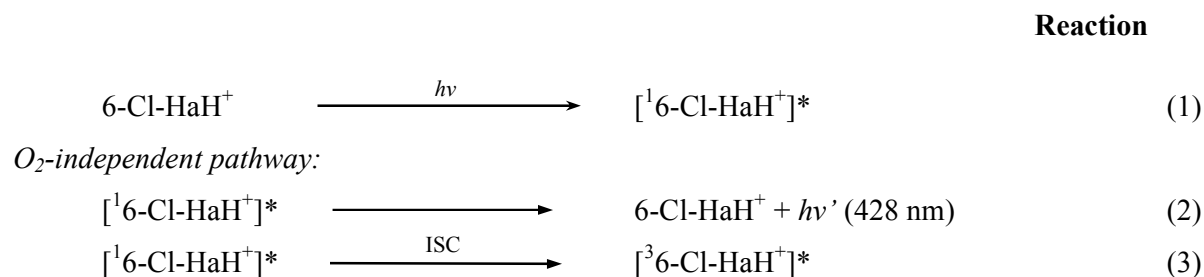
**Table 2:** Quantum yields of 6-Cl-HaH<sup>+</sup> consumption ( $\Phi_R$ ) and H<sub>2</sub>O<sub>2</sub> production ( $\Phi_{H_2O_2}$ ) upon UVA (350 nm) excitation of 6-Cl-HaH<sup>+</sup> acidic aqueous solutions (pH 4.0) in absence and in presence of different reactive oxygen species (ROS) scavengers.

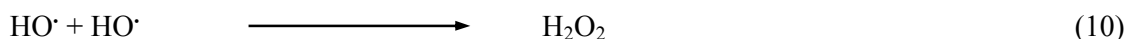
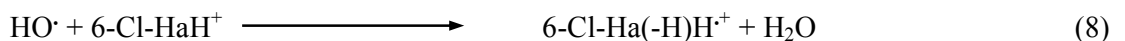
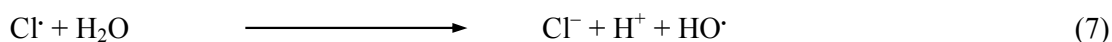
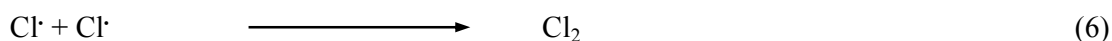
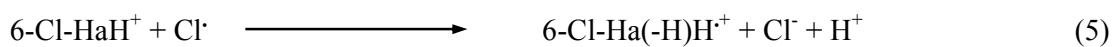
Entry	Experimental condition	<sup>a</sup> $\Phi_R$	<sup>a</sup> $\Phi_{H_2O_2}$	<sup>a</sup> $\Phi_{H^+}$	<sup>a</sup> $\Phi_{Cl^-}$
1	$\Phi_R$ (argon)	$7.9 \times 10^{-3}$	$1.0 \times 10^{-3}$	$14.5 \times 10^{-3}$	$9.4 \times 10^{-3}$
2	$\Phi_R$ (air)	$7.8 \times 10^{-3}$	$1.8 \times 10^{-3}$	$14.4 \times 10^{-3}$	$6.1 \times 10^{-3}$
3	<sup>a</sup> $\Phi_R$ (air, + propan-2-ol)	$7.2 \times 10^{-3}$	$1.7 \times 10^{-3}$	--	--
4	<sup>a</sup> $\Phi_R$ (air, + SOD)	$6.4 \times 10^{-3}$	$6.8 \times 10^{-3}$	--	--
5	<sup>a</sup> $\Phi_R$ (air, + NaN <sub>3</sub> )	$6.4 \times 10^{-3}$	$3.1 \times 10^{-3}$	--	--
6	<sup>a</sup> $\Phi_R$ (air, + catalase)	$7.6 \times 10^{-3}$	$0.2 \times 10^{-3}$	--	--

<sup>a</sup> Error of  $\pm 0.3$  on the mantissa shown.

### Proposed mechanism of reactions

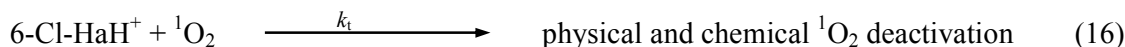
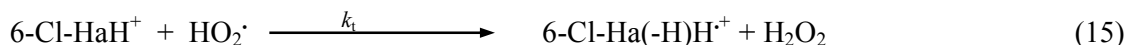
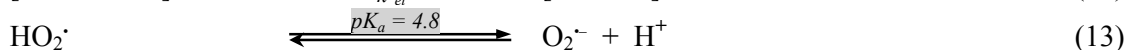
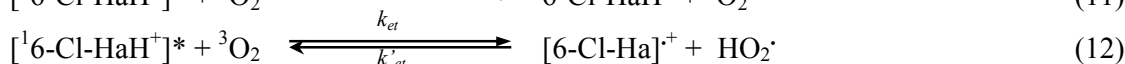
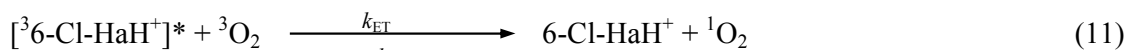
According to the results above described the following reaction scheme is proposed for the first steps of the photochemical reaction of 6-Cl-HaH<sup>+</sup> (a representative example of chloroharmines) in aqueous solution, at pH 4.0:





Other radical species, such as  $\text{Cl}_2\cdot^-$ , might be formed and, consequently, additional reactions would then contribute to the total  $6\text{-Cl-HaH}^+$  consumption. Additional experiments are needed to further investigate this hypothesis.

*O<sub>2</sub>-dependent pathway:*



Based on the reaction of other related  $\beta\text{Cs}$ , the radicals  $[6\text{-Cl-Ha}]^+$  and  $6\text{-Cl-Ha(-H)H}^+$  would follow two competitive dimerization pathways previously described for norharmane, harmane and harmine in dichloromethane solution<sup>76</sup>: (i) the reaction with another molecule of  $6\text{-Cl-HaH}^+$  in its ground state and/or (ii) the annihilation with another radical  $[6\text{-Cl-Ha}]^+$ . In this regards,  $[\text{HaH}^+]\cdot$  might follow similar processes.

## CONCLUSIONS

Data presented herein make this study relevant from the biological point of view. These particular approaches represent basic research that provides, at a molecular level, relevant information that can be a stepping stone on the way of understanding the biological role of these alkaloids and/or to suggest possible biological functions: (i) due to the quite low efficiency showed by these compounds to photoinduce ROS formation, instead of being photo-triggered active compounds against invasive microorganism via ROS (as it has been suggested)<sup>77</sup>, they can act in ROS-mediated intracellular signalling. (ii) The quite high  $k_t^{\Delta}$  values observed ( $\sim 10^7 - 10^8$ ) make halogenated  $\beta\text{Cs}$  good candidates to act as antioxidant in living systems, under photoinduced oxidative stress. (iii) Chloro- $\beta\text{C}$  alkaloids found in nature, such as Bauerine, has a methyl group

bound to the pyrrolic nitrogen (N-9).<sup>11</sup> Having in mind that under physiological pH conditions the neutral species of chloro- $\beta$ Cs are present in more than 50 - 70% (*vide supra*), such a methylation might act as a protective response to thermal hydrolysis showed by neutral non-methylated-chloroharmines, where the hydrogen atom at N-9 seems to play a key role in the mentioned reaction. Certainly, this hypothesis should be further considered.

On the other hand, this study will also provide the chance to further explore other fundamental aspects. As an example, in the search of a better photosensitizer (more effective, at a low dose, absorbing in the far UVA and/or visible region) the herein investigated chloroharmines showed quite distinctive photophysical and photochemical properties that make them good candidates that deserve to be further inspected from this point of view.

**Electronic supplementary information (ESI) available:** Spectrophotometric titration of 6-chloroharmine (6-Cl-Ha), 8-chloroharmine (8-Cl-Ha) and 6,8-dichloroharmine (6,8-diCl-Ha), thermal stability of chloroharmine derivatives under acidic and alkaline conditions, composition (% character) of relevant Molecular Orbitals of chloroharmine derivatives, experimental and calculated electronic spectra of 8-Cl-Ha and 8-Cl-HaH<sup>+</sup>, 6,8-diCl-Ha and 6,8-diCl-HaH<sup>+</sup>, evolution of the  $\beta$ Cs and H<sub>2</sub>O<sub>2</sub> concentrations in irradiated air-equilibrated aqueous solutions, comparative analysis of the effect of HCl and HClO<sub>4</sub> on the photodegradation of 6-Cl-HaH<sup>+</sup>, triplet state kinetics, and evolution of the  $\beta$ Cs and H<sub>2</sub>O<sub>2</sub> concentrations in irradiated air-equilibrated aqueous solutions in the presence of ROS scavengers. See DOI: 10.1039/b000000x/

## ACKNOWLEDGEMENTS

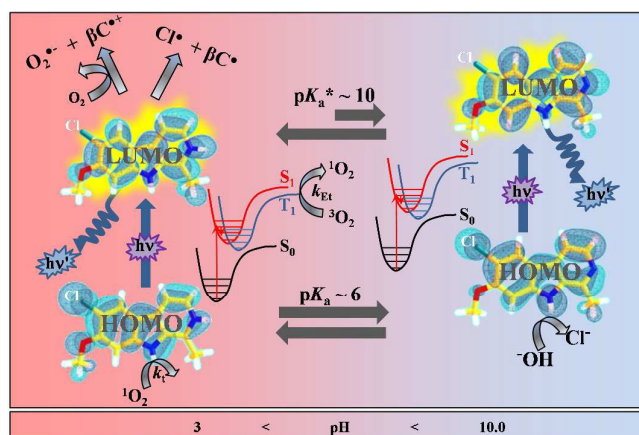
The present work was partially supported by CONICET (PIP 11220120100072CO), ANPCyT (PICT 2012-0423, 2013-2536 and 2014-2856), UBA (X088), UNSAM (E103), MinCyT-DAAD Cooperation Program (DA/11/15), DAAD (Equipment Grants for Higher Education and Comparable Scientific and Research Institutions in Developing Countries) and 3PA. FAORS and JGY thank CONICET for doctoral research fellowships. MMG, MPD, EW, REB and FMC are research members of CONICET. Authors deeply thank C. G. Alberici (CONICET) for his technical support, Dr. P. R. Ogilby for his contributions in singlet oxygen and laser flash photolysis experiments (performed at Center for Oxygen Microscopy and Imaging, University of Aarhus, Denmark) and Dr. B. Epe for his careful reading of the manuscript and for his invaluable editorial suggestions which greatly enhanced its quality.

## REFERENCES AND NOTES

1. Susilo, R.; Rommelspacher, H. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1987, 335(1), 70-76.
2. Spijkerman, R.; van den Eijnden, R.; van de Mheen, D.; Bongers, I.; Fekkes, D. *Eur Neuropsychopharmacol* 2002, 12(1), 61-71.
3. Torreilles, J.; Guerin, M. C.; Previero, A. *Biochimie* 1985, 67(9), 929-947.
4. Pfau, W.; Skog, K. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 2004, 802(1), 115-126.
5. Breyer-Pfaff, U.; Wiatr, G.; Stevens, I.; Jörg Gaertner, H.; Mundle, G.; Mann, K. *Life Sciences* 1996, 58(17), 1425-1432.
6. Kam, T.-S.; Sim, K.-M.; Koyano, T.; Komiyama, K. *Phytochemistry* 1999, 50(1), 75-79.
7. Hemmateenejad, B.; Abbaspour, A.; Maghami, H.; Miri, R.; Panjehshahin, M. R. *Analytica Chimica Acta* 2006, 575(2), 290-299.
8. Herraz, T.; Galisteo, J. *Journal of Agricultural and Food Chemistry* 2003, 51(24), 7156-7161.
9. Stachell, S. J.; Stockwell, S. A.; Van Vranken, D. L. *Chemistry & Biology* 1999, 6(8), 531-539.
10. Siderhurst, M. S.; James, D. M.; Rithner, C. D.; Dick, D. L.; Bjostad, L. B. *J Econ Entomol* 2005, 98(5), 1669-1678.
11. Ramesh, S.; Rajan, R.; Santhanam, R. *Freshwater Phytopharmaceutical Compounds*, CRC Press, 2013; 250 p.
12. Davidson, B. S. *Chemical Reviews* 1993, 93(5), 1771-1791.
13. Dembitsky, V. M.; Tolostikov, G. A. *Chemistry for Sustainable Development* 2003, 11, 451-466.
14. Fattorusso, E.; Tagliatalata-Scafati, O. *Modern Alkaloids: Structure, Isolation, Synthesis, and Biology*, Wiley, 2008; 689 p.
15. Rinehart, K. L.; Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Mascal, M.; Holt, T. G.; Shield, L. S.; Lafargue, F. *Journal of the American Chemical Society* 1987, 109(11), 3378-3387.
16. Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Rinehart, K. L. *Journal of the American Chemical Society* 1984, 106(5), 1526-1528.
17. Debitus, C.; Laurent, D.; Pais, M. *Journal of Natural Products* 1988, 51(4), 799-801.
18. Blunt, J. W.; Lake, R. J.; Munro, M. H. G.; Toyokuni, T. *Tetrahedron Letters* 1987, 28(16), 1825-1826.
19. Aiello, A.; Fattorusso, E.; Magno, S.; Mayol, L. *Tetrahedron* 1987, 43(24), 5929-5932.
20. Larsen, L. K.; Moore, R. E.; Patterson, G. M. L. *Journal of Natural Products* 1994, 57(3), 419-421.
21. Lake, R. J.; Brennan, M. M.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *Tetrahedron Letters* 1988, 29(18), 2255-2256.
22. Rashid, M. A.; Gustafson, K. R.; Boyd, M. R. *Journal of Natural Products* 2001, 64(11), 1454-1456.
23. Huber, K.; Brault, L.; Fedorov, O.; Gasser, C.; Filippakopoulos, P.; Bullock, A. N.; Fabbro, D.; Trappe, J.; Schwaller, J.; Knapp, S.; Bracher, F. *Journal of Medicinal Chemistry* 2012, 55(1), 403-413.
24. Gonzalez, M. M.; Pellon-Maison, M.; Ales-Gandolfo, M. A.; Gonzalez-Baró, M. R.; Erra-Balsells, R.; Cabrerizo, F. M. *Organic and Biomolecular Chemistry* 2010, 8(11), 2543-2552.
25. Gonzalez, M. M.; Vignoni, M.; Pellon-Maison, M.; Ales-Gandolfo, M. A.; Gonzalez-Baró, M. R.; Erra-Balsells, R.; Epe, B.; Cabrerizo, F. M. *Organic and Biomolecular Chemistry* 2012, 10(9), 1807-1819.
26. Gonzalez, M. M.; Rasse-Suriani, F. A. O.; Franca, C. A.; Pis Diez, R.; Gholipour, Y.; Nonami, H.; Erra-Balsells, R.; Cabrerizo, F. M. *Organic and Biomolecular Chemistry* 2012, 10, 9359-9372.
27. Vignoni, M.; Rasse-Suriani, F. A. O.; Butzbach, K.; Erra-Balsells, R.; Epe, B.; Cabrerizo, F. M. *Organic & Biomolecular Chemistry* 2013, 11(32), 5300-5309.
28. Vignoni, M.; Erra-Balsells, R.; Epe, B.; Cabrerizo, F. M. *Journal of Photochemistry and Photobiology B: Biology* 2014, 132(0), 66-71.
29. Gonzalez, M. M.; Denofrio, M. P.; Garcia Einschlag, F. S.; Franca, C. A.; Pis Diez, R.; Erra-Balsells, R.; Cabrerizo, F. M. *Physical Chemistry Chemical Physics* 2014, 16, 16547-16562.
30. Yañuk, J. G.; Alomar, M. L.; Gonzalez, M. M.; Simon, F.; Erra-Balsells, R.; Rafii, M.; Cabrerizo, F. M. *Physical Chemistry Chemical Physics* 2015, 17(19), 12462-12465.
31. Tarzi, O. I.; Ponce, M. A.; Cabrerizo, F. M.; Bonesi, S. M.; Erra-Balsells, R. *Arkivoc* 2005, (vii), 295-310.
32. Tarzi, O. I.; Erra-Balsells, R. *Journal of Photochemistry and Photobiology B: Biology* 2006, 82(2), 79-93.
33. Tarzi, O. I.; Erra-Balsells, R. *Journal of Photochemistry and Photobiology B: Biology* 2005, 80(1), 29-45.
34. Hrsak, D.; Holmegaard, L.; Poulsen, A. S.; List, N. H.; Kongsted, J.; Denofrio, M. P.; Erra-Balsells, R.; Cabrerizo, F. M.; Christiansen, O.; Ogilby, P. R. *Physical Chemistry Chemical Physics* 2015, 17(18), 12090-12099.
35. Gonzalez, M. M.; Salum, M. L.; Gholipour, Y.; Cabrerizo, F. M.; Erra-Balsells, R. *Photochemical & Photobiological Sciences* 2009, 8(8), 1139-1149.
36. Gonzalez, M. M.; Arnbjerg, J.; Paula Denofrio, M.; Erra-Balsells, R.; Ogilby, P. R.; Cabrerizo, F. M. *Journal of Physical Chemistry A* 2009, 113(24), 6648-6656.
37. Cabrerizo, F. M.; Arnbjerg, J.; Denofrio, M. P.; Erra-Balsells, R.; Ogilby, P. R. *ChemPhysChem* 2010, 11(4), 796-798.
38. Alomar, M. L.; Gonzalez, M. M.; Erra-Balsells, R.; Cabrerizo, F. M. *Journal of Photochemistry and Photobiology B: Biology* 2014(136), 26-27.
39. Ponce, M. A.; Tarzi, O. I.; Erra-Balsells, R. *Journal of Heterocyclic Chemistry* 2003, 40(3), 419-426.
40. Cabrerizo, F. M.; Thomas, A. H.; Lorente, C.; Dántola, M. L.; Petroselli, G.; Erra-Balsells, R.; Capparelli, A. L. *Helvetica Chimica Acta* 2004, 87(2), 349-365.
41. Braslavsky, S. E. *Pure and Applied Chemistry* 2007, 85(7), 1437-1449.
42. Cabrerizo, F. M.; Dantola, M. L.; Thomas, A. H.; Lorente, C.; Braun, A. M.; Oliveros, E.; Capparelli, A. L. *Chemistry and Biodiversity* 2004, 1(11), 1800-1811.
43. Thomas, A. H.; Cabrerizo, R.; Vignoni, M.; Erra-Balsells, R.; Cabrerizo, F. M.; Capparelli, A. L. *Helvetica Chimica Acta* 2006, 89(6), 1090-1104.
44. Meech, S. R.; Phillips, D. *Journal of Photochemistry* 1983, 23(2), 193-217.
45. Marti, C.; Jürgens, O.; Cuenca, O.; Casals, M.; Nonell, S. *Journal of Photochemistry and Photobiology A: Chemistry* 1996, 97(1&2), 11-18.
46. Arnbjerg, J.; Johnsen, M.; Frederiksen, P. K.; Braslavsky, S. E.; Ogilby, P. R. *The Journal of Physical Chemistry A* 2006, 110(23), 7375-7385.
47. Hohenberg, P.; Kohn, W. *Phys Rev* 1964, 136(3B), B864-B871.
48. Kohn, W.; Sham, L. J. *Phys Rev* 1965, 140(4A), A1133-A1138.
49. Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*, Oxford University Press, 1989.
50. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; J. A. Montgomery, J.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Keith, T.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision A1*, Gaussian, Inc Wallingford CT 2009.
51. Bauernschmitt, R.; Ahlrichs, R. *Chem Phys Lett* 1996, 256(4-5), 454-464.
52. Casida, M. E.; Jamorski, C.; Casida, K. C.; Salahub, D. R. *J Chem Phys* 1998, 108(11), 4439-4449.
53. Stratmann, R. E.; Scuseria, G. E.; Frisch, M. J. *J Chem Phys* 1998, 109(19), 8218-8224.
54. Barone, V.; Cossi, M. *J Phys Chem A* 1998, 102(11), 1995-2001.
55. Cossi, M.; Barone, V. *J Chem Phys* 2001, 115(10), 4708-4717.

56. Mennucci, B.; Tomasi, J. *J Chem Phys* 1997, 106(12), 5151-5158.
57. Gorelsky, S. I.; Lever, A. B. P. *J Organomet Chem* 2001, 635(1-2), 187-196.
58. Gorelsky, S. I. AOMix program, revision 681, <http://wwwsg-chemnet/>.
59. O'Boyle, N. GaussSum 225 program documentation ([http://gausssumsourceforge.net/GaussSum\\_UVVis\\_Convolutionpdf](http://gausssumsourceforge.net/GaussSum_UVVis_Convolutionpdf)).
60. Tomas Vert, F.; Zabala Sanchez, I.; Olba Torrent, A. *Journal of Photochemistry* 1983, 23(3), 355-368.
61. The small or null effect induced by the chlorine atom placed at C-8 described in (iii) and (iv) was suggested to be a consequence of the short distance between the chlorine atom and the pyrrolic NH group, leading place to the formation of a hydrogen-bridge-like-interaction. Thus, two opposite effects would operate simultaneously: a weak electron-donor effect (mesomeric effect) induced by the chlorine atom together with its spatial interaction with the hydrogen of the NH group by a hydrogen-bond-like interaction.
62. TD-DFT calculations do not produce "pure" H→L or H-1→L excitations as there is a mixing of around 80 - 90 % of the main excitation with 20-10% of the minor contribution.
63. This is in good agreement with the enhancement on the basicity of the pyridinic nitrogen observed in excited bCs.
64. With the exception of 6,8-diCl-Ha (i.e., the neutral form) highly probable due to its fast chemical decomposition observed under alkaline pH conditions.
65. Note that we have no direct evidence to further distinguish the zwitterionic from the anionic species. Although this, based on the literature, we will refer to this species as zwitterion (Z).
66. Turro, N. J., editor. *Modern Molecular Photochemistry*: Menlo Park, 1978.
67. Plummer, B. F.; Steffen, L. K.; Braley, T. L.; Reese, W. G.; Zych, K.; Van Dyke, G.; Tulley, B. *Journal of the American Chemical Society* 1993, 115(24), 11542-11551.
68. Varela, A. P.; Burrows, H. D.; Douglas, P.; da Graça Miguel, M. *Journal of Photochemistry and Photobiology A: Chemistry* 2001, 146(1-2), 29-36.
69. Becker, R. S.; Ferreira, L. F. V.; Elisei, F.; Machado, I.; Latterini, L. *Photochemistry and Photobiology* 2005, 81(5), 1195-1204.
70. Mesaros, M.; Tarzi, O. I.; Erra-Balsells, R.; Bilmes, G. M. *Chemical Physics Letters* 2006, 426(4-6), 334-340.
71. Note that, in aerated aqueous solutions, no transients with lifetimes in the microsecond to second timescale were observed by Varela *et al.* This is in agreement to the fact that lifetimes of the transients in aerated solutions observed in this work are shorter than the excitation pulse used by these authors.
72. Wolfbeis, O. S.; Fuerlinger, E. *Z Phys Chem (Wiesbaden)* 1982, 129, 171-183.
73. Note that for 8-Cl-Ha and 6,8-diCl-Ha a similar set of experiments was not done due to the instability of these two chloroharmines in the time-window of the photochemical experiments.
74. Downum, K. R. *New Phytologist* 1992, 122(3), 401-420.
75. Mercader, A. G.; Duchowicz, P. R.; Fernández, F. M.; Castro, E. A.; Cabrerizo, F. M.; Thomas, A. H. *Journal of Molecular Graphics and Modelling* 2009, 28(1), 12-19.
76. Erra-Balsells, R.; Frasca, A. R. *Tetrahedron* 1983, 39, 33-39.
77. de Meester, C. *Mutation Research/Reviews in Genetic Toxicology* 1995, 339(3), 139-153.

## GRAPHICAL ABSTRACT



In water, chloroharmines follow very distinctive thermal and photochemical pH- and O<sub>2</sub>-dependent-reaction pathways.