Electronic Supplementary Information (ESI)

PHOTOSENSITIZED CLEAVAGE OF PLASMIDIC DNA BY NORHARMANE, A NATURALLY OCCURRING β-CARBOLINE.

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Contents: **Page**

1. Photocleavage of DNA by norharmane performed at pH 4.7.

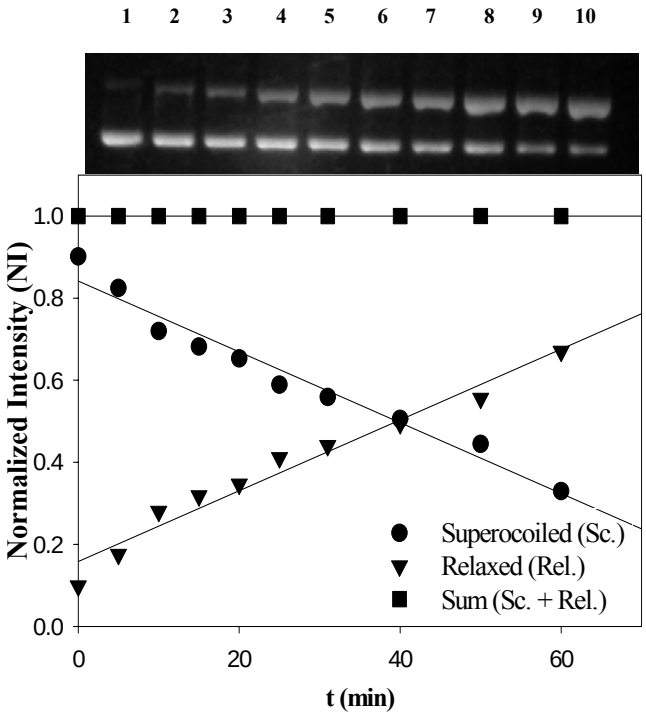

Figure S1. Evolution of normalized relative intensity of pGEM-3z plasmid forms (Sc. and Rel.) as a function of the irradiation time in the presence of norharmane. Experiment performed at pH = 4.7 where $nHoH^+$ is present. Inset: Electrophoretic run: Lane 1 to 10 = solution of $nHoH^+$ and pGEM-3z irradiated during 0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes, respectively. Note that the increase in the relaxed form concentration correlated very well with the decrease in the supercoiled form concentration.

2. Photocleavage of DNA by norharmane performed at pH 7.4.

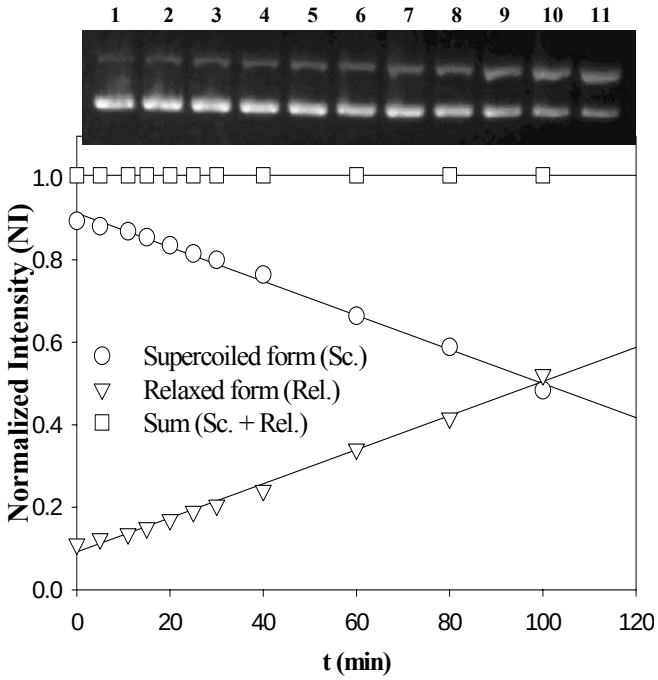

Figure S2. Evolution of normalized relative intensity of pGEM-3z plasmid forms (Sc. and Rel.) as a function of the irradiation time in the presence of norharmane. Experiment performed at pH = 7.4 where a mixture of both $nHoH^+$ and nHoN forms are present. Inset: Electrophoretic run: Lane 1 to 11 = solution of both $nHoH^+$ and nHoN and plasmidic DNA irradiated during 0, 5, 10, 15, 20, 25, 30, 40, 60, 80 and 100 minutes, respectively. Note that the increase in the relaxed form concentration correlated very well with the decrease in the supercoiled form concentration.

3. Photocleavage of DNA by norharmane performed at pH 10.2.

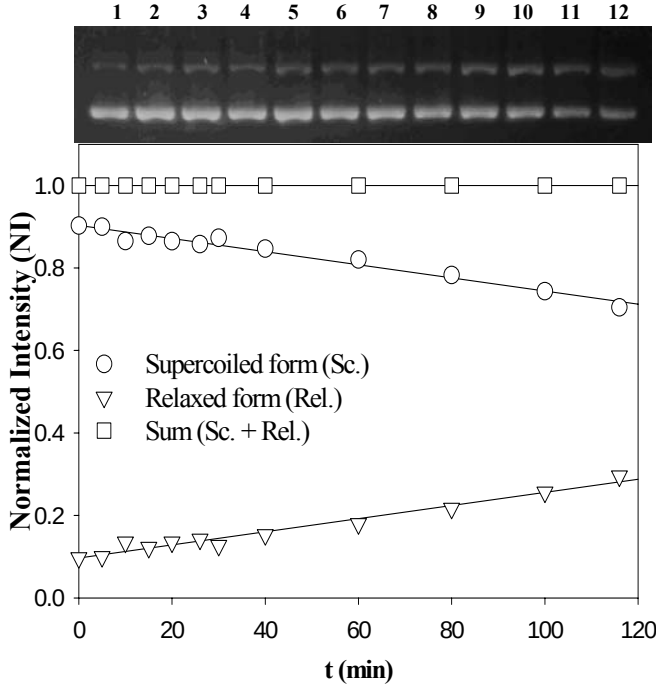

Figure S3. Evolution of normalized relative intensity of pGEM-3z plasmid forms (Sc. and Rel.) as a function of the irradiation time in the presence of norharmane. Experiment performed at pH = 10.2 where nHoN is present. Inset: Electrophoretic run: Lane 1 to 12 = solution of nHoN and plasmidic DNA irradiated during 0, 5, 10, 15, 20, 25, 30, 40, 60, 80, 100 and 116 minutes, respectively. Note that the increase in the relaxed form concentration correlated very well with the decrease in the supercoiled form concentration.

4. Determination of the binding constant (K_G) at pH 4.4.

The association constant (K_G) between $nHoH^+$ (βC) and plasmidic DNA (B) was analyzed by UV-vis spectrophotometric titration. Assuming a 1:1 stoichiometry for the complexes K_G can be estimated by using the Benesi-Hildebrand equation:

$$\frac{1}{\Delta A} = \frac{1}{(\varepsilon_{\beta C \bullet B} - \varepsilon_{\beta C})} \bullet \frac{1}{[\beta C]_o} + \frac{1}{K_G \bullet (\varepsilon_{\beta C \bullet B} - \varepsilon_{\beta C}) \bullet [\beta C]_o} \bullet \frac{1}{[B]} \qquad \textbf{(eq. S1)}$$

where $\varepsilon_{\beta C \bullet B}$ and $\varepsilon_{\beta C}$ are the molar absorption coefficients of $nHoH^+$-DNA complex (βC•B) and $nHoH^+$ (βC), respectively, at the titration wavelength. ΔA is the change of absorbance relative to the completely free $nHoH^+$ at this wavelength.

The plots of the experimental data obtained at pH 4.4, according to equation S1, showed good linearity (see Figure S4), confirming the 1:1 stoichiometry of the complex. K_G obtained from the slope and intercept of these plots were 4.5 (± 0.5) x 10^4 M^{-1}.

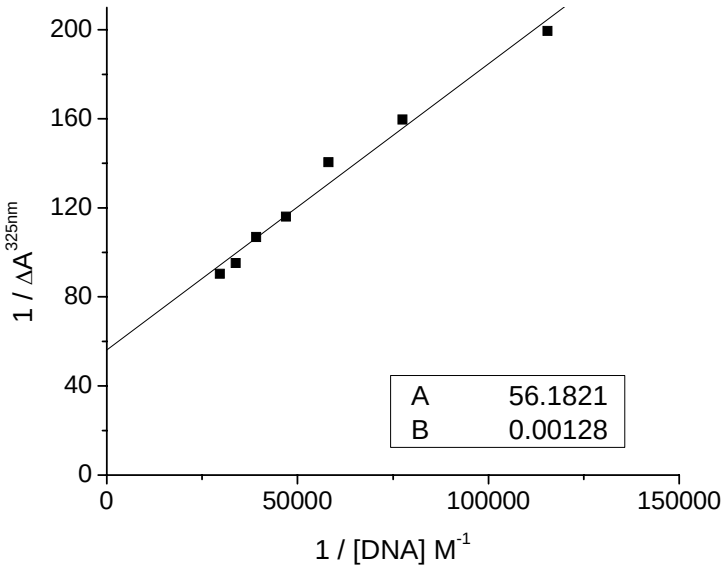

Figure S4. A representative example of the Benesi-Hildebrand plot for $nHoH^+$ / plasmidic DNA system.

5. Numerical Support.

In this discussion, we are providing a numerical estimation of the extent of the DNA photocleavage reaction observed in the experiments performed under physiological pH conditions (pH = 7.4).

In general, all the photophysical and photochemical processes in which the excited state of a photosensitizer ([M]*) is generated and / or deactivated can be described in four main steps or equations: (eq. S2 1) absorption of the incident light by M; (eq. S3 2) fluorescence emission deactivation of M*; (eq. S4 3) all the deactivation pathways of M* other than quenching process with a quencher (Q); and (eq. S5 4) deactivation pathway of M* due to quenching with Q:

$$M + h\nu \xrightarrow{v = P_A} M^* \qquad (S2)$$

$$M^* \xrightarrow{v = k_F^0 [M^*]} M + h\nu' \qquad (S3)$$

$$M^* \xrightarrow{v = \Sigma k_i [M^*]} M \qquad (S4)$$

$$M^* + Q \xrightarrow{v = k_q [M^*] [Q]} M + Q^* \qquad (S5)$$

where P_A is the rate of the absorbed photon flux, k_F^0 is the intrinsic fluorescence rate constant, k_i represents the rate constants of each deactivation pathway different from the quenching process and k_q is the rate constant of the bimolecular quenching.

Taking into account equations S2 to S5, the following equation can be written:

$$\frac{d[M^*]}{dt} = P_A - k_F^0 [M^*] - \Sigma k_i [M^*] - k_q [Q] [M^*] \qquad (S6)$$

Under continuous irradiation and assuming a steady-state regime $-d[M^*]/dt \approx 0$. Thus, the concentration of [M]* can be calculated from equation (S7):

$$[M]^* = \frac{P_A}{k_F^0 + \Sigma k_i + k_q [Q]} \qquad (S7)$$

Considering that $P_A = (1 - 10^{-A}) P_0$, where P_0 is the rate of the incident photon flux and A is the

absorbance at the excitation wavelength (350 nm). Then equation (S7) can be re-written in terms of the fraction of P_0 absorbed by de sensitizer:

$$[M]^* = (1 - 10^{-A})\ C \qquad (S8)$$

where C represents $P_0 / (k_F^0 + \Sigma\ k_i + k_q\ [Q])$.

As it was discussed in the main text of this work the single excited state of the protonated form of norharmane ($^1[nHoH^+]^*$) is the specie responsible for the DNA photocleavage reaction under all the pH conditions analyzed. Therefore, the calculation of the steady-state concentration of $^1[nHoH^+]^*$ produced upon continuous irradiation of the solutions at different pH, would provide a very good estimation on the relative extent of the reaction that should be expected.

(a) $^1[nHoH^+]^*$ steady-state concentration at pH 4.7: Under this pH condition more than a 99 % of norharmane is present in its protonated form ($nHoH^+$). Thus, the whole incident light (350 nm) is absorbed by $nHoH^+$ to yield $[nHoH^+]^*$. Taking into account that the total absorbance (A_T) of the solution at 350 nm was 0.3 (measured in a quartz cell of 4 mm optical path length); then, from eq. (S8):

$$[nHoH^+]^* = 0.5 \text{ x } C \qquad \text{(at pH 4.7)}$$

(b) $^1[nHoH^+]^*$ steady-state concentration at pH 10.2: On the contrary, at pH = 10.2 more than a 99 % of norharmane is present in its neutral form (nHoN). This means that, at 350 nm, the whole incident light is absorbed by nHoN to yield [nHoN]*. However, even in pH 10.2 aqueous solutions, [nHoN]* is readily protonated, by rapid proton exchange with the solvent[1,2]. Thus, the photochemical and photophysical behavior of nHoN occurs mainly from the excited-state of the protonated form of norharmane ($^1[nHoH^+]^*$), instead of the excited state of the neutral form ($^1[nHoN]^*$). It was demonstrated that, in the case of norharmane, only a fraction ~ 28 % of the S_1 of nHoN goes on with the protonation yielding $[nHoH^+]^*$[3,4]. Taking into account that A_T was 0.3 (4 mm optical path length), then:

$$[nHoH^+]^* = 0.28 \text{ x } [nHoN]^* = 0.28 \text{ x } 0.5 \text{ x } C = 0.14 \text{ x } C \qquad \text{(at pH 10.2)}$$

This value corresponds to a 28 % decrease in the $[nHoH^+]^*$ steady-state concentration, in comparison with the value obtained at pH 4.7 (0.5 x C). As consequence, should be observed a ~30 % decrease of the DNA photocleavage reaction at pH 10.2 in comparison with the reaction at pH 4.7. The

experimental results described in the Discussion section of the present paper are in good agreement with this calculation (i.e., the initial relaxation rates of the supercoiled DNA (d[Sc]/dt) observed were -8.2 (± 0.9) x 10^{-3} NI × min^{-1} and -2.3 (± 0.5) x 10^{-3} NI × min^{-1} at pH 4.7 and 10.2, respectively; indicating a decrease of 28 %).

(c) $^1[nHoH^+]$ steady-state concentration at pH 7.4:* In aqueous solution norharmane shows an acid-base equilibrium with a pK_a value of 7.2. Thus, at pH 7.4 a mixture of both protonated ($nHoH^+$) and neutral (nHoN) form of norharmane are present in the irradiated solution (i.e., ~ 38 % and 62 % of $nHoH^+$ and nHoN, respectively). Despite the fact that this experiment was also done by using a solution with a total absorbance (A_T) of 0.3 (4 mm optical path length), the fraction of the incident light that is absorbed by each norharmane species should be taken into account when calculation $[nHoH^+]$* steady-state concentration is attempted.

On the basis of the pK_a (7.2) and the A_T (0.3), at pH = 7.4 the initial concentration of $nHoH^+$ and nHoN are 85 μM and 135 μM, respectively. Taking into account both the corresponding molar absorption coefficient (ε), at the excitation wavelength, of each species (i.e., ε = 2776 M^{-1} cm^{-1} and 3807 M^{-1} cm^{-1} for $nHoH^+$ and nHoN, respectively), and the optical path length, the contribution to the A_T of $nHoH^+$ and nHoN can be calculated (i.e., 0.09 and 0.21, respectively).

Eq. (S8) can be used to calculate the steady-state concentration of $^1[nHoH^+]$* also under this pH condition. For this purpose eq. (S8) should be slightly modified to take into account the contribution of $nHoH^+$ and nHoN to the total $^1[nHoH^+]$* concentration:

$$[nHoH^+]^* = (1 - 10^{-0.09}) \times C + (1 - 10^{-0.21}) \times 0.28 \times C = 0.30 \times C \qquad \text{(at pH 4.7)}$$

This latter value corresponds to a 60 % decrease in the $[nHoH^+]$* steady-state concentration in comparison with the value obtained at pH 4.7 (0.5 x C). This fact should induce a ~60 % decrease of the DNA photocleavage reaction at pH 7.4 in comparison with the reaction performed at pH 4.7. This prediction agrees with the experimental results described main text, where d[Sc]/dt values observed were -8.2 (± 0.9) x 10^{-3} NI × min^{-1} and -4.2 (± 0.5) x 10^{-3} NI × min^{-1}, at pH 4.7 and 7.4, respectively (i.e., a decrease of 54 %).

6. References.

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3 M. M. Gonzalez, M. L. Salum, Y. Gholipour, F. M. Cabrerizo and R. Erra-Balsells, *Photochem. Photobiol. Sci.*, 2009, **8**, 1139–1149.
4 M. M. Gonzalez, J. Arnbjerg, M. P. Denofrio, R. Erra-Balsells, P. R. Ogilby and F. M. Cabrerizo, *J. Phys. Chem. A*, 2009, **113**, 6648–6656.