

Electronic Supplementary Information

for

**Drug-DNA Complexation as the Key Factor
in Photosensitized Thymine Dimerization**

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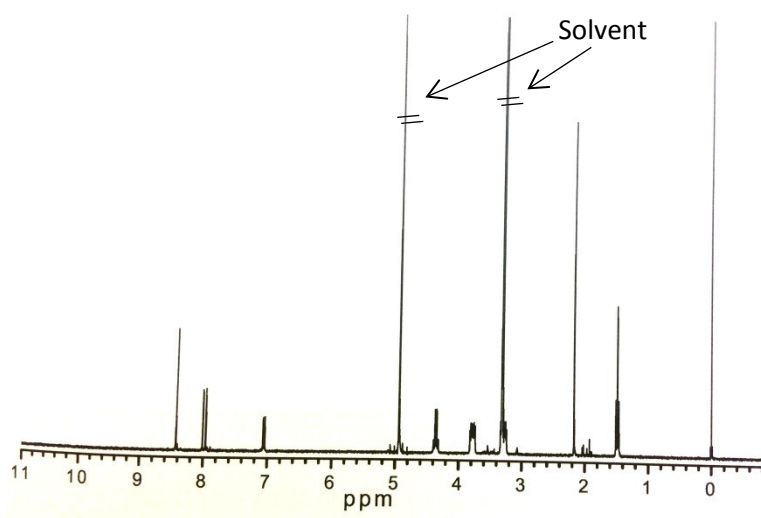
Content: Experimental procedures

Experimental Procedures

All the spectroscopic measurements were performed at room temperature using quartz cells of 1 cm optical path, if not otherwise stated.

Materials. Norfloxacin (NFX), ofloxacin (OFX) and enoxacin (ENX) were obtained from Sigma-Aldrich). Rufloxacin (RFX) hydrochloride was from Bracco (Milan, Italy). Supercoiled circular pBR322 DNA was obtained from Roche Diagnostics (Barcelona, Spain). The DNA repair enzyme T4 endonuclease V (endo V) and its buffer were purchased from AbBcn S.L. (Barcelona). Other chemicals were of reagent grade and used as received. The sodium phosphate buffer (PB, 1 mM) was prepared from reagent-grade products using deionized water. The pH of the solutions was measured through a glass electrode and adjusted with NaOH to be pH 7.4.

Synthesis of 7-(4-Acetyl-1-piperazinyl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (ANFX). A solution of 1-ethyl-6-fluoro-7-(1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (NFX) (150 mg, 0.47 mmol) in Ac₂O (25 mL) was refluxed for 5 h. The solution was cooled to room temperature and concentrated. The residue was dissolved in water, neutralized to pH 7.4, and extracted with CH₂Cl₂. The organic phase was concentrated to dryness. The ¹H-NMR spectrum was mainly coincident with that reported by Koga *et al.*¹



¹H-NMR (CD₃OD, 300 MHz) spectrum of ANFX

Emission measurements. The fluorescence of the samples (FQ at 5 × 10⁻⁵ M in 1 mM PB aqueous media with and without the presence of DNA) was registered upon excitation at 350 nm. The DNA binding constant (K_a) was determined using the fluorescence quenching method.^{2,3} The FQ (10⁻⁵ M) fluorescence was quenched by DNA after excitation at 350 nm. The DNA concentrations were determined spectrophotometrically taking into account a molar extinction coefficient ε_{258 nm} = 6700 cm⁻¹ M⁻¹.^{2,3} Equation 1 was selected to determine the drug-DNA interactions from fluorescence quenching data:^{2,3}

$$F_0/F = 1 + K_{sv} [Q] \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of the quencher respectively, $[Q]$ is the quencher concentration (DNA from 10^{-5} to 2×10^{-3} M in base pair) and Stern-Volmer quenching constant (K_{sv}). Hence, a K_a of ca. $0.4 \times 10^3 \text{ M}^{-1}$ was determined for ANFX, which is more than 10 times lower than the obtained for NFX, ENX, OFX and RFX (K_a ca. $3 \times 10^3 \text{ M}^{-1}$ for all FQ, which is close to the described values ²⁻⁴

The phosphorescence measurements were performed using 5×10^{-5} M fluoroquinolones in 2 mM PB aqueous media with and without the presence of increasing amounts of DNA (from 1 mM to 10 mM in base pair) and in 2 mM PB/ETG (1/2, v/v). The samples were placed in a quartz tube and cooled at 77K prior registering their spectra.

Laser flash photolysis (LFP) was run using a homemade set up using a Nd:YAG laser ($\lambda_{exc} = 355 \text{ nm}$) with single pulses were ca. 10 ns duration. The detecting light source employed was a pulsed Xe lamp. The LFP apparatus consisted of the pulsed laser, the Xe lamp, a monochromator, and a photomultiplier made up of a tube, housing, and power supply. The output signal from the oscilloscope was transferred to a personal computer.

The FQ samples (5×10^{-5} M) were purged with N_2O to remove formation of solvated electrons. The triplet lifetime norfloxacin and ANFX (ca. 4 μs and 10.5 μs , respectively) were not reduced by DNA additions. In this context, considering that thymine in DNA must be ca. 1 mM at 2 mM in base pair of DNA, the triplet energy transfer rate constant between these drugs and the thymine of DNA must be lower than $10^{-7} \text{ M}^{-1} \text{ s}^{-1}$.

Femtosecond transient absorption spectroscopy. Transient absorption spectra were recorded using a typical pump-probe system. The femtosecond pulses were generated with a compact regenerative amplifier that produces pulses centered at 800 nm (τ_{pulse} 100 fs approx., 1 mJ/pulse). The output of the laser was split into two parts to generate the pump and the probe beams. Thus, tunable femtosecond pump pulses were obtained by directing the 800 nm light into an optical parametric amplifier. In the present case, the pump was set at 320 nm and passed through a chopper prior to focus onto a rotating cell (0.8 mm optical path) containing the aqueous solutions of 3×10^{-4} M NFX in the presence and absence of 1 and 2 mM DNA (in base pair). The white light used as probe was produced after part of the 800 nm light from the amplifier travelled through a computer controlled 8 ns variable optical delay line and impinge on a CaF_2 rotating crystal. This white light is in turn, split in two identical portions to generate reference and probe beams that then are focused on the rotating cell containing the sample. The pump and the probe are made to coincide to interrogate the sample. A computer controlled imaging spectrometer is placed after this path to measure the probe and the reference pulses and obtain the transient absorption decays/spectra.

Photosensitized Damage to DNA. All the DNA and drug stock solutions were prepared in water. Samples containing 5 μL (40 μM in base pair) of supercoiled circular DNA (pBR322, 4361 base pair) in absence or presence of the drugs (5 μL from 40 to 80 μM) were employed in

the experiments. The samples were irradiated using a multilamp photoreactor with lamps emitting in the 300-400 nm range with a maximum at 350 nm. Next, they were incubated for 1 h at 37 °C with an excess of T4 endonuclease V. The samples were loaded on a 0.8% agarose gel containing ethidium bromide. After electrophoresis, the relative abundance of supercoiled DNA (form I) and relaxed DNA (form II) was quantified by densitometry.

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- (3) Sortino, S.; Condorelli, G. *New J. Chem.* **2002**, 26, 250.
- (4) Vijan, L. E. *Optoelectron. Adv. Mat.* **2009**, 3, 60.