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Photonucleophilic Aromatic Substitution of 6-Fluoroquinolones in Basic Media: Triplet Quenching by Hydroxide Anion

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Photoreaction of 1-ethyl-6-fluoro-7-(1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (norfloxacin, NFX) and other 6-fluoroquinolones in aqueous solution gives rise to the corresponding 6-hydroxy derivatives. Although two mechanisms have been proposed for this photonucleophilic aromatic substitution, direct evidence for any of them is still missing. Obtaining such evidence requires work in basic media, where intramolecular electron transfer from the piperazine ring to the quinolone system is the almost exclusive singlet deactivation pathway. To overcome this problem, the 4′-N-acetyl derivative of norfloxacin (ANFX) has been employed in the present paper due to the lower availability of the N lone pair. The photochemical and photophysical properties of ANFX have been studied in aqueous solutions at pH between 7.4 and 13. As expected, fluorescence of ANFX is not significantly quenched in basic media. Furthermore, the excited triplet state (λ_{max} = 620 nm) reacts with hydroxide anions with a rate constant of $(0.3 \pm 0.1) \times 10^6$ M^{-1} s^{-1}. This supports a direct attack by hydroxide anions to the excited triplet state with subsequent release of fluoride as the operating mechanism. The fact that the reaction is inhibited by the presence of naproxen (a water-soluble naphthalene derivative) as triplet quencher clearly confirms the mechanistic assignment.

Introduction

Owing to the high energy of the C−F bond, its photochemical cleavage in fluoroaromatics is a relatively rare event.[1,2] Under reductive conditions, defluorinated products are obtained, presumably via loss of fluoride from the aromatic radical anions; however, in some cases products of formal photonucleophilic substitution have been reported.[3−7] This is particularly the case for some quinolones with fluorine substituents.[8,9]

Fluoroquinolones (FQs) are widely used antibacterial agents; however, they exhibit photosensitivity side effects.[10] In general, these drugs are responsible for undesired sunlight-induced dangerous cutaneous reactions,[11−13] and some of them can even operate as photomutagenicand photocarcinogenic agents.[13−16] Although this has stimulated a number of photochemical and photobiological studies, the processes involved in the photosensitization are not clearly established, and the photophysical and photochemical properties of FQs are not completely understood. Thus, it has been found that photodegradation of FQs in aqueous solution leads mainly to defluorination and cleavage of the cyclic amino substituent at C-7.[8,9,17−19] These processes strongly depend on the reaction medium, especially on the pH[8,17,20] and on the presence of salts.[20−23] The photochemical reactions of FQs with cell components (for instance,

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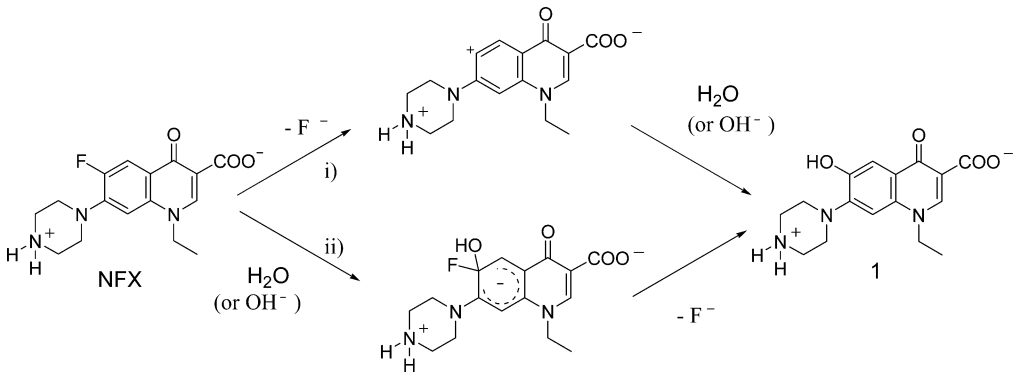
SCHEME 1. Possible Mechanistic Pathways for the Photodegradation of NFX



photosubstitution of fluoride by the nucleophilic groups present in biomacromolecules) could be in the origin of the observed photobiological effects.

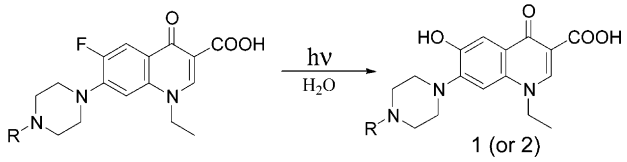
In this context, the photochemistry of 1-ethyl-6-fluoro-7-(1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (norfloxacin, NFX) and other 6-monofluoro derivatives in nucleophilic media has been previously studied.[8,17,20] The main photoreaction observed in neat water at neutral pH is photosubstitution of the fluorine atom to give 6-hydroxyquinolones.[8] Two different reaction pathways have been proposed for this photoreaction:[8,24] (i) heterolytic C−F cleavage to give an aryl cation and (ii) water addition, followed by loss of fluoride anion (Scheme 1).[25] Both mechanisms have been suggested to occur from the triplet excited state on the basis of the decreasing photodegradation quantum yield in the presence of oxygen. Moreover, although pathway ii has been favored, direct mechanistic evidence is still missing. In neutral aqueous solutions, FQs are mainly present as zwitterions; however, at higher pH intramolecular electron transfer can occur from the deprotonated piperazine ring to the quinolone system, providing an efficient energy-wasting channel for deactivation of the singlet excited state. Thus, in basic media neither fluorescence emission nor the typical transient T−T absorption can be detected. This has precluded performing systematic product and mechanistic studies in the presence of increasing concentration of hydroxide anions,[24] as it has been done in related photosubstitutions to discriminate between uni- and bimolecular kinetics.[2]

In the present work, evidence has been obtained for nucleophilic quenching of triplet FQs by hydroxide by means of steady-state and laser flash photolysis (LFP) studies. To overcome the problem of singlet deactivation in alkaline solutions, the 4′-N-acetyl derivative of norfloxacin (ANFX) has been employed, due to the lower availability of its 4′-N lone pair.

Results

Photochemistry of Acetylated Norfloxacin (ANFX). ANFX (60 μM) was irradiated with a multilamp photoreactor, at λ_{max} 350 nm (Gaussian distribution) in deaerated aqueous solutions at pH = 7.4. Under these conditions, ANFX was found to be photolabile, giving rise to its 6-hydroxy derivative **2** as the only photoproduct (Scheme 2). The same results were obtained in the absence or presence of phosphate buffer (PB) from 10 to 100 mM. A similar 6-hydroxy derivative **1** has been reported as the major photoproduct of NFX in aqueous solution.[8] In basic medium (pH ca. 12) ANFX was also photolabile, while NFX was unreactive (Figure 1A); compound **2** was again the major photoproduct. Photodegradation of ANFX was studied at different pH values. All experiments were performed in the presence of 0.1 M $NaClO_4$. The results (Figure 1B) show that at pH above 10 a clear acceleration of the reaction took place.

SCHEME 2. Irradiation Products of (A)NFX in Neutral Aqueous Solution under Anaerobic Conditions



NFX and 1: R = H
ANFX and 2: R = $COCH_3$

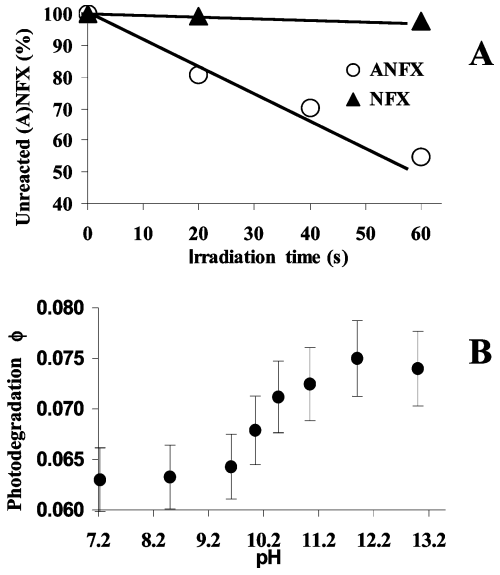


FIGURE 1. (A) Time course of photolysis of aqueous solutions of (A)NFX (60 μM) at pH = 12 under anaerobic conditions. (B) Quantum yields (ϕ) of ANFX photodegradation at different pH. Both series of experiments were performed in the presence of $NaClO_4$ (0.1 M).

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(25) A reviewer has suggested that an additional possibility could be reversible formation of the aryl cation−fluoride ion pair.

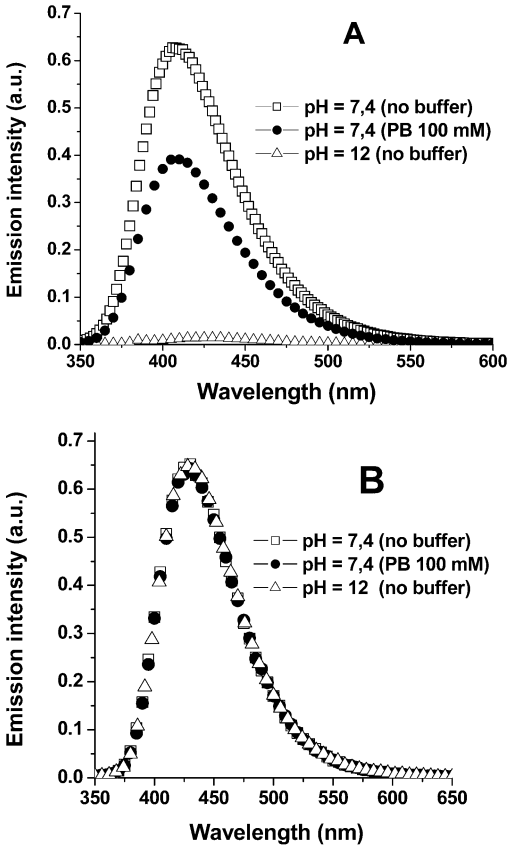

FIGURE 2. Fluorescence spectra of NFX (A) and ANFX (B) in aqueous solution at pH 7.4 (with and without 100 mM PB) and at pH 12.

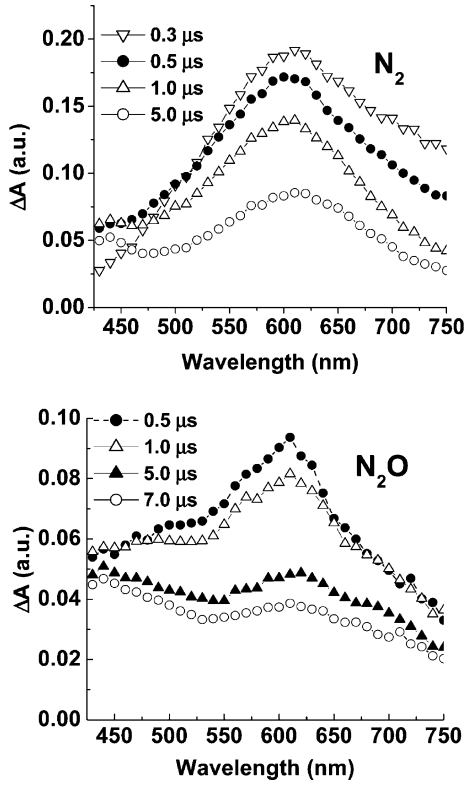

FIGURE 3. Transient absorption spectra of 6×10^{-5} M ANFX in aqueous 10 mM PB solutions (absorbance 0.3 at 355 nm) obtained after laser excitation, under nitrogen (top panel) and N_2O (bottom panel).

Fluorescence Measurements. The emission spectrum of ANFX in aqueous solution at pH 7.4 showed a broad band with maximum at λ_{max} 427 nm (Figure 2). This maximum is red-shifted around 17 nm with respect to NFX. The fluorescence quantum yield (ϕ_F) of ANFX was 0.13, similar to that reported for NFX ($\phi_F = 0.12$).[24]

The emission of ANFX at pH 12 was similar to that obtained at pH = 7.4, not only in the λ_{max} but also in the quantum yield. This is in sharp contrast with the behavior of NFX, which does not exhibit significant fluorescence at pH higher than 11.[21] The lifetime of ANFX singlet excited state was 2 ns at both neutral and basic pH; by contrast, the fluorescence lifetime of NFX has only been determined under neutral conditions and found to be in the range $\tau = 1.5-2.3$ ns.[21,24]

Fluorescence measurements were also performed in the presence of different amounts of PB buffer (from 10 to 100 mM). No significant changes were observed for ANFX, while NFX singlet excited state was quenched by PB as previously reported.[24] This agrees well with the concept that only fluoroquinolones with two hydrogens in the protonated 4′ nitrogen of the piperazinyl ring can interact with the phosphate dianion, resulting in static quenching.[24] This is not possible in the case of ANFX.

Identification of the Major Transient Intermediates. Transient absorption spectra of ANFX in 10 mM PB aqueous solutions at pH = 7.4 were obtained upon 355 nm laser excitation, under both nitrogen and N_2O atmospheres (Figure 3). In general, they were very similar to those previously described for NFX,[24] with two major components:

(a) The solvated electron, a broad and structureless absorption band with a flat maximum at 720 nm, which is efficiently scavenged under N_2O saturated solutions,[26] whose spectrum was obtained by difference between the transient absorptions of ANFX under nitrogen and N_2O 100 ns after the laser pulse (see Supporting Information). Its lifetime was found to be 0.28 μs, a value close to that previously obtained in the laser flash photolysis of NFX ($\tau_e = 0.25$ μs).[27] According to the literature, this is assumed to result from a biphotonic process.[27]

(b) A broad band with λ_{max} 620 nm, which can be attributed to the ANFX triplet excited state because, in addition to showing a similar transient absorption spectrum to that described for NFX triplet excited state,[24] it was quenched by oxygen with a rate constant of $(2.1 \pm 0.4) \times 10^9$ M^{-1} s^{-1}. The assignment was confirmed by energy transfer to naproxen (NP, a water-soluble naphthalene derivative), that was found to occur with a rate constant of $(2.0 \pm 0.4) \times 10^9$ M^{-1} s^{-1}. Decay of ^{3}ANFX was parallel to the formation of ^{3}NP at 430 nm (Figure 4). As expected, the same rate constant was obtained for quenching of ^{3}NXF by NP.

Reaction of Triplet ANFX with Hydroxide Anions. LFP experiments were performed at different pH values (from 7.4 to 13.4) in order to investigate the reactivity of ^{3}ANFX with hydroxide anions (Figure 5). The

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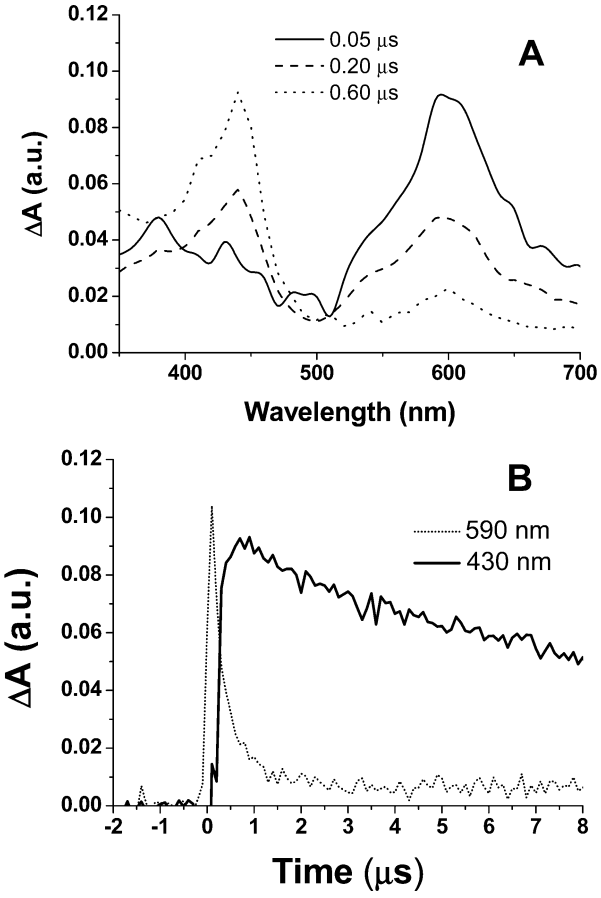

FIGURE 4. (A) Absorbance changes observed upon laser flash photolysis (LFP) of an aqueous solution of ANFX under N_2O, in the presence of 2 mM NP as the triplet quencher. (B) Decay and growth traces at 430 and 590 nm.

shapes of the transient absorption spectra did not show observable changes, but the triplet lifetime decreased with increasing pH. The rate constant for ^{3}ANFX quenching by OH^- was $(0.3 \pm 0.1) \times 10^6$ M^{-1} s^{-1}. It was not possible to do similar studies for NFX, as no transient absorption spectrum was observed for this compound at pH higher than 11.

The influence of phosphate anions was also investigated. At 10 mM PB the triplet lifetime of ANFX was 7.00 ± 0.15 μs, while under the same conditions ^{3}NFX lived clearly shorter (2.3 μs).[24] At higher PB concentrations (up to 100 mM), at pH 7.4, no significant ^{3}ANFX quenching was observed. This contrasts with the case of ^{3}NFX, which is quenched by PB with a rate constant of 8×10^7 M^{-1} s^{-1}.[24]

Photochemistry of ANFX in the Presence of a Triplet Quencher. Irradiation of deaerated solutions of ANFX in 10 mM PB was performed with the laser beam at 355 nm in the presence and absence of NP (0.5 mM) to disclose the possible role of the excited triplet state in the photoreaction pathway. Under these conditions (where NP does not absorb), substantial quenching of triplet ANFX (ca. 87%) occurs; by contrast, less than 5% fluorescence quenching is observed. The results showed that photodegradation was much slower when NP was present (quantum yield 0.011 vs 0.063).

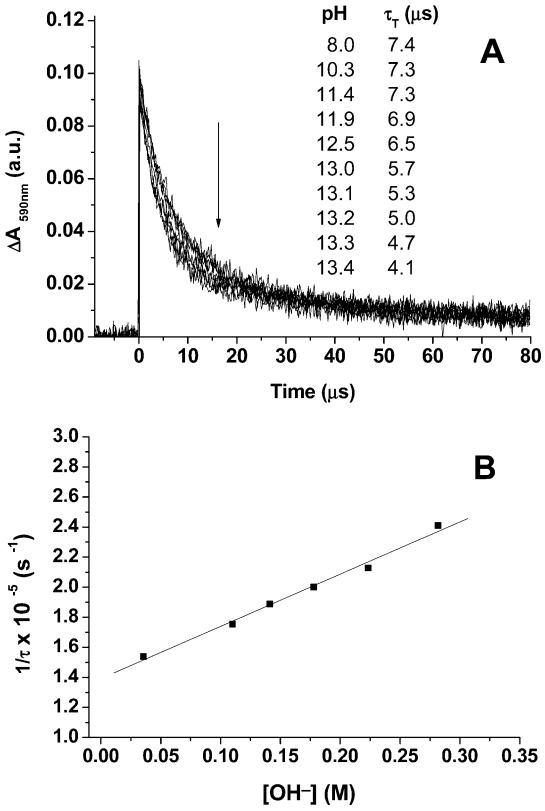

FIGURE 5. (A) Decay profiles of the signal at 590 nm obtained after laser excitation of ANFX aqueous solutions at different pH values. (B) Reciprocal lifetime versus OH^- concentration. Ionic strength was kept constant (1.5 M $NaClO_4$) in all the experiments.

Discussion

Acetylated norfloxacin (ANFX) is photolabile at pH = 7.4 under anaerobic conditions. Its major photoproduct is the corresponding 6-hydroxy derivative **2** (see Scheme 2). This compound is analogous to that described for the parent compound NFX. However, important differences have been found when irradiation was performed in basic aqueous solutions (pH = 12). Thus, while NFX was shown to be stable to light, ANFX was quickly photodegraded. A key factor to explain this fact would be the availability of the 4′-N lone pair of the piperazinyl ring. In NFX, it is free at pH = 12 but not at pH = 7.4, due to protonation. Conversely, in the case of ANFX the lone pair is blocked by resonance with the acyl group at both pHs.[28] Hence, intramolecular electron transfer from this nitrogen to the fluoroquinolone ring system would provide an efficient energy-wasting channel, being responsible for the photostability of NFX in basic media. This hypothesis is supported by fluorescence studies. The intensity of the emission spectrum of ANFX did not change with increasing alkalinity of the medium (OH^- concentration from 10^{-7} to 10^{-1} M). Similar quantum yields ($\Phi_F = 0.13$) and lifetimes (τ 2 ns) were obtained at pH = 7.4 and at pH 12. This clearly shows that the ANFX singlet excited state is not affected by the presence of hydroxide anions. However, in the case of NFX the fluorescence quantum

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yield decreased with pH (from 0.12 at pH = 7.4 to less than 0.001 at pH ca. 12). This fact was previously observed and rationalized as due to different intrinsic radiative constants of the fluoroquinolone anion and the zwitterionic form.[21] In view of the present results, this effect can be better understood by means of the above-mentioned intramolecular electron transfer from the 4′-N piperazinyl group to the fluoroquinolone ring system in its singlet excited state.

On the other hand, it has been postulated that defluorination of NFX in aqueous solutions at neutral pH occurs from its triplet excited state. This is in agreement with the fact that oxygen and several anions, such as phosphate buffer, are able to quench the NFX triplet excited state and decrease NFX photodegradation rates.[24]

In this context, two reaction mechanisms have been proposed: (i) heterolytic cleavage of triplet NFX, followed by nucleophilic trapping of the resulting aryl cation, or (ii) direct nucleophilic attack of OH^- or H_2O to ^{3}NFX, to give subsequent release of fluoride. The lack of NFX signal in alkaline medium precluded the possibility of providing direct LFP evidence in favor of the second reaction pathway. The use of ANFX could be a valuable tool for this purpose, as both fluorescence and T−T absorption are observable at higher pH values.

In the LFP experiments with ANFX from pH = 7.4 to 13.4, a transient absorption spectrum was detected that was assigned to the triplet excited state based on its quenching not only by oxygen but also by NP. The fact that similar intensities of the T−T band of ANFX were observed in the whole range indicates the formation of ANFX triplet with similar quantum yields. Although the hydrated electron was also observed as transient, its origin is assumed to be biphotonic;[27] thus, this species should not play any role in the photoreaction mechanism upon lamp irradiation.

On the other hand, the photoreaction was faster at higher pH values, and the triplet was quenched by OH^- anions with a measurable rate constant of $(0.3 \pm 0.1) \times 10^6\ M^{-1}\ s^{-1}$. This is in agreement with photosubstitution following pathway ii, involving direct attack of the nucleophile to the excited triplet. Accordingly, photolysis of ANFX in the presence of the triplet quencher NP proceeded much more slowly than in the absence of this quencher. The fact that the photodegradation rates follow a plateau behavior in the pH range 7−10 suggests that, under these conditions, water is the actual nucleophile. Taking into account the relative concentrations of H_2O and OH^- at pH = 10 (55 M vs 10^{-4} M, respectively), the rate constants for water attack to triplet ANFX must be at least 3 orders of magnitude lower than that of hydroxide.

Finally, the mechanistic insight gained in the present work allows a better understanding of previous observations on the photochemistry and photophysics of norfloxacin, enoxacin, and related monofluorinated quinolones, which lead also to 6-hydroxyquinolones by photosubstitution of fluoride anion in neutral media and are unreactive under basic conditions.

Conclusions

The photodegradation of ANFX in basic media, under anaerobic conditions, involves photonucleophilic substitution of fluoride anion. This process is explained by direct attack of OH^- to the excited triplet state, to give a cyclohexadienyl anion, with subsequent release of fluoride. Inhibition of this process by addition of a triplet quencher is in agreement with the involvement of the ANFX triplet excited state. On the other hand, the 4′-N of the piperazinyl ring plays an important role in the photochemistry and the photophysics of these compounds. When its electron lone pair is fully available, the singlet excited state of the fluoroquinolone system is quenched through intramolecular electron transfer. This precludes observation of fluorescence or any LFP transient and leads to photostability.

Experimental Section

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 Ac_2O (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 CH_2Cl_2. The organic phase was concentrated to dryness. The ^{1}H NMR spectrum was essentially coincident with that previously reported by Koga et al.[29]

Irradiation Procedures. Irradiations were performed in a multilamp photochemical reactor equipped with six lamps emitting in the 310−390 nm range, with a maximum at 350 nm. Samples of different pH were obtained by dropwise addition of some stock solutions of NaOH (from 10 to 0.01 M); pH values were measured by means of a glass electrode. $NaClO_4$ (0.1 M) was present in all the solutions. The photoreactions were performed under anaerobic conditions and monitored by HPLC on an analytical C18 column (25 × 0.4 cm, mean particle size 5 μm) with a flow rate of 0.7 mL/min and a mixture of acetonitrile/water/trifluoracetic acid 15/84.9/0.1 as eluent. Photolysis of aqueous solutions of (A)NFX (60 μM) at pH 7.4 was performed with and without PB (10−100 mM). Moreover, irradiations of (A)NFX in aqueous solutions at pH ∼ 12 were carried out.

For quantum yield measurements, irradiations were performed at different pH values (7.2−13.2) and the photodegradation of ANFX was also followed by HPLC. The quantum yields were obtained by comparison with the value reported for NFX photodegradation (0.06),[29] which was used as actinometer.

On the other hand, irradiation of deaerated solutions of ANFX in 10 mM PB in the presence and absence of NP (0.5 mM) was performed with the laser beam at 355 nm as the light source. Under these conditions there is no remarkable quenching of the ANFX fluorescence by NP (less than 5%) and 99% of the light is absorbed by the ANFX ground state.

Irradiation of ANFX: Isolation of 1-Ethyl-1,4-dihydro-6-hydroxy-4-oxo-7-(4-acetyl-1-piperazinyl)quinoline-3-carboxylic Acid (2). ANFX (80 mg, 0.21 mmol) was dissolved in water (2 L) and the solution was irradiated for 6 h. The resulting solution was extracted with CH_2Cl_2 and the organic layer was concentrated down to 50 mL. After the organic layer was cooled in an ice bath, a solution of diazomethane (216 mg, 4 mmol) in diethyl ether (100 mL) was added dropwise. The mixture was stirred at room temperature overnight. Then it was concentrated under vacuum, and the residue was purified by silica gel chromatography (eluting with 85/15 CH_2Cl_2/MeOH solution) and subsequently by semipreparative HPLC, with 74.9/0.01/25 $H_2O/CF_3COOH/CH_3CH$ as eluent. In this way, compound 2 was characterized as its methyl ester. ^{1}H NMR

(29) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.

(CD_3OD) $\delta = 1.57$ (t, $J = 7.1$ Hz), 2.18 (s, 3H), 3.46 (m, 2H), 3.54 (m, 2H), 3.80 (m, 4H), 4.03 (s, 3H), 4.70 (q, 2H), 7.20 (s, 1H), 7.70 (s, 1H), 9.10 (s, 1H). ^{13}C NMR (CD_3OD) $\delta = 14.9$, 21.2, 42.6, 47.4, 49.9, 50.5, 50.9, 53.3, 105.7, 106.9, 109.37, 119.6, 136.6, 147.8, 149.9, 151.8, 161.64, 166.0, 171.9. HRMS Calcd for $C_{19}H_{23}N_3O_5$: 373.1638. Found: 373.1632.

Fluorescence Measurements. The procedure to determine the fluorescence quantum yields and the fluorescence lifetimes was to fix the absorbance of the solutions at 0.1 at the excitation wavelength of 340 nm. The fluorescence quantum yield of quinine bisulfate in 1 N H_2SO_4 ($\phi_F = 0.546$) was used as standard. Samples of different pH were obtained by dropwise addition of some stock solutions of NaOH (from 10 to 0.01 M) to obtain each pH value, which was measured by means of a glass electrode.

Laser Flash Photolysis Measurements. A pulsed Nd:YAG laser was used for the excitation at 355 nm. The single pulses were ~10 ns duration and the energy was from 10 to 1 mJ/pulse. A pulsed xenon lamp was employed as detecting light source. The laser flash photolysis 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.

All samples of ANFX and NFX were dissolved in the different aqueous solutions to have an absorbance of 0.3 at 355 nm. Solutions were deaerated by bubbling nitrogen (when specified) or N_2O. Naproxen (NP) was used as quencher for the triplet states of both fluoroquinolones through an energy transfer mechanism. The experiment was carried out with solutions of the FQ in 10 mM PB aqueous solutions at neutral pH with increasing amounts of the quencher, ensuring that no changes in the pH are induced. As NP does not absorb at 355 nm (see Supporting Information) under these conditions, more than 99% of the light was absorbed by the FQ. Moreover, possible quenching effects of PB and OH^- concentration were investigated. Thus, as in the case of NP, increasing amounts of PB (10, 25, 50, and 100 mM) were used, keeping the pH at 7.4. On the other hand, nonbuffered aqueous solutions were also used at different pHs; in all these cases the ionic strength was maintained by addition of 1.5 M $NaClO_4$. Samples of different pH were obtained by dropwise addition of some stock solutions of NaOH (from 10 to 0.01 M); pHs were measured by means of a glass electrode. The OH^- concentrations were determined from the experimental pH values. For this purpose, k_w was taken to be 13.95, which is the value of the water ionization in the presence of 1.5 M $NaClO_4$ at room temperature.[30] The rate constants of triplet excited-state quenching by OH^-, oxygen, and NP were determined by use of the Stern−Volmer equation ($1/\tau = 1/\tau_0 + k$[quencher]. Concentrations between 0.5 and 2 mM were used for NP and 1.27 and 0.27 mM for oxygen (concentrations of pure O_2 gas and air at room temperature, respectively).

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Supporting Information Available: General experimental procedures, NMR spectra (^1H, ^{13}C, DEPT) for the new compound, UV spectra of ANFX and NP, the transient spectrum of the hydrated electron, and raw kinetic data, as well as equations used for calculations of the quenching rate constants (PDF). This material is available free of charge via the Internet at http:// pubs.acs.org.

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