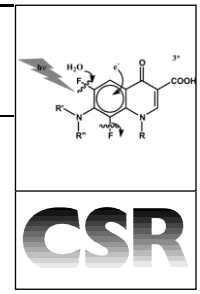
Photophysics and photochemistry of fluoroquinolones



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The photobehavior of fluoroquinolone antibiotics, one of the most successful classes of drugs in therapeutic applications, has recently been the object of increasing interest due to the finding of their phototoxic and photocarcinogenic properties. The main results obtained for a series of structurally related, representative fluoroquinolone drugs is reviewed. Both activation of oxygen and various degradation pathways have been identified and the effects of medium and structure have been rationalized. The results can help in the understanding of the photochemistry occurring in biological environments and in the assessing of the correlation between structural characteristics and biological photodamage.

1 Introductory

Fluoroquinolone antibiotics (FQs) are one of the most successful classes of drugs. They are considered to be well tolerated drugs with relatively limited adverse effects,[1,2] so that the use of several compounds of the series in therapy is increasing. In spite of this, they belong to one of the two classes of drugs (the other one is the NSAIDs, Non Steroidal Anti-Inflammatory Drugs) that induce phototoxicity as a significant side effect. In general, the mechanisms underlying the phototoxic reactions are not known, and one prerequisite for a deeper understanding is the exact knowledge of the photochemistry of the drugs. In the last years, a large number of photochemical and photophysical studies has been devoted to FQs, and the results, beyond their usefulness for formulating hypotheses on the phototoxic mechanism, revealed unexpected facets of the photoreactivity of heterocycles. Indeed it was shown that for a large number of these drugs the main photoprocess is heterolytic defluorination, an unexpected reaction with fluoroaromatics due to the strength of the aromatic C–F bond (dissociation energy *ca*. 120 kcal mol^{-1}).

In this paper we present an overview of the photophysical and photochemical properties of structurally related, representative fluoroquinolone drugs. Evidence about the excited states involved in the photodegradation, the fragmentation modes operative (indicated in Scheme 1) and the intermediates occurring is discussed. A rationalization of the photoreactivity of the drug molecules and the medium effects is presented, an important step in the understanding of the photodegradative paths occurring in biological environments and in the correla-

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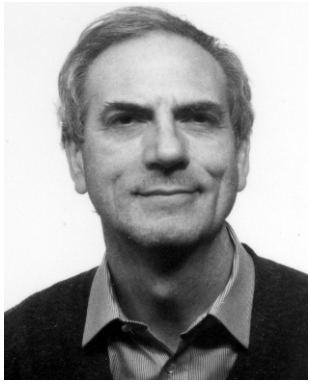

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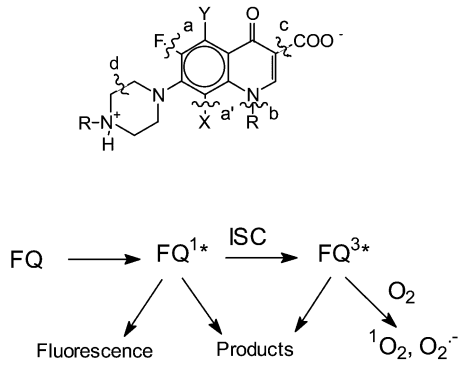


Scheme 1 Relevant processes to fluoroquinolone photochemistry.

tion between structural characteristics and phototoxic potential.

1.1 Structure and phototoxicity

The currently used drugs have been optimised through a very extensive exploration of the structure–activity relationships evolving from some earlier discovered antibacterials such as nalidixic acid (NA) and oxolinic acid (OA), *i.e.* quinol-4-one- or 1,8-naphtyridin-4-one-3-carboxylic acids. In many cases a fluoro and an amino substituents have been introduced and most of the drugs used nowadays are 1-alkyl-7-dialkylamino-6-fluoro-4-quinolonecarboxylic acids (or the corresponding 1,8-naphthyridine derivatives) with various substituents (3rd generation FQs; the most commonly used drugs are listed in Chart 1, where the abbreviations are also indicated). The fluorine atom at C-6 and the dialkylamino chain at C-7 appear to be important for optimal efficacy. A remarkable phototoxic potential, though varying greatly within the series, seems to be quite general.

1.2 Protonation equilibria

The presence of multiple proton binding sites in FQs makes rather complex the pattern of acid–base equilibria. By several techniques, mainly potentiometry, UV and NMR spectroscopy[3] and, more recently, spectrofluorimetry,[4] it was shown that the carboxylate group and the 4′-amino of the piperazine ring are the most significant proton binding sites from the biological point of view (p*K* in the pH range 5–9). In Scheme 2 the interconversion between the four relevant forms in aqueous medium is shown for pefloxacin (PEF). Two of the structures are neutral protonation isomers, *i.e.* the form with undissociated carboxyl substituent and the zwitterion bearing protonated 4′-N and dissociated 3-carboxyl group. Although the ratio of the concentrations of the different forms appears to be very differentiated in the various derivatives,[3] at neutral pH the largely prevailing species is always the zwitterion. However, the cationic form and the anionic form predominate in acidic and in basic solutions, respectively.

2 Excited states

2.1 Absorption spectra

In neutral aqueous medium FQs (*i.e.* essentially their zwitterionc forms) are characterized by an intense absorption band with a maximum in the 260–300 nm region, cited below as "short-wavelength band", with molar absorption coefficient

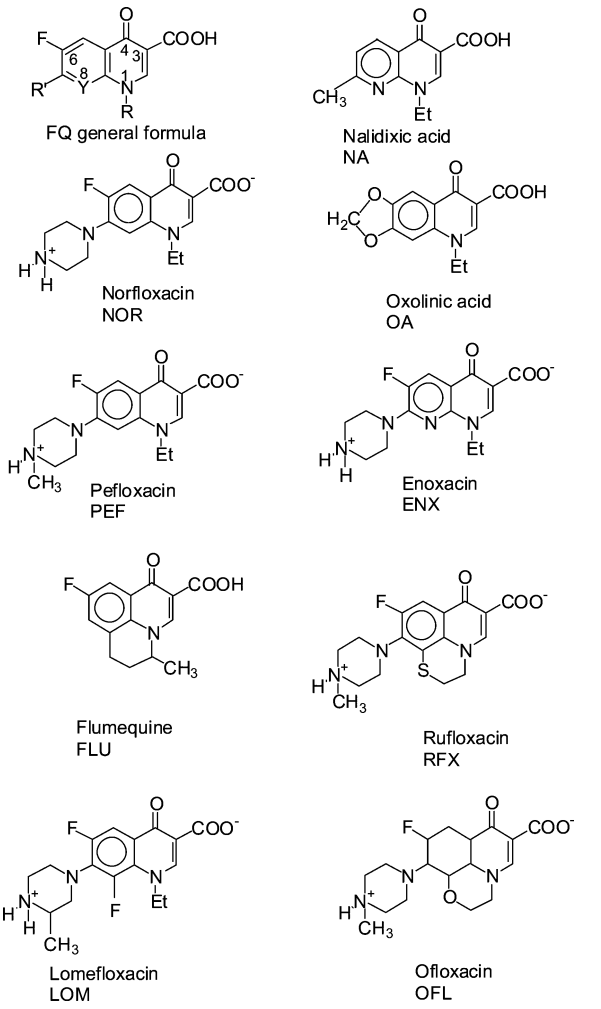
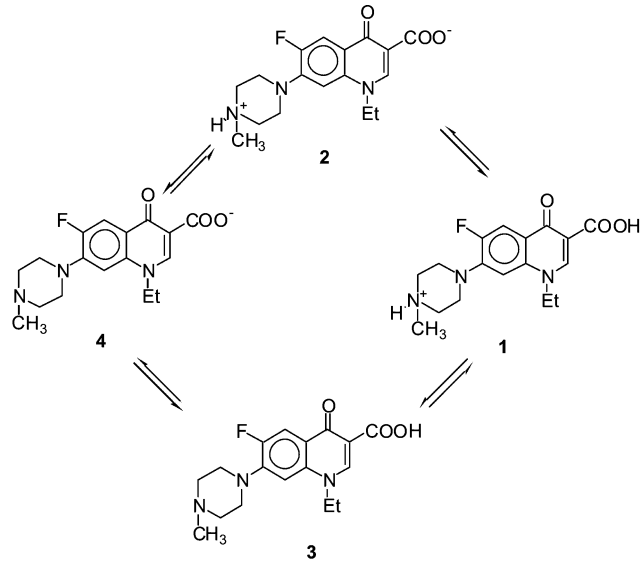


Chart 1 Structural formulae of quinolone derivatives and abbreviations.



Scheme 2 Protonation equilibria of pefloxacin.

ε_{max} *ca.* 2.0–2.5 × 10^4 M^{-1} cm^{-1} , a weaker band centered at 320–340 nm , cited as "long-wavelength band", with ε_{max} *ca.* 10^4 M^{-1} cm^{-1} and a weak tail extending beyond 350 nm. The position and the intensity of the bands below 250 nm more critically depend on the molecular structure. Figure 1 shows the spectra of representative members of the FQ family in 5 × 10^{-3} M phosphate buffer at pH 7.4.

According to the above described acid–base equilibria, the change of pH induces large changes in the absorption features of

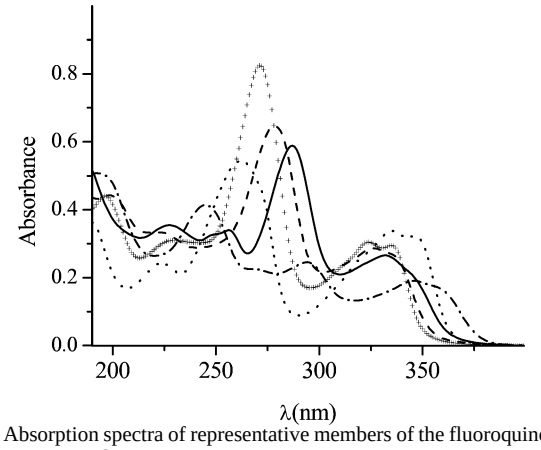


Fig. 1 Absorption spectra of representative members of the fluoroquinolone family in 5×10^{-3} M phosphate buffer at pH 7.4, 20 °C, cell path 1 cm. **---**, Rufloxacin 2.40×10^{-5} M; ——, Ofloxacin 2.52×10^{-5} M; **+++**, Norfloxacin 2.79×10^{-5} M; **---**, Lomefloxacin 2.71×10^{-5} M; ·····, Enoxacin 2.53×10^{-5} M.

FQs, qualitatively dependent on the molecular structure.[5–7] For example, on going from acidic to alkaline conditions, the long wavelength band in norfloxacin (NOR) shifts to the red and increases in intensity, while the short wavelength band shifts to the blue and decreases in intensity. The behavior of the short wavelength absorption at pH < 3 is complicated by the presence of the additional protonation sites at the 1-N and 1′-N centers.[5,6] The pH induced changes in the absorption spectrum of the corresponding 1,8-naphtyridinone enoxacin (ENX) are shown in Figure 2.[7]

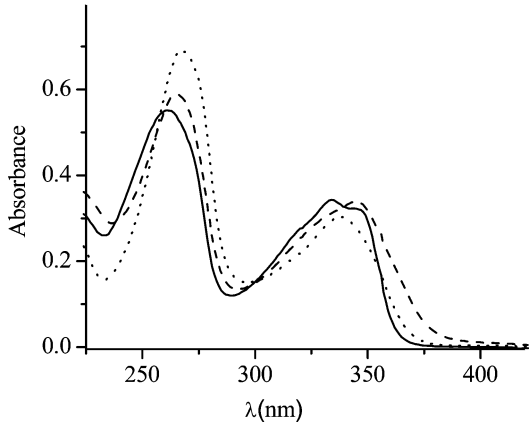


Fig. 2 Absorption spectra of 2×10^{-5} M Enoxacin in aqueous buffered solutions as a function of pH: ·····, pH 3.5; **---**, pH 12.0; ——, pH 7.4 . T = 20 °C, cell path 1 cm. Data from ref. 7.

In organic solvents the absorption features of FQs are similar to those in neutral aqueous medium.[8,9] On the contrary strongly solvent dependent absorption is exhibited by flumequine (FLU), a FQ not containing the piperazinyl substituent.[8] This fact points to a crucial role of the piperazine nucleus in determining the character of the lowest excited states.

2.2 Theoretical calculations

Quantum chemical ZINDO/S (Zerner Intermediate Neglect of Differential Overlap/Spectroscopic) calculations on both the neutral and the zwitterionic forms of rufloxacin (RFX) were performed and the solvatochromic effect of water was estimated.[10] The lowest excited singlets (forbidden nπ* states) of the zwitterion and of the neutral molecule were calculated to be at 23580 and 25900 cm^{-1}, corresponding to absorption at 424 and 386 nm, respectively, whereas the lowest lying singlets with sizeable oscillator strength (f = 0.1) were located at 30770 and 33670 cm^{-1} (325 and 297 nm), respectively.

By the AM1 COSMO model[8] it was shown that the change in the dipole moment of NOR and ofloxacin (OFL) upon electronic excitation is small in the gas phase, whereas it is very large in the aqueous phase, in agreement with a substantial charge redistribution. Changes in the bond lengths and in the dihedral angle CCNC around the carbon 7 were calculated for the excited state. The importance of the piperazinyl substituent in this respect is testified by the fact that FLU does not exhibit such behavior.[8]

2.3 Fluorescence properties

The pH induced changes in the absorption features of FQs are dramatically reflected in the fluorescence emission.[4–7] Significant fluorescence is only present in neutral and moderately acidic solutions, the emission being much weaker in basic and strongly acidic media. This behavior is in agreement with the existence of significant fluorescence from cationic and zwitterionic forms and poor emission ability from anions. Similarly protonated structures involving the centers of low basicity 1-N or 1′-N also do not emit appreciably.[5–7] In Figure 3A,B the

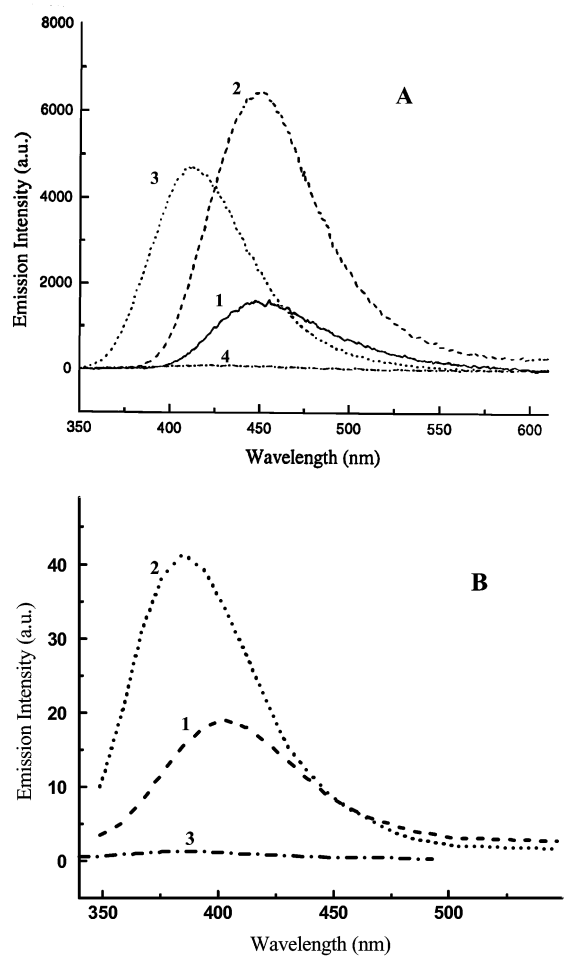


Fig. 3 Fluorescence spectra at room temperature: **A** (data from ref. 5, reproduced by permission of the American Society for Photobiology) Norfloxacin at pH 1.2 (**1**), 3.4 (**2**), 7.4 (**3**) and 10.8 (**4**); **B** (data from ref. 7.) Enoxacin at pH 3.5 (**1**), 7.4 (**2**), 12.0 (**3**).

fluorescence spectra of NOR[5] and ENX[7] are represented as a function of the pH of the solution. In Figure 4A,C the pH dependence of the emission quantum yields is shown.

The variations in the fluorescence quantum yields of ENX (Figure 4C) with pH fairly well parallel the corresponding changes in the absorption spectra. This indicates that during the lifetime of the emitting state the existing prototropic equilibria

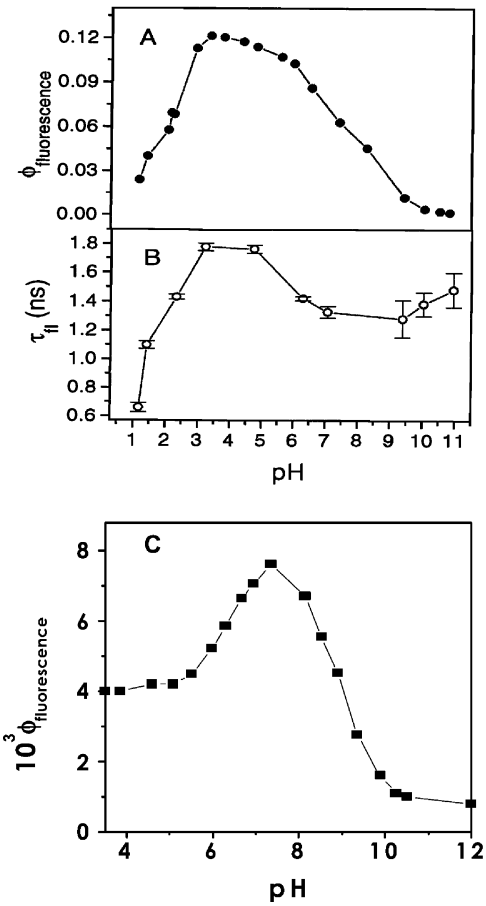


Fig. 4 pH dependence of the fluorescence quantum yield (**A**) and lifetimes (**B**) of Norfloxacin, (data from ref. 5, reproduced by permission of the American Society for Photobiology); pH dependence of the fluorescence quantum yield of Enoxacin (**C**), from ref. 7.

do not change, in spite of the lower basicity of the piperazinyl 4′-nitrogen in the excited state, which is expected on the basis of the pH effects on the absorption spectra.[7]

In the case of NOR it was shown that the pH also influences the fluorescence lifetime (Figure 4B).[5] At alkaline pH the decrease in lifetime only partially accounts for the decrease in quantum yield, clearly indicating that the FQ anion has an intrinsic radiative constant lower than that of the zwitterion. The fluorescence properties of the neutral forms of FQs in different media are summarized in Table 1.[5,7,8,13] In aqueous solutions at neutral pH the emission spectra are broad and structureless and exhibit large Stokes shifts. The emission quantum efficiencies are generally ≤ 10–15%, except in ENX where considerably lower values were found. The fluorescence lifetimes appear to be rather short (~ 1 ns or lower for NOR, ENX, lomefloxacin (LOM) and *ca*. 4–5 ns for OFL and RFX). In organic solvents the fluorescence intensity is much weaker than in aqueous solution, the lifetime longer and, close to the main emission band, an additional band appears in the region 350–400 nm, roughly the mirror image of the absorption.[8] These properties were attributed to the existence of a ground state equilibrium between the neutral zwitterion and the molecular form: the zwitterion exhibits the best emissive properties, predominates in water and is responsible for the visible emission band, while the molecular form is less fluorescent, prevails in organic solvents and is responsible of the near UV emission band.

Table 1 Fluorescence properties of the neutral forms of FQs in various media (see Chart 1 and 2 for structures and abbreviations; PB = phosphate buffer pH 7.2–7.4). Errors in quantum yields and lifetimes are typically within 10%

Compound	Medium	$\lambda_{max,f}$/nm	Φ_f	τ_f/ns	Ref.
NOR	PB 0.01 M	410	0.11	1.5	13
	PB 0.1 M	410	0.075	104	5
	H_2O	436	0.16	2.3	8
	$CHCl_3$	419	0.008	3.27	8
ENX	PB 0.01 M	380	0.01	< 0.5	7
RFX	PB 0.01 M	470	0.075	4.5	10
OFL	PB 0.01 M	460	0.1	4.0	13
	H_2O	475	0.2	5.86	8
	$CHCl_3$	455	0.004	14.2	8
LOM	PB 0.01 M	415	0.08	1.0	13
	PB 0.1 M			0.91	
	H_2O			1.5	
FLU	H_2O	375	0.034	0.73	8
	$CHCl_3$	372	0.036	0.71	

The large Stokes shift of the emission spectra of FQs in neutral aqueous media was attributed to a change in the geometry of the emitting state with respect to the ground state of the zwitterion, in view of the existence of a strong out of plane geometry of the piperazinyl group which may turn to planarity in the excited state due to a change in the molecular dipole moment.[10] Accordingly, it was proposed that a twisted intramolecular charge-transfer (TICT) state is populated by electron transfer from the 1′-N lone pair (donor) to the aromatic system (F at carbon 6 and O at carbon 4 acting as acceptors).[8] Such a TICT mechanism satisfactorily accounts for the peculiar behavior of NOR and OFL, compared to that of FLU, in the kinetics of the nanosecond scale reorganization of water molecules around the excited probes in sodium 1,4-bis[2-ethylhexyl]sulfosuccinate(AOT)/H_2O/heptane reversed micelles,[8] as well as in the reverse solvatochromism in mixed solvents.[8]

2.4 Effect of ions on the electronic transitions

Co-dissolved ionic species influence both ground and excited states of FQs, present as ions or zwitterions in water, as a consequence of either non-specific medium effects or specific-bimolecular interactions.

Anions. The fluorescence intensity of NOR, ENX, LOM at neutral pH depends slightly on the concentration of dissolved sodium salts of sulfite, chloride, hydrogen carbonate.[13] Specific effects were observed with phosphate ions (see below). The emission intensity decreases by increasing the concentration of the phosphate buffer up to 0.1 M according to a linear relationship (Figure 5, slope K_a ~ 10–15 M^{-1}).[13] By applying the Stern–Volmer equation ($I/I_0 = 1 + K_a$[Quencher] with $K_a = k_q\tau_0$), these values imply that the shortlived excited singlets of these FQs ($\tau_0 \leq 10^{-9}$ s^{-1}) are quenched with bimolecular rate

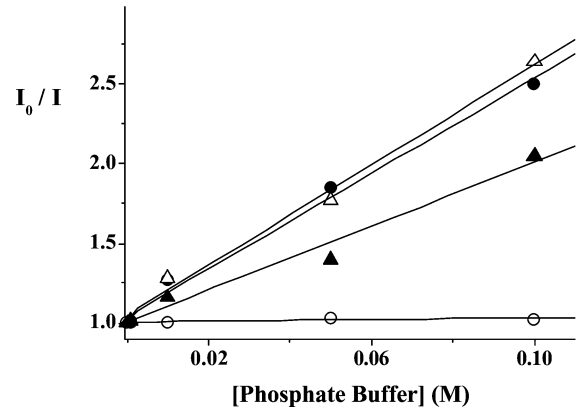
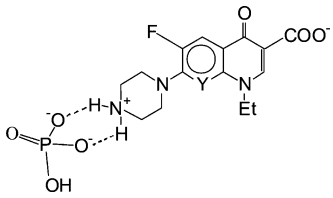


Fig. 5 Fluorescence intensity quenching by phosphate buffer salts at pH 7.4. K_a: 10 M^{-1} LOM (▲), 16 M^{-1} NOR (Δ), 15 M^{-1} ENX (●), ≤ 1 M^{-1} OFL, RFX (○). Data from ref. 13.

constants $k_q \geq 10^{10}$dm^3 mol^{-1} s^{-1}, *i.e.* larger than the diffusion limit (in water, 6.5×10^9 dm^3 mol^{-1} s^{-1}). A quenching mechanism mainly of "static" origin was hypothesized.[13] In agreement with such a mechanism no significant influence of varying the salt concentration in the same range was observed on the excited state lifetimes of the studied FQs. A ground state association between the phosphate dianion and the positively charged 4′-NH_2^+ moiety with two hydrogen bonds was proposed (Scheme 3). This interaction could preclude fluorescence either by increasing the CT character of the FQ excited state and channelling energy into a radiationless path or by promoting proton release from the 4′-N to the phosphate dianion able to act as proton acceptor.[7] The quenching effect was consistently found to be small with OFL and RFX, being 4′-N methylated in these derivatives and therefore not capable of such strong binding.[13]



Scheme 3 Hypothesized structure of ground state associates of fluoroquinolones with phosphate dianions (Y = CH, N, CF).

Cations. Metal cation complexation was largely studied with the aim of understanding the mechanism of action, bioavailability and cellular penetration of quinolones. It was also shown to represent the basis of useful, rapid and sensitive fluorimetric methods for the quantification of FQs in aqueous solutions.[5,14] Indeed FQ solutions undergo strong changes in the fluorescence properties (generally an intensity enhancement), if added with multicharged species such as Al^{3+}, Mg^{2+}, Ca^{2+}, Fe^{3+},[13,14] and Cu^{2+} as well as with Sc^{3+}, Ga^{3+}, In^{3+},[2] Tb^{3+}.[14] The formation of complexes between the inorganic cations and the FQs in the ground state is supported by changes in the excitation spectra.[4,15,16] The cation emission may appear by intramolecular energy transfer from the FQ.[14] The stoichiometry of the binding depends on the nature of the metal ion and on the ionization state of the quinolone. The most likely binding sites were indicated to be the 3-carboxy moiety, the adjacent 4-keto group and the 4′-N of the piperazine ring.[4,14–18]

Magnesium and calcium complexation of 7-piperazinyl-FQs leads to the formation of 1:1 and 2:2 associates.[17] With NOR, drug–metal complexes with 1:2 stoichiometry were evidenced at alkaline pH.[18] The effect of cation complexation on the NOR emission, singlet oxygen formation and drug photodegradation efficiencies were studied at different pHs with the aim of finding a correlation with the phototoxic potential of this drug.[18] It was shown that the protonated FQ form does not appreciably interact with the metal ions, likely due to an intramolecular H-bond between the 4-keto and 3-carboxy moieties. On the contrary the FQ zwitterion and the anion are able to bind the divalent metal cations with subsequent increase of the emission quantum yield and decrease of the production of singlet oxygen. Decrease of the internal conversion, intersystem crossing or photoreactivity by cation complexation was proposed in order to account for the observed fluorescence enhancement.[18]

2.5 Triplet state

Molecules containing the 4-quinolone or the 4-naphthyridinone moiety efficiently convert to the triplet state upon light absorption. Phosphorescence emission was indeed detected at low temperature in NOR with λ_{max} at 500 nm[5] and in ENX at 450 nm.[7] From the onset of the phosphorescence band the triplet energy in these derivatives can be estimated to be close to 260–280 kJ mol^{-1}.

The triplet states of quinolones were further characterized by laser flash-photolysis and the relevant data from the literature are collected in Table 2. The triplet–triplet (T–T) absorption spectra are characterized by λ^{max}_{T-T} in the interval 500–650 nm. The reported molar triplet absorption coefficients, ε_T^{max}, are in the range 4000–14000 M^{-1} cm^{-1}, depending on the molecular structure and the methods used for their determination. Since the estimation of the quantum yields for triplet formation crucially depends on ε_T values, the comparison of the results obtained in different laboratories is limited to the product $\varepsilon^{max}_T \Phi_T$.

As for non amino-substituted quinolones, FLU at pH 8 and NA at pH 9.2 have very high intersystem crossing efficiency, *i.e.* $\Phi_T = 0.9$[19] and ≥ 0.6,[20] for the anionic 3-carboxylate form FLU^- and NA^-, respectively. In the case of amino-derivatives, the zwitterionc forms of FQs convert to the triplet state with somewhat lower efficiency. For OFL a $\Phi_T = 0.33$ was reported at pH 7,[6] and for RFX a $\Phi_T = 0.36$ was determined.[11] Lower limits were given for ENX and NOR ($\Phi_T \geq 0.5$), for RFX ($\Phi_T \geq 0.4$) and OFL ($\Phi_T \geq 0.3$) in ref. 13† A much lower triplet quantum yield was estimated for LOM ($\Phi_T \approx 0.1$–0.2).[13] The interaction of the phosphate anions with the FQs in the ground state, described previously, leads to a substantial reduction in the triplet quantum yield at high concentrations of salts (> 0.01 M).[13]

Under the conditions of laser flash photolysis, T–T annihilation or self-quenching can come into play, so that the triplet lifetimes τ_T are strongly dependent on the experimental

† Φ_T estimations for ENX and RFX represent a revision of previously reported higher values,[7,10] based on underestimated triplet molar absorption coefficnents.

Table 2 Triplet state properties of neutral FQ in various media (see Chart 1 and 2 for structures and abbreviations; PB = phosphate buffer, pH 7–7.4 except when otherwise indicated)

Compound	Medium	T–T Absorption λ^{max}_{T-T}/nm	Quantum yield for triplet formation Φ_T	$\varepsilon^{max}_T \Phi_T$	Triplet lifetime τ_T/μs	Ref.
NOR	$NaHCO_3$ 0.001 M	620	≥ 0.5	3400	1.3	13
ENX	$NaHCO_3$ 0.001 M	520	≥0.5	3500	0.85	13
	PB 0.01 M	520		3500	0.09	7
RFX	PB 0.01 M	640	≥ 0.4	2800	10[a]	10,13
		640	0.36	2100	7	11
OFL	PB 0.01 M	620	≥ 0.3	2300	1.8	13
		610	0.33	3600	40	6
LOM	$NaHCO_3$ 0.001 M	500	≤ 0.2	900	≈ 0.1	13
FLU	PB 0.02 M pH 8	575	0.9	12600	~10	19
NA	Buffered H_2O pH 9.2	620	≥ 0.6	5400	~100	20
CPX	$NaHCO_3$ 0.001 M	610			1.5	30

[a] Extrapolated at infinite dilution.

conditions.[10,11,19,20] The value of τ_T is quite high for NA^- (~100 μs),[20] is in the range 1–15 μs for FLU^{-19} and other FQs,[7,10,11,13]‡ and is much smaller (~100 ns) for LOM[13] (see Table 2).

2.6 Triplet state quenching

Quenching by oxygen of the triplet state is characterized by rate constants in the range $2–3 \times 10^9$ M^{-1} s^{-1}, typical for aromatic molecules. The possible reactions are energy transfer with formation of singlet oxygen [eq. (1)], electron transfer to form the superoxide anion and the FQ cation radical [eq. (2)], physical quenching [eq. (3)]. Indeed singlet oxygen and the superoxide anion radical were actually observed in light irradiated FQ solutions (see Section 3.3).

$$^3FQ + {}^3O_2 \rightarrow FQ + {}^1O_2 \quad (1)$$

$$^3FQ + {}^3O_2 \rightarrow FQ^{\cdot+} + O_2^{\cdot-} \quad (2)$$

$$^3FQ + {}^3O_2 \rightarrow FQ + {}^3O_2 \quad (3)$$

Other triplet state quenchers were found to be active either by energy transfer or charge transfer mechanisms (data collected in Table 3). They include β-carotene with RFX,[10] 2-acetonaphtone with OFL,[6] di-*tert*-butylnitroxide (DTBN) with ENX.[7] Tryptophan, tyrosine, *N*-acetylcysteine and 2-deoxyguanosine quench the triplet states of FLU^{-19} and NA^{-20} with clear evidence for the occurrence of electron transfer.

NOR and ENX triplet states in neutral aqueous medium were quenched by inorganic buffer salts, like phosphate and sulfite, with bimolecular rate constants of $10^8–10^9$ M^{-1} s^{-1} (see Table 3). This process, responsible of a substantial reduction of the FQ triplet lifetime at normally used buffer concentrations (for example in ≥ 0.01 M phosphate or sulfite buffers), was interpreted as due to electron transfer from the buffer salt anions to the FQ excited state (see Section 4.3).[7,13] The thermodynamic balance of the reaction, calculated to be exo-ergonic or thermoneutral, justifies the high values of the observed bimolecular rate constants.[13]

3 Transient intermediates and metastable species

3.1 FQ radical anions and cations

Quenching of the triplet state of the quinolone-like derivatives by electron transfer leads to the concomitant formation of transients, assigned to the respective FQ reduced species. The reduced form of the nalidixate anion, *i.e.* the NA^{2-} radical anion, absorbs at 650 nm with a molar absorption coefficient of 3000 M^{-1} cm^{-1},[20] while a band maximum at 570 nm with absorption coefficient of 2500 M^{-1} cm^{-1} was assigned to the reduced form of the flumequine anion FLU^{2-}.[19] Reaction of the two radical dianions with oxygen leads to the formation of the superoxide anion, which was characterized by its reaction with ferricytochrome C.[19,20]

Pulse radiolysis experiments with OFL showed the absorption maximum of the corresponding anion radical at 410 nm (ε_{max} = 3000 M^{-1} cm^{-1}) and cation radical at 770 nm (ε_{max} = 5000 M^{-1} cm^{-1}).[6] The absorption maxima of the anion radicals of ENX and NOR were located at 670–700 nm.[13] Indeed dynamic quenching by electron transfer of the respective triplet states by inorganic buffer salts, like sulfite and phosphate at concentrations ≥ 0.01 M (rate constants in Table 3), led to the concomitant formation of a new absorption band in the above region, as shown in Figures 6 and 7. In Figure 8 the spectral features of the ENX anion, formed by reaction with sulfite and phosphate as electron donors, are shown.[13]

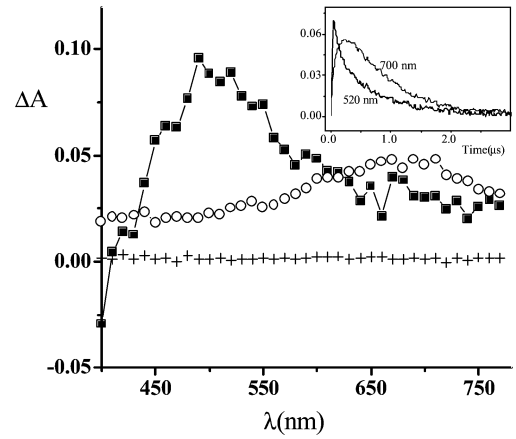


Fig. 6 Transient absorbance changes (ΔA) observed in 6×10^{-5} M Enoxacin in Ar-saturated phosphate buffer 0.01 M pH 7.4 after laser excitation at 355 nm, cell path 1 cm : ■, end of pulse; ○, 450 ns delay; +, 4 μs delay after pulse. Inset: time profile of ΔA at 700 and 520 nm. Data from ref. 7.

Addition of water to the FQ triplet was proposed to be at the basis of the photosubstitution of the fluorine atom by a hydroxyl group, as observed for NOR, ENX and ciprofloxacin (CPX) (see Section 4.2). This reaction was suggested to proceed through a cyclohexadienyl anion with a lifetime of a few

‡ For triplet OFL two significantly different lifetime values were reported, *ca*. 40 μs[6] and 1.8 μs.[13] This discrepancy can be explained by different experimental conditions.

Table 3 Bimolecular rate constants for quenching of the triplet state of FQs by various quenchers in various media (see Chart 1 and 2 for structures and abbreviations; PB = phosphate buffer)

		k_q/s^{-1} quencher								
Triplet state	Medium	O_2	DTBN	β-carotene	HPO_4^{2-}/HPO_4^-	SO_3^{2-}/HSO_3^-	aceto-naphtone	tyrosine	tryptophan	Ref.
NOR	$NaHCO_3$ 0.001 M, pH7.4	2.7×10^9								13,29
	Buffered H_2O pH 7.4				8×10^7	2.6×10^8				
ENX	$NaHCO_3$ 0.001 M, pH7.4	2.4×10^9								13
	PB 0.01 M, pH 7.4	2.7×10^9	5.8×10^8							7
	Buffered H_2O pH 7.4				9.8×10^8	4.8×10^8				13,29
RFX	PB 0.01 M, pH 7.4	1.7×10^9	4.7×10^8	2.1×10^9						10
OFL	PB 0.01 M, pH 7.4	1.7×10^9								13
	PB 0.01 M, pH 7.2	3×10^9					2.5×10^9			6
	Buffered H_2O pH 7.4					$1. \times 10^8$				13,29
LOM	Buffered H_2O pH 7.4				$< 10^7$	$> 10^8$				13
CPX	Buffered H_2O pH 7.4				2.2×10^7	$2. 6 \times 10^8$				30
FLU	PB 0.02 M pH 8	3×10^9						0.9×10^8	2.6×10^8	19
NA	Buffered H_2O pH 9.2	3.2×10^9						1.4×10^9	2.5×10^9	20

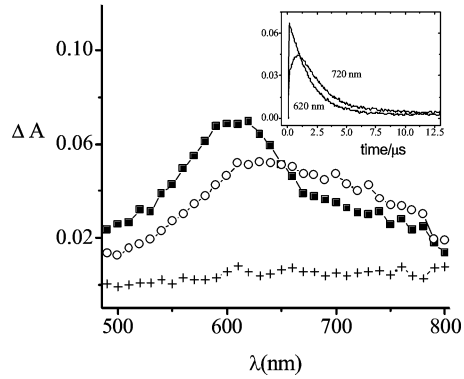


Fig. 7 Transient absorbance changes (ΔA) observed in 2.7×10^{-4} M Norfloxacin in Ar-saturated phosphate buffer 0.01 M pH 7.4, after laser excitation at 355 nm, cell path 1 cm : ■, 0.15 μs delay ; ○, 1 μs delay; +, 8 μs delay. Inset: time profile of ΔA at 720 and 620 nm. Data from ref. 13.

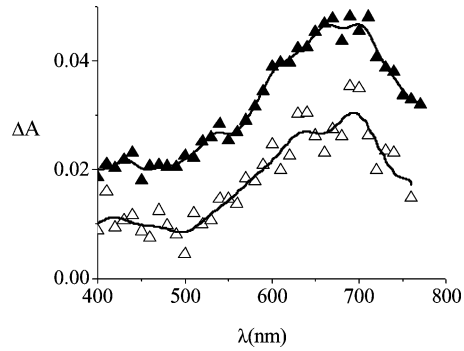


Fig. 8 Transient absorbance changes (ΔA) observed in a 6.5×10^{-5} M Ar-saturated solution of Enoxacin after laser excitation at 355 nm, cell path 1 cm: in phosphate buffer 0.01 M pH 7.4 at 450 ns delay (▲); in sulfite buffer 0.01 M pH 7.1 at 430 ns delay (△). Data from ref. 13.

microseconds, compatible with the absorption changes observed at $\lambda > 700$ nm upon laser excitation of NOR in argon saturated water solutions (Figure 9).[13]

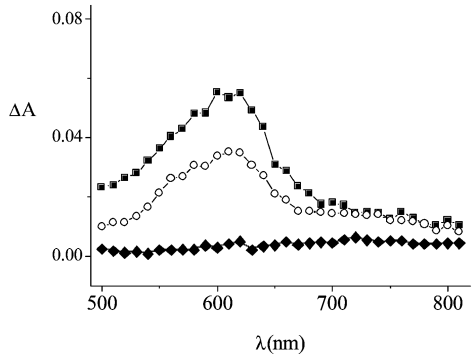


Fig. 9 Transient absorbance changes (ΔA) observed in 2.7×10^{-4} M Norfloxacin in Ar-saturated water solution after laser excitation at 355 nm, cell path 1 cm : ■, 0.25 μs delay; ○, 1.3 μs delay; ◆, 15 μs delay . Data from ref. 13.

3.2 Solvated electrons

The production of solvated electrons in neutral aqueous medium was observed by excitation at 355 nm. The process appears to be predominantly two-photon under moderate laser energy conditions.[12] FQ derivatives bearing an electron donating substituent in position 8 also exhibit monophotonic contributions to photoionization: the reported quantum yields were 0.2 for OFL,[6] 0.09[11] or 0.034[12] for RFX; with NOR , ENX and LOM the monophotonic path, if any, has a quantum yield below 0.009.[12]

3.3 Singlet oxygen and superoxide anion

Energy transfer to molecular oxygen was observed with FQs and structurally related compounds [eq. (1)].[5,6,10,21,22,23] A systematic investigation of the quantum yields Φ_Δ with many FQ derivatives was performed by Martinez *et al.*[23] and the results are reported in Table 4. The rate constants for the reaction of singlet oxygen with ground state FQs k_q are also reported. The best singlet oxygen sensitizers appear to be NA both in the deprotonated (Φ_Δ = 0.15) and protonated form (Φ_Δ = 0.24),[21] FLU (Φ_Δ = 0.34) and cinoxacin (CNX)(Φ_Δ = 0.16),[23] as well as RFX (Φ_Δ = 0.32).[10] Apart from the last derivative, generally, FQs containing the piperazinyl moiety seem to exhibit singlet oxygen quantum yields < 0.1 at neutral pH, pointing to a scarce ability of the zwitterionic triplet states to transfer energy to molecular oxygen. Oxygen quenching by electron transfer [eq. (2)] appears to be a more significant mechanism in many cases (see below).

Table 4 Singlet oxygen quantum yield (Φ_Δ), rate constant of singlet oxygen quenching by FQ ($k_q/10^6$ $M^{-1}s^{-1}$) in neutral aqueous medium and rate of generation of the superoxide anion ($k_{O_2^{\cdot-}}$/arbitrary units min^{-1}) in DMSO for FQs

Compound	Φ_Δ[a]	$k_q/10^6$ $M^{-1}s^{-1}$[a]	Ref.	$kO_2^{\cdot-}$/a. u. min^{-1}[b]	Ref.
NOR	0.081	1.8 ± 0.1	23	0.25	23
PEF	0.045	12 ± 1	23		
ENX	0.061	1.4 ± 0.2	23	1.7	23
RFX	0.32		10		
OFL	0.076	5.6 ± 0.6	23	0.06[c]	23
	0.13		6		
LOM	0.072	18 ± 3	23	0.17	23
FLX	0.029	6.9 ± 0.7	23	0.36	23
CPX	0.092	5.2 ± 0.2	23	0.20	23
CNX	0.16	1.8 ± 0.2	23		
NA	0.15	250	21	10.1	23
FLU	0.34	3.4 ± 0.4	23		

[a] Data in neutral phosphate buffer, 50 mM in D_2O (ref. 23), 0.01 M 90/10 D_2O/H_2O (ref. 10), 0.01M in D_2O (ref. 6). [b] In these EPR experiments the spin trap DMPO was used; the initial rates of generation of the DMPO/·OOH adduct signal were measured and corrected for the number of photons absorbed (ref. 23). [c] An upper limit of 0.2 for the quantum yield of superoxide formation by OFL was given in ref. 6.

The effect of pH on the singlet oxygen production was studied in detail for NOR.[5] The highest Φ_Δ was observed at pH 8–9, while the production decreases both at lower and higher pH values. When the data are corrected for the reaction of NOR itself with singlet oxygen, whose rate below pH 7 depends strongly on pH, a continuous increase in the quantum yield is observed from acid to alkaline pHs, the value Φ_Δ = 0.2 being reached at pH 13. On the contrary below pH 4 the corrected quantum yield of singlet oxygen production appears to be fairly constant (Φ_Δ = 0.08), while the fluorescence quantum yield strongly decreases. This behavior, combined with the pH dependence of the fluorescence quantum yields was interpreted by an increase of the intersystem crossing (ISC) efficiency in the anion and a substantial constancy of the ISC efficiency in the cation, with respect to the zwitterions.[5] However care should be taken in the interpretation of such data, because both of the corrections involved and of a possible role of the electron transfer channel [eq. (2)] in the triplet state quenching, differentiated in the various FQ protonation forms.

The photogeneration of superoxide by FQs under steady state photolysis was assessed by detection of H_2O_2, formed by disproportionation of $O_2^{\cdot-}$ with its conjugated acid form $HO_2^{\cdot}$,

and reduction of cytochrome c in *in vitro* studies.[24] It was also recently demonstrated using EPR and a spin trap technique in dimethyl sulfoxide (DMSO).[23] The rates of superoxide production by several FQs at neutral pH are reported in Table 4.

4 Photochemistry

The interest in the photobiological effect of fluoroquinolones has stimulated a number of photochemical investigations over the last decade. Detailed product studies are now available for a significant number of FQs, and general conclusions can be inferred.

FQs, as in general polynuclear (hetero)aromatics, are not expected to photorearrange,[25] nor are these compounds expected to abstract hydrogen, in contrast to quinolines, since the lowest triplet state is not a $n\pi^*$ state in these derivatives.[26] This is indeed the case and, apart from chemical processes involving the intervention of some photogenerated reactive form of molecular oxygen (Sections 3 and 4.1), the occurrence of reactions after photoexcitation is due to processes involving a substituent. Various types of photochemically labile molecular sites have been recognised, among which the carbon–fluorine bond is particularly important. The relevant processes are discussed in Sections 4.2–4.4 according to the moiety involved, as indicated in Scheme 1. As it will appear, reactions involving previous photoinduced electron transfer represent a significant fraction of the recognised chemistry.

4.1 Reaction with reactive oxygen species

In Section 3.2 it has been indicated that FQs generate both singlet oxygen ($^1O_2$) and superoxide anion (O_2^{-}). It is not clear, however, whether this results in a self-sensitised oxidation, *i.e.* any photoproducts arise *via* attack of an activated form of dioxygen onto a FQ. The total (physical + chemical) rate constants for the quenching of singlet oxygen (k_q) are in the order of 10^6–10^7 $M^{-1}s^{-1}$ in aqueous medium (10^7 $M^{-1}s^{-1}$ in methanol) and vary very little with the structure (see Table 4).[23] These values are close to those observed with anilines under the same conditions[27] and indeed the conjugated π system of the 1-alkylquinolone moiety is strictly related to that of an aniline derivative. A 6-amino group is not a prerequisite, since also with FLU the quenching rate remains similar. A somewhat higher value was reported for the non-fluorinated derivative NA.

In the case of NOR the quenching rate constant k_q has also been measured in D_2O and it has been found that it is similar to that in MeOH under basic conditions but decreases under acidic and, more strongly (by a factor of 20), under neutral conditions where the zwitterion is present.[5] It appears that, while quenching is purely physical in methanol, a fraction of it involves a chemical reaction in water.[23] The chemistry occurring has not been clarified, but analogy with the case of *N*-alkylanilines[28] suggests that attack at the *N*-alkyl chain occurs.

On the other hand, alkyl chain degradation may involve other forms of activated dioxygen, *e.g.* reaction between the FQ radical cation and superoxide anion as proposed for RFX,[11] or non dioxygen-based reactions, such as hydrogen abstraction by the triplet excited state of the drug or by hydroxyl radicals (see Section 4.5).

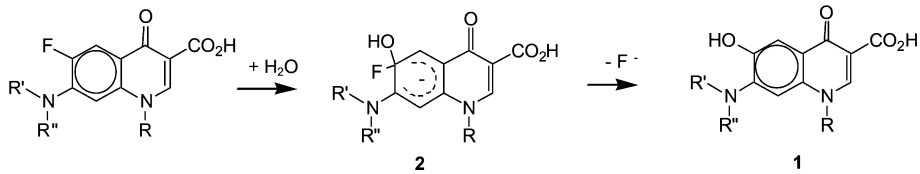
4.2 Defluorination

As indicated in the introduction, the large majority of the presently used quinolone antibacterials contain at least one fluorine atom (in position 6); in some cases further fluorine or chlorine atoms may be present at positions 5 and 8 (Chart 1). With many of the investigated FQs it has been found that the main photoreaction occurring under neutral conditions is defluorination. However, the quantum yield of this process depends on the structure and varies over several orders of magnitude (reported values from 0.55 to < 0.001) and furthermore two different photoreactions, involving different mechanisms, actually occur.

With 6-*mono*fluoroderivatives the process is photosubstitution of the fluorine atom by a hydroxyl group (cleavage 'a' in Scheme 1) to give the corresponding 6-hydroxyquinolones **1** (Scheme 4), as demonstrated for NOR, ENX, CPX[13,29,30] In principle, this reaction may involve either unimolecular fragmentation of the C–F bond in the excited state or water addition followed by fluoride anion loss. The latter mechanism (shown in Scheme 4) has precedent in several aromatic photosubstitution reactions *via* the triplet state.[31] A support for this mechanism cannot be found in the dependence of the quantum yields on [OH^-], since a change in pH would affect the ionic equilibria of the substrates themselves, and this has a strong effect on the formation of the triplet state. However, an indication comes from the fact that a 0.1 M Cl^- concentration does not lead to introduction of a chlorine atom,[13] as would be expected if a cation were formed. Indeed, for NOR and ENX flash photolysis investigations support that the reaction proceeds from the triplet state and involves a long-lived intermediate. A transient with τ = 3.6 μs, reasonably identified as the cyclohexadienyl anion **2** (Scheme 4) formed by addition of the solvent (Scheme 4), is apparent in the case of NOR (see Figure 9). A similar transient is barely appreciable for ENX (λ_{max} > 650 nm).[13]

Consistent with this mechanism, the quantum yield of the defluorination reaction Φ_{-F^-} is proportional to the electrophilicity of the heterocyclic ring, which is mainly determined by the substituent in position 8. Indeed, Φ_{-F^-} changes by a factor > 100 in the series ENX (0.13) > CPX, NOR (0.06–0.007) >> RFX, OFL ($\leq$ 0.001), in a way that is strictly parallel to the change in electrophilicity induced by the substituent in 8, *i.e.* electron withdrawing aza substitution in ENX, no substituent in CPX and NOR,[13,29,30] electron donating alkoxy or thioalkoxy groups in OFL, RFX.[10,31–33] With the two last derivatives, no hydroxyquinolones analogous to **1** (Scheme 4) have been isolated,[32,33] though in the first case fluoride liberation has been monitored,[32] and different reactions occur (see Sections 4.4 and 4.5). These are much less efficient than defluorination in the cases above and it seems possible to state the rule that FQs bearing an additional electron-donating substituent (besides the standard amino group in position 6) have a decomposition quantum yield well below 10^{-2}, probably around 10^{-3}.

Conversely, the presence of a *second halogen* X *in position 8* makes the molecule much more sensitive and it is the C–X bond



2 **1**

Scheme 4 The process of photosubstitution in 6-*mono*fluoroquinolone derivatives.

at that position that is fragmented (cleavage 'a'' in Scheme 1). This increased photosensitivity has been early noted[34] and quantitative studies on a typical example of this class, LOM, have shown that quantum yield is much higher in water (Φ_{-F^-} = 0.55).[29,35,36] Fleroxacin (FLX) (Chart 2) reacts at a rate only

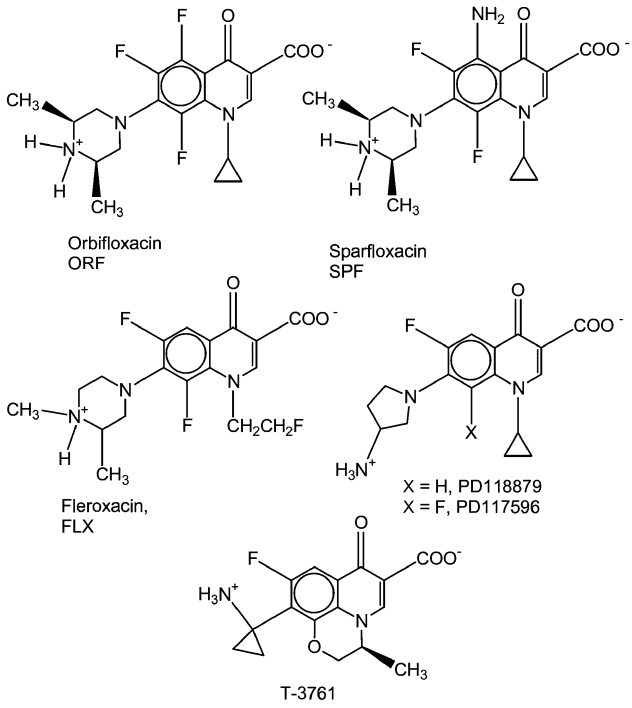
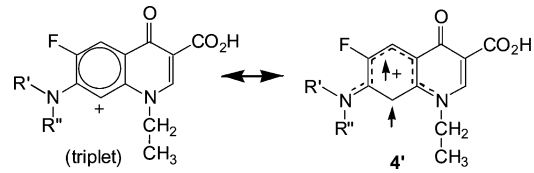


Chart 2 Structural formulae of quinolone derivatives and abbreviations.

slightly lower than LOM[37] and defluorination at position 8 has been noted also with orbifloxacin (ORF)[38–40] and sparfloxacin (SPF).[41] The final products formed and the transients observed also change. A transient in the microsecond time range which may be attributed to the triplet state is weak and shorter-lived in the case of LOM.[13] Thus, either the singlet excited state reacts directly in competition with ISC or the triplet state is formed and reacts more efficiently than the other FQ triplet states. Concerning the mode of reaction, various pieces of evidence support that unimolecular fragmentation to give an aryl cation (intermediate **4** in Scheme 5) occurs in this case and is favoured by the better mesomeric stabilisation of the cation formed in position 8.[32] No direct evidence for the formation of such a species has been obtained yet, but the product distribution supports such a mechanism.

The cation formed (**4**, Scheme 5) does not react indiscriminately as it would be expected if it were a localized σ cation (Scheme 5). With both LOM and ORF (for the latter FQ, see Scheme 7 below) it has been found that the chloride anion reacts in position 8 (product **3**, Scheme 5, path 'a''), but water does not add (*no* path 'b'), and, contrary to the case of 6-monofluoro derivatives, no hydroxyquinolone is formed.[35,40]

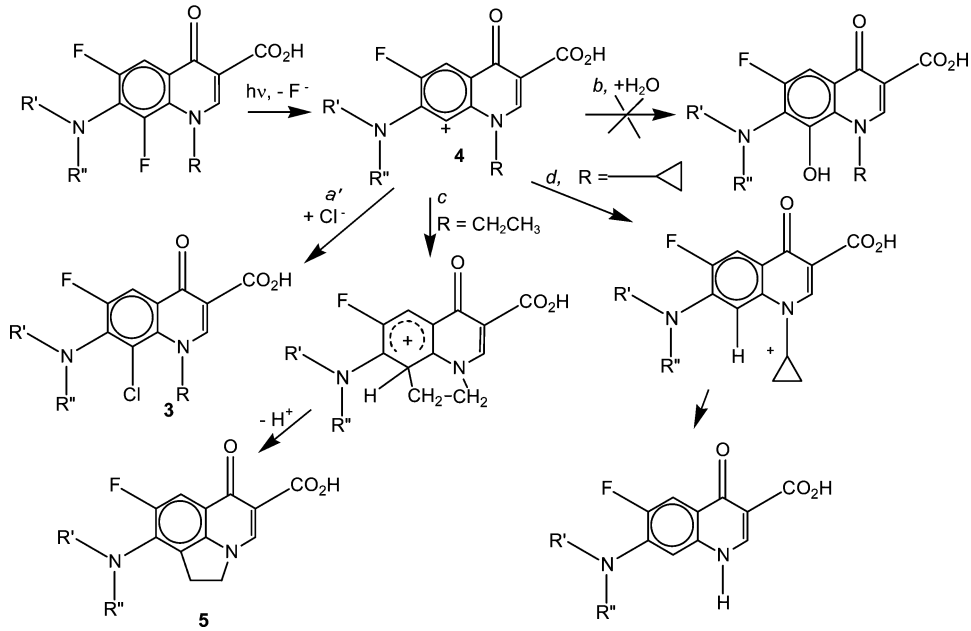
The same chemistry has been found with a simple model, 4-aminophenyl cation, for which extended calculations showed that, while the singlet state has an empty σ orbital at unsaturated C-1, the triplet state, which is the lowest one, has one of the unpaired electrons in the σ orbital at C-1 and the other one delocalised over the π system, with the charge mainly on the nitrogen atom. This gives to this species a radical—triplet carbene (rather than cationic), character at the unsaturated carbon.[42] The *triplet* multiplicity is thus reasonably also relevant to the lowest—and reactive—state of the cation derived from LOM, best represented by structure **4′** (Scheme 6).



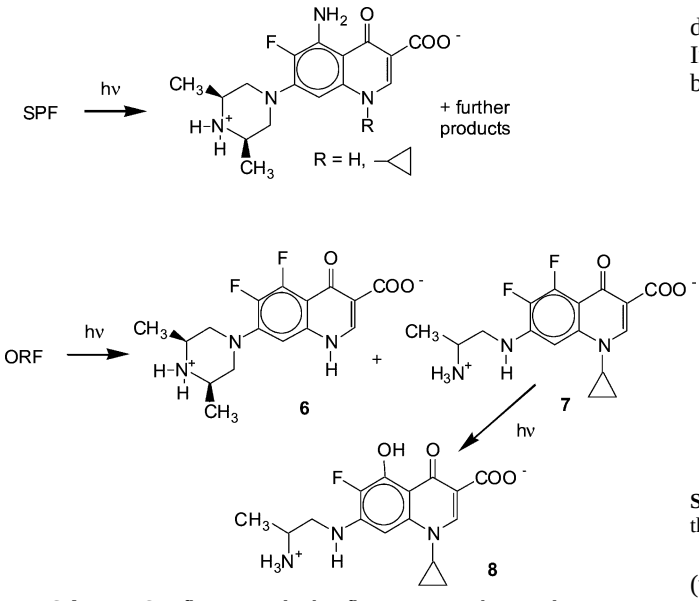
Scheme 6 Mesomeric structures in the triplet aryl cation derived from triplet mediated defluorination of lomefloxacin. Electron spin is indicated by arrows.

Accordingly, the main fate of the cation of LOM in neat water is a 'carbene' reaction, *i.e.* insertion in the neighbouring C–H bond (position β in the *N*-ethyl group), finally leading to the formation of a fused five-membered ring (product **5**, Scheme 5, path c), with no competing reaction with water.[29,36] As one may expect, a chlorine atom is even more easily cleaved, as shown with the 8-chloro analogue of LOM.[35]

Defluorination from position 8 takes place also in the case of ORF (Scheme 7)[38–40] and the carbocation attacks either *i*) the *N*-cyclopropyl group, which is lost in one of the products (compare Scheme 5, path *d*, reasonably because the intermediate diradical



Scheme 5 Photoinduced unimolecular fragmentation in 6,8-difluoroquinolone derivatives.



Scheme 7 Sparfloxacin and orbitofloxacin main photoproducts.

formed by attack at the three-membered ring initiates the fragmentation of that moiety) or *ii*) the dimethylpiperazinyl chain, which is degraded in the second one (actually the main product, **7** in Scheme 7). Noteworthy, the last compound undergoes secondary photosubstitution of a hydroxy for a fluoro group at position 5 (product **8**, Scheme 7), showing that photosubstitution *via* the triplet state addition–elimination mechanism (compare Scheme 4) can occur also at position 5, indeed in preference with respect to position 6.

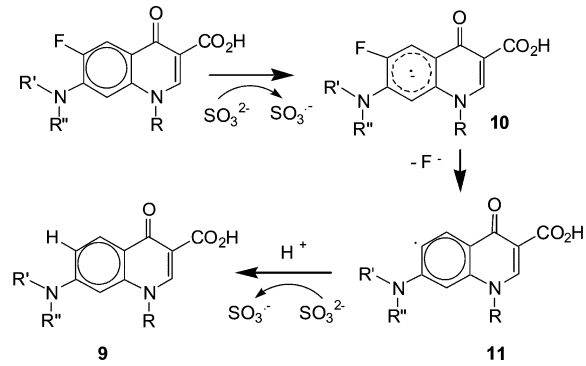
Again selective defluorination at position 8 is observed with SPF (Scheme 7). The two products obtained have both undergone reductive defluorination, and one of them has also lost the cyclopropyl group.[34] It is important to notice that loss of the *N*-alkyl chain (cyclopropyl with both ORF and SPF, cleavage 'b' in Scheme 1) occurs only when coupled with formation of a cation at position 8 (compare the negative case of CPX above). This is not a primary photoprocess, but rather results from the reaction of the photogenerated cation.

For another 6,8-difluoroquinolone, the aminopyrrolidino derivative PD 117596 (Chart 2), the quantum yield of decomposition has been reported to be 0.021 (under conditions, however, where CPX had a corresponding quantum yield of 0.0007, see in contrast the recent value of 0.07[30]) and is much more reactive that the analogous monofluoro derivative PD118879. The mode of photoreaction for these FQs has not been yet established.[43]

Another difference between 6-monofluoro and 6,8-difluoro derivatives is that defluorination is quenched by dioxygen for the former ones, but only marginally for the latter ones (11% effect with LOM). Consistently with this fact, defluorination involves the relatively long-lived (microsecond) lowest triplet state with 6-monofluoro derivatives, the lowest excited singlet state (or a much shorter-lived triplet state) with the 6,8-difluoro ones. However, with FQs reluctant to defluorination, such as OFL, the photo-decomposition quantum yield (which is in the order of 0.001) slightly increases in air equilibrated solution (see further Section 4.5).[32]

4.3 Electron transfer-induced defluorination

The above defluorination reactions result from irradiation in neat water or when the pH is adjusted to neutrality by addition of small amounts of sodium bicarbonate. The use of solutions buffered with different anions leads to electron transfer induced defluorination under some conditions. Thus, in sulfite buffer several FQs (LOM, NOR, ENX, CPX) undergo reductive defluorination to give products of structure **9** (Scheme 8).[13,29,30] In the case of LOM, the process occurs selectively at position 8 but represents only a part of the overall photodegradative path

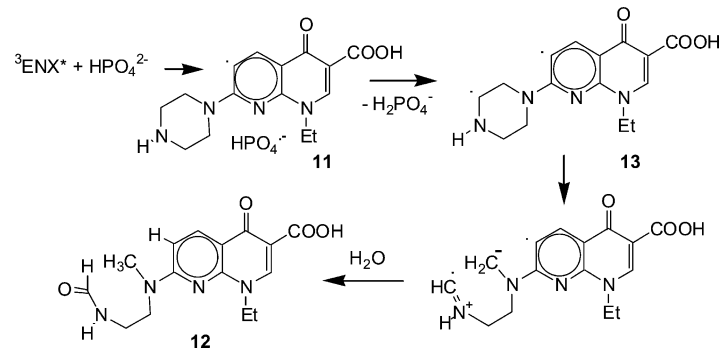


Scheme 8 Electron transfer induced defluorination of fluoroquinolones in the presence of sulfite.

(which in this case also gives product **5**, Scheme 5), whereas with 6-monofluoro derivatives such as NOR and ENX this becomes the only photoprocess at $[SO_3^{2-}] \geq 0.01$ M. Under such conditions, the triplet state is quenched by the salt and a new transient is revealed at 670–700, which is identified as the radical anion (see Section 2.6). The proposed mechanism involves fluoride loss from the radical anion (**10**, Scheme 8) and reduction of the aryl radical (**11**, Scheme 8).[13,29]

Notably, the triplet state of NOR, CPX and ENX is quenched also by phosphate.[13] In the case of the first two FQs, the quenching occurs at a lower rate than with sulfite, as one may expect given the less favourable thermodynamics, though with easily reducible ENX k_q with phosphate is even larger than k_q with sulfite (see Table 3). The radical anion is identified also under these conditions, as shown in Figure 8, where the transients formed with both buffers are compared. However, the end products in phosphate buffer result from reductive ring defluorination accompanied by oxidative degradation of the piperazine side-chain, giving a *N*-methyl-*N′*-formylaminoethylamino group[13,44] (product **12** in Scheme 9). A mechanism is proposed in the scheme for the case of ENX. The first step from the radical anion is fluoride loss, but then a relatively poor reducing agent such as phosphate does not reduce the aryl radical **11** (compare Scheme 8 with Scheme 9). Rather, hydrogen transfer within the radical pair occurs, due to the known property of the co-formed phosphate radical anion. C–C bond cleavage in the resulting diradical (**13**, Scheme 9) and water addition leads to the observed product. Products of this type are the main ones in neutral phosphate buffer for NOR, ENX and CPX, are formed to a small extent in neat water with ENX and LOM and correspond to the main product for ORF (product **7** in Scheme 7).

One should also notice that the group of reactions presented in this subsection, in contrast to the addition–elimination and cation insertion reactions involved in the defluorination processes discussed in Section 4.2, involve *radical* intermediates, *i.e.* very reactive species with an unpaired electron. Since most photochemical studies on drugs, particularly in more biologically minded laboratories are routinely carried out in phosphate buffer, one should be aware that under such circumstances the photochemistry of FQs changes completely, and that in this case radicals may be formed, in contrast to neat water. This is an example where transferring experimental procedures typical of ground state chemistry to photochemistry is no trivial matter. Phosphate buffer is in most cases a perfect choice for assessing the thermal stability of a drug in solution, but not for studying the photostability of FQs, where the effect of the buffer salts is not limited to the stabilization of the pH. Therefore, when comparing relative efficiencies of photochemical or photobiological phenomena, it must be checked

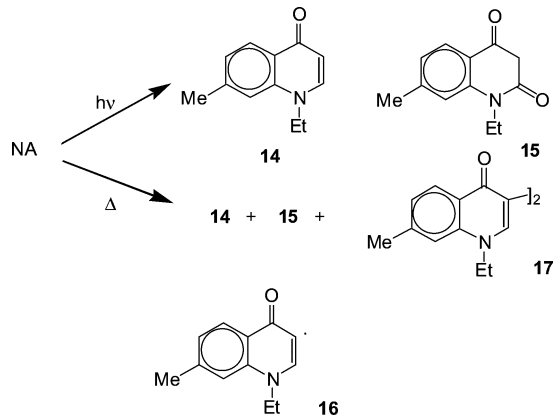


Scheme 9 Reductive ring defluorination and oxidative degradation of the piperazine side-chain in fluoroquinolones in presence of phosphate.

whether the experimental conditions allow such comparison. Likewise, the dramatic dependence of the reactivity on the medium makes it difficult to predict the photochemical behaviour of the drug *in vivo*, since this will depend on the localisation of the molecule when absorbing light and on the presence of potential quenchers, *e.g.* phosphate derivatives of carbohydrates.

4.4 Decarboxylation

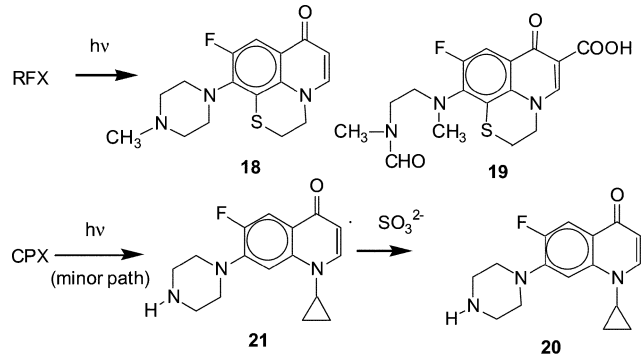
Decarboxylation (cleavage 'c' in Scheme 1) seems to be the main photoprocess for non-fluorinated quinolones. NA, a 1,8-naphthyrid-4-one-3-carboxylic acid which has been the precursor of this antibacterial series, is photodecarboxylated to naphthyridone **14** and naphthyridin-2,4-dione **15** , possibly *via* disproportionation of radical **16** resulting from single electron oxidation of the carboxylate and carbon dioxide loss (Scheme 10). Thermal decarboxylation yields the dimer in position 3 (product **17**, Scheme 10) along with the previous compounds.[45]



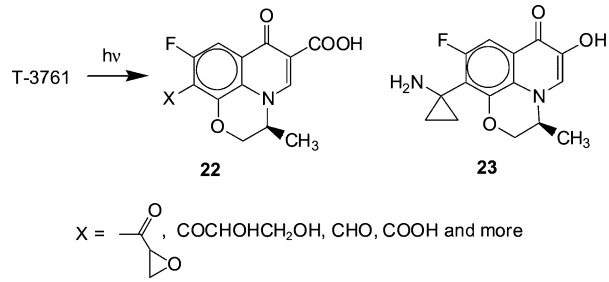
Scheme 10 Photocarboxylation in the non-fluorinated quinolone nalidixic acid.

Among FQs, this process is limited to derivatives in which a strongly electron donating substituent precludes defluorination. RFX, as mentioned above, reacts only slowly and does not defluorinate. One of the products isolated results from decarboxylation[33] (product **18**, Scheme 11). Decarboxylation is a minor photoprocess with CPX, as shown by the isolation of a small amount of product **20** in the presence of sulfite (Scheme 11). This reasonably results from reduction of the free radical formed by decarboxylation (**21**, Scheme 11),[30] which under non reducing conditions couples to dimeric structures, as suggested by mass spectroscopic evidence, though the products have not been isolated.[46]

Oxidative decarboxylation (to yield 3-hydroxyquinolone **23**, Scheme 12) is one of the processes in the case of T-3761 (Chart 2, Scheme 12), which in contrast to most FQs bears a (1-aminomethyl) rather than a *N*,*N*′-dialkylamino group in



Scheme 11 Isolated rufloxacin and ciprofloxacin photoproducts.

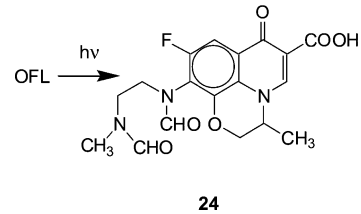


Scheme 12 Isolated T-$_{3761}$ photoproducts.

position 7, but, like RFX has an electron donating substituent in position 8.[47]

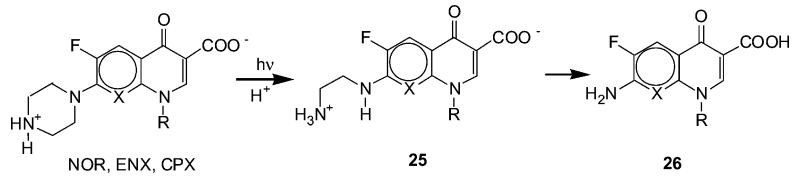
4.5 Side-chain degradation

The defluorination process of FQs, typical for neutral aqueous solution, does not take place under acidic conditions nor, as already mentioned, with FQs bearing electron donating groups. Under such conditions inefficient degradation of the alkylamino side chain (quantum yields usually in the order of 0.001, cleavage 'd' in Scheme 1) occurs and leaves the heterocyclic fluorine intact. Typical are the cases of electron-donating substituted FQs like OFL (product **24** in Scheme 13)[29,48] and



Scheme 13 Isolated ofloxacin photoproducts.

RFX (product **18** in Scheme 11).[33] Also in the case of T-3761, which as seen above has a C rather than a N bonded substituent at position 7, the side chain is oxidised in most of the photoproducts (structure **22** in Scheme 12).[46] *N*-Demethylation may occur also *via* electron transfer to dioxygen.[11] However, NOR, ENX and CPX, all of which defluorinate in neutral



Scheme 14 Photodegradation of the piperazine side-chain of norfloxacin, enoxacin and ciprofloxacin in acidic solution.

solution (*vide supra*), undergo in acidic solution stepwise degradation of the piperazine side-chain until this latter is reduced to an amino group (products **25** and **26** in Scheme 14).[30,35,49] The mechanism of this class of reactions has not yet been clarified, nor is this easy in view of their low quantum yield. Possibilities are hydrogen abstraction either by some excited state (the triplet state of the FQ or of some impurity, such as an aromatic ketone) or by hydroxyl radicals arising through direct oxidation of water by the drug. Activation of residual dioxygen (*vide supra*) is difficult to exclude, even in a de-aerated solution when long irradiation times are required, and obviously relevant to air-equilibrated solutions. In this respect, it is noteworthy that the photoreaction of OFL, involving side chain oxidation, is slightly more efficient in the presence than in the absence of oxygen (Table 5), contrary to what is observed with most FQs, where defluorination is more efficient.

Table 5 Quantum yield of photodecomposition for some FQ in water (see Chart 1 and 2 for structures)

Compound	Φ_{-FQ}	
	Ar-flushed	*Air-equilibrated*
NOR	0.06	0.01
ENX	0.13	0.08
LOM	0.55	0.49
OFL	0.0012	0.0016

5 Conclusions. General trends in the photoreactivity of fluoroquinolones and relation to photostability and phototoxicity

From the data that have been accumulated in the last decade, it appears that FQs are considerably photoreactive and the processes occurring depend on structure and medium. As discussed in Section 4, general trends have been recognised and rationalised. Some of the reactions observed find analogy in the literature (*e.g.* aromatic photosubstitution) while other ones have little precedent (*e.g.* heterolytic fragmentation and the behaviour of the aryl cation). In general, the photochemistry of heterocycles in water has received limited attention up to now whereas the work on FQs exemplifies that this is an intellectually rewarding topic and reveals new facets of organic photochemistry.

The observed phototoxicity may depend on various causes.

1. Since many FQs react rather efficiently, toxic photoproducts may be formed to an extent sufficient to cause a serious effect.

2. Since FQs activate dioxygen, forming both singlet oxygen and superoxide anion, a dioxygen related effect may operate.

3. The (triplet) excited state of FQs is a highly oxidising species. Photoinduced oxidation of various anions occurs efficiently and leads to organic and inorganic radicals.

4. The (triplet) excited state of FQs is a strong electrophile and bears a good nucleofugal group such as the fluoride. This leads to more or less efficient photosubstituion *via* an addition–elimination mechanism.

5. The excited state of FQs bearing a further halogen atom in position 8 undergoes unimolecular fragmentation to yield an aryl cation.

Properties 2 to 4 are a consequence of the characteristics of the ($\pi\pi^*$) triplet state of these molecules which easily takes part in electron transfer processes (both as reductant, with dioxygen, and as oxidant with anions) and acts as a good electrofile. Thus it appears that introducing a fluorine atom in 6 makes the drug somewhat photoreactive (and potentially phototoxic), despite the favourable effect of this group on the pharmacological profile of the drug. The degree to which the molecule is photolabile is modulated by further substituents, *e.g.* electron donating groups limit the electrophilicity and thus decrease the efficiency of the aromatic photosubstitution by a factor of 10 to 100. This should be considered among the other known structure–activity relationships when predicting the toxic potential of a drug. On the other hand, a halogen atom in position 8 makes the molecule quite photolabile and such drugs must possess positive characteristics sufficient to counterbalance this adverse effect.

From the point of view of understanding the mechanism of phototoxicitiy, FQs afford a unique possibility. In fact, different FQs, while maintaining the same basic structure—and presumably having similar association ability to the cell components—show an actually diversified photochemical behaviour when different substituents are introduced. Different paths are followed (nucleophilic substitution, 'carbene' reactions, radical reactions *etc.*) which depend on the medium characteristics (solvent, presence of anions, presence of dioxygen) in a different way. By correlating the photochemistry with the phototoxic effect and the respective medium dependence, one should be able to correlate the initial photochemical reaction and the final biological effect and to arrive at a realistic picture of the mechanism of phototoxicity.

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