Fluoroquinolones as potential photochemotherapeutic agents: covalent addition to guanosine monophosphate†

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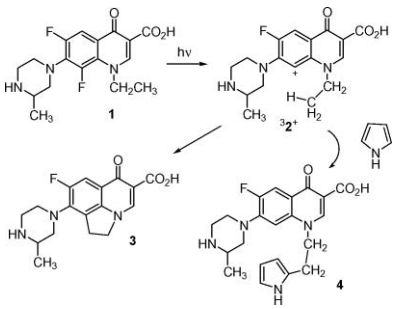
The triplet aryl cation photochemically generated from fluoroquinolones bearing a fluoro atom at position 8 attacks guanosine monophosphate ($k_r > 10^9$ $M^{-1}s^{-1}$) and forms covalent adducts. The reaction is a model for the implementation of oxygen-independent photochemotherapy

Photoinduced DNA damage in the presence of xenobiotics may cause serious disorders and skin cancer. On the other hand, a targeted effect that results in the selective killing of tumour cells offers an appealing therapeutic method, which indeed is increasingly applied in clinical practice. Such an action may be obtained by the generation of singlet oxygen—known to attack DNA bases—*via* a localized sensitizer, as in photodynamic therapy.[1] An alternative is the use of heterocycles that intercalate and undergo photo-cycloaddition, as is the case for psoralens in UV-A therapy (PUVA) for cutaneous T-cell lymphoma and psoriasis.[2] The presently approved photo-activated drugs are limited in number and structure type, making the introduction of new systems highly desirable. In particular this holds for those with an oxygen-independent activity, because oxygen often has a low partial pressure in tumour cells and is consumed during the treatment. This results in a non-linear dependence of the effect on laser intensity and in some cases in the failure of the treatment.[3] An ideal drug should bind selectively and undergo a very fast photoreaction, so that only biomolecules in close proximity are attacked and the action is as specific as possible.

Looking for new potential photoactivated drugs with such characteristics we considered fluoroquinolone antibacterials, known to complex either mammalian or bacterial DNA topoisomerase, thus blocking the transcription process,[4] and to be photoreactive.[5] In particular those bearing a halogen substituent at position 8, such as lomefloxacin **1**, react efficiently (Φ *ca.* 0.5) and are strongly photogenotoxic.[6] These molecules undergo heterolysis of the C_8–F bond to give the corresponding carbocation $^3\mathbf{2}^+$ in the *triplet* state.[7] If such a high energy intermediate proves to be able to attack DNA, it would represent a model for new potential photochemotherapeutic agents. Although in water cation $^3\mathbf{2}^+$ undergoes intramolecular insertion into the *N*-ethyl chain yielding compound **3**,[8] some bimolecular reactions are known. In particular, we found that in the presence of pyrrole product **4** is formed (Scheme 1).[9] Apparently, cation $^3\mathbf{2}^+$ undergoes



Scheme 1 Photochemistry of lomefloxacin **1** in neat water and in the presence of pyrrole.

hydride transfer and attacks the electron-rich heterocycle *via* the β-carbon of the chain. This suggested that DNA bases may be alkylated in the same way. We thus decided to investigate the photochemistry of **1** in the presence of the most nucleophilic nucleotide, guanosine monophosphate (dGMP) performing steady state and time-resolved experiments.

Laser flash photolysis under N_2O[10] led to the formation of the triplet state of lomefloxacin, $^3\mathbf{1}$, whose decay was *not* affected by the presence of dGMP (λ_{max} = 360 nm, $k_{triplet} = 2.5\times10^7$ s^{-1}). On the contrary, the triplet cation $^3\mathbf{2}^+$ (λ_{max} = 490 nm, $k_{cation} = 8.3\times10^6$ s^{-1}), formed by C_8–F bond cleavage in $^3\mathbf{1}$,[9] reacted with dGMP (see Fig. 1–3). Varying the dGMP concentration in the range 2×10^{-4}–2×10^{-3} M we obtained the bimolecular reaction rate constant $k_r = (4.8 \pm 1.5)\times10^9$ M^{-1} s^{-1}.

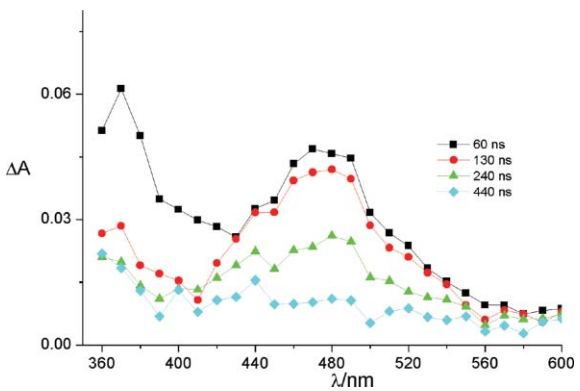


Fig. 1 Transient spectra following excitation at 355 nm of a N_2O saturated 1.4×10^{-4} M solution of **1** in 1.0×10^{-3} M $NaHCO_3$ buffer of pH 7.2 at 25 °C. Laser pulse energy 4.25 mJ, cell path 1 cm.

Likewise, HPLC monitoring evidenced that new photoproducts different from **3** were formed by irradiation of **1** in the presence of dGMP (2×10^{-3} M, N_2 flushed solution). HPLC at the end of the reaction (see the ESI†), showed that the yield of product

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† Electronic supplementary information (ESI) available: HPLC traces and HPLC/mass data of the photoreactions of **1** in the presence of dGMP. See DOI: 10.1039/c0ob00056f

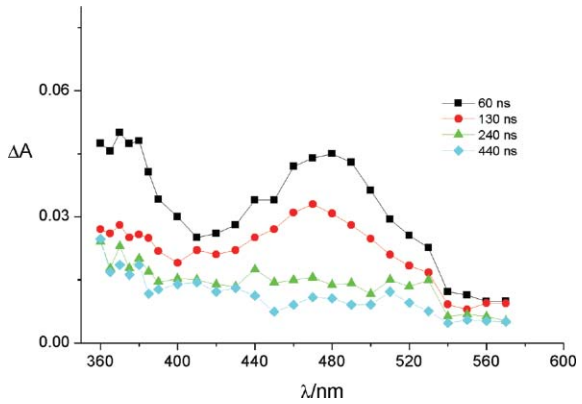


Fig. 2 Transient spectra following excitation at 355 nm of a N_2O saturated 1.4×10^{-4} M solution of **1** in 1.0×10^{-3} M $NaHCO_3$ buffer of pH 7.2 at 25 °C in the presence of 1×10^{-3} M dGMP. Laser pulse energy 4.25 mJ, cell path 1 cm.

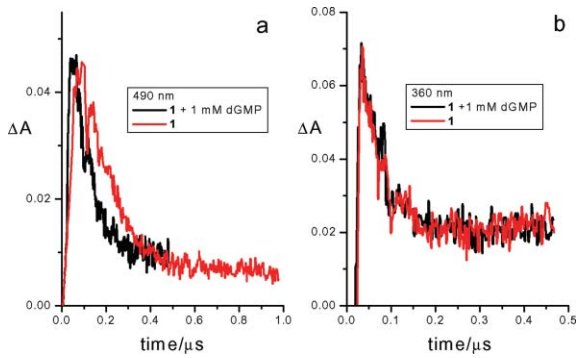
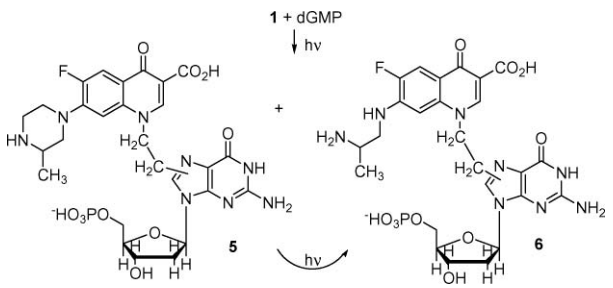


Fig. 3 Decay of absorbance changes following excitation at 355 nm of a N_2O saturated 1.4×10^{-4} M solution of **1** in 1.0×10^{-3} M $NaHCO_3$ buffer of pH 7.2 at 25 °C: (a) at 490 nm, (b) at 360 nm. Red lines, **1**; black lines, **1** in the presence of 1×10^{-3} M dGMP. Laser pulse energy 4.25 mJ, cell path 1 cm.

3 decreased with the concomitant appearance of three new pairs of products absorbing at 350 nm and thus containing the fluoroquinolone ring. Analysis of the mass spectrum showed that all of them were covalent adducts resulting from addition of a dGMP molecule to a fragment of **1** after loss of F^-. Thus, formula **5** was assigned to a pair of peaks with m/z 679 ($M+H^+$, Scheme 2), consistent with the analysis of MS/MS fragmentation spectra evidencing the characteristic loss of the deoxyribosephosphate moiety (m/z 483, M^+–196) and of the carboxyl group (see the ESI†). These are apparently a pair of regioisomers and at the moment we are unable to determine the position of attack.[11] A more polar pair of peaks had m/z 653 ($M+H^+$) and showed again loss of the sugar phosphate as daughter ion (m/z 457, M^+–196). The 26 au difference from **5** was thus located in the fluoroquinolone moiety. Analogy with previous results on the photochemistry of fluoroquinolones[5] suggests that fragmentation of the piperazine moiety is involved (structure **6**). Such a fragmentation is characteristic of fluoroquinolones bearing a single fluoro atom at position 6, where a stepwise degradation of the side-chain takes place.[5] Thus, **6** is a secondary photoproduct from **5**. Indeed, the proportion of this product was larger in experiments with a low (1×10^{-3} M) concentration of dGMP, where the conversion was more extensive, consistently with the fact that it resulted from secondary photolysis.



Scheme 2 Products of the addition of **1** to dGMP

Low conversion experiments (<30% in deoxygenated solutions) were carried out at increasing dGMP concentrations. The yield ratio of product **3** in the absence *vs.* in the presence of dGMP, [**3**]°/[**3**], linearly depended on the dGMP concentration. The plot in Fig. 4 afforded the slope $K_{SV} = k_r/k_{cation} = 468$ M^{-1}. With the k_{cation} value obtained from the flash photolysis experiment, k_r calculated from the steady state data was 3.9×10^9 $M^{-1}s^{-1}$, in remarkably good agreement with the flash photolysis determination. Thus, the *same* phenomenon was evidenced by the two techniques: the competition of the intermolecular reaction of triplet cation $^3\mathbf{2}^+$ with dGMP to give **5** with the intramolecular C–H insertion to give **3** (Scheme 3). The cation appeared to react exclusively as an electrophile, not as oxidant, as indicated by the absence of 8-oxoguanosine typically formed under oxidative conditions.

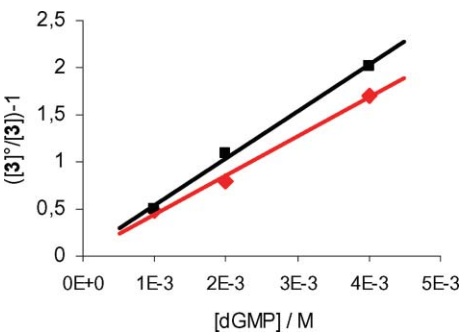
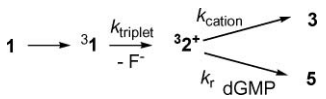
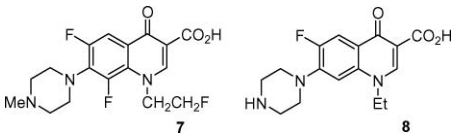


Fig. 4 Stern–Volmer plot for the dependence of the yield of product **3** on the dGMP concentration (diamond); same for the main product from **7** (square).



Scheme 3 competing reactions of triplet cation $^3\mathbf{2}^+$.

The investigation was extended to another 6,8-difluoroquinolone, fleroxacin, undergoing analogous triplet mediated photofragmentation.[12] Flash photolysis of fleroxacin (**7**) revealed the formation of the corresponding triplet cation and this intermediate was similarly trapped by dGMP, affording analogous products. In this case a reaction rate constant value of $k_r = 2.3\times10^9$ M^{-1} s^{-1} was measured (see ESI) consistent with the 2.8×10^9 M^{-1} s^{-1} value obtained from steady-state experiments ($K_{SV} = k_r/k_{cation} = 420$ M^{-1}, Fig. 4).



7 8

A different behaviour was observed with norfloxacin (**8**) that lacks the fluorine at position 8 and does not undergo a unimolecular photofragmentation. Here only the intact triplet was revealed at delays ≥60 ns after the laser pulse and was quenched by dGMP at a rate (k_q = 7.7×10^7 M^{-1}s^{-1}) similar to that by the inorganic phosphate anion.[13] Photoproduct analysis did not reveal any covalent photoadduct. The triplet quenching process is conceivably attributable to hydrogen abstraction from the sugar residue or interaction with the phosphate moiety and differs from the above addition to the heterocyclic base.

These data demonstrate the *covalent* addition of a fragment arising from a photoexcited drug (here cation ^{3}**2**$^+$) to a DNA base. To our knowledge, there is no precedent for this mechanism, which is different from the direct attack by the drug excited state.[2,14] Ongoing biological experiments will ascertain the consequences of this photoprocess in the cell. The present data in solution encourage the development of new potential photochemotherapeutic drugs with the fluoroquinolone skeleton. Thus, one takes advantage of the available knowledge on the structural features controlling human gyrase complexation on one hand and of those controlling photoreactivity on the other. The literature suggests how to address the two activities separately and offers viable approaches towards selectivity and thus reduced side effects.[15] In this frame the high absorptivity in UV-A, the efficient cleavage, the absence of oxygen effects on both the drug triplet and the cation, the specific reactivity of the latter with guanosine (k_r ~ 4×10^9 M^{-1}s^{-1}, several orders of magnitude higher than that of other electrophiles considered for this function, *e.g.* quinone methides),[16] the lack of competing reaction with water or the DNA backbone are all promising features.

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