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Synthesis and photo DNA-damaging activities of fluoroquinolone analogues

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Abstract—Fluoroquinolone (FLQ) analogues were synthesized and their DNA photocleaving abilities were assayed. The photo-bioactivities of the fluoroquinolones were dependent on the carbonyl moieties attached to quinolone ring.

Fluoroquinolones (FLQs), such as enoxacin, lomefloxacin and fleroxacin, are a class of compounds widely used as broad-spectrum antimicrobial agents.[1] FLQs are also known to exhibit phototoxic, photomutagenic and photocarcinogenic activities and sometimes cause undesirable side effects in patients upon exposure to light, especially light in the UV-A region.[2] To clarify the source of these side effects, the behaviour of FLQs under irradiation conditions has been studied extensively by several groups and the origins of these side effects have been increasingly elucidated. Some of these drugs, such as ofloxacin, are relatively photostable and these drugs are thought to activate oxygen by a photosensitization pathway, which is very common in phototoxicities of drugs.[3] On the other hand, among FLQs, lomefloxacin and fleroxacin, which have two fluorine atoms on aromatic ring exhibit considerably potent phototoxicities. These compounds generate a carbene under irradiation conditions by heterolytic cleavage of the C–F bond at the C8 position, and the carbene is thought to be responsible for their remarkable phototoxicities (Fig. 1).[3a,4] Recent developments in molecular design of FLQs have been aimed at preventing these undesired phototoxicities,[4a] On the other hand, from a different standpoint, this phototoxicity is also attractive for developing agents for photochemotherapy. In the course of our study to develop drugs having DNA photo-damaging activity, we became interested in FLQs. Although some FLQs are known to have potent photo DNA-damaging activity, the molecular design for developing FLQs having more potent photo-bioactivities has not been clarified. In this letter, we synthesized several FLQ derivatives **1a**–**h** (Fig. 2), and found that imide **1f** has about 10-times greater photo DNA-damaging ability than does the parent compound, FLQ acid **1a**.

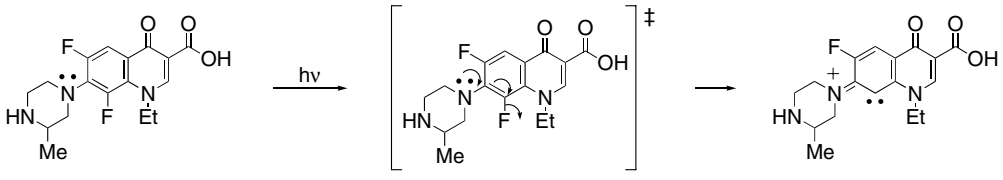

Figure 1. Photogeneration of an aromatic carbene from *Lomefloxacin*.

Keywords: Fluoroquinolone; DNA damage; Photo-irradiation; Carbene; Hydrogen-bonding.
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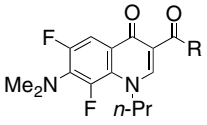
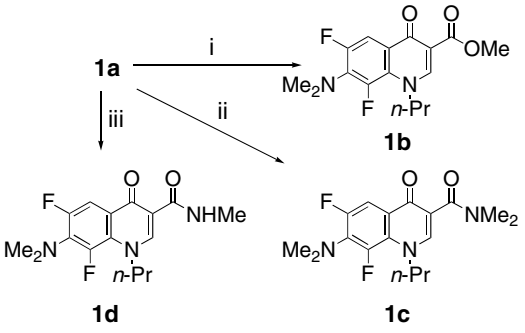
FLQ acid **1a** was prepared by the literature procedure with slight modification from commercially available 2,3,4,5-tetrafluorobenzoyl chloride.[5] Acid derivatives **1b–d** were synthesized from acid **1a** using conventional methods as shown in Scheme 1.

Amide **1e** was also synthesized from acid **1a** via mixed pyrocarbonate and converted to imide **1f** as depicted in Scheme 2.

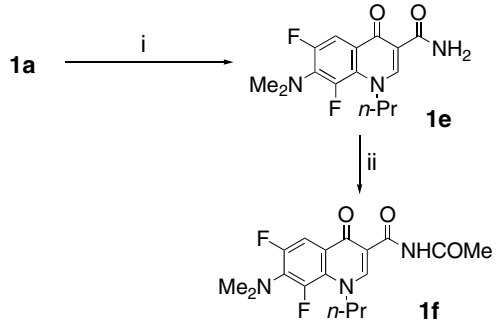


1a: R = OH; **1b**: R = OMe; **1c**: R = NMe_2;
1d: R = NHMe; **1e**: R = NH_2;
1f : R = NHCOMe; **1g**: R = H; **1h**: R = Me

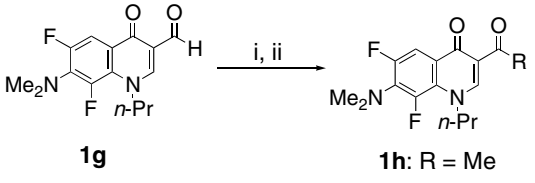
Figure 2.



Scheme 1. Reagents and conditions: (i) EDC, MeOH, DMAP/CH_2Cl_2, 40 °C, 80%. (ii) $HNMe_2$·HCl, PyBOP, *i*-Pr_2EtN/DMF, rt, 83%. (iii) $MeNH_2$HCl, PyBOP, *i*-Pr_2EtN/DMF, rt, 67%.



Scheme 2. Reagents and conditions: (i) $ClCO_2Et$, Et_3N/CH_2Cl_2, rt, then NH_4OAc, Et_3N/DMF, rt, 75% (2 steps). (ii) NaH, CH_3COCl, *n*-Bu_4NI/C_6H_6, 0 °C, 35%.



Scheme 3. Reagents and conditions: (i) MeMgBr/THF, 0 °C, 95%. (ii) MnO_2/CH_2Cl_2, rt, 51%.

Ketone **1h** was prepared from aldehyde **1g**[6] by treating with MeMgBr followed by oxidation reaction as shown in Scheme 3.

Photochemical properties, λ_{max}, molar absorption coefficient ε and decomposition time of FLQ **1a–h** are summarized in Table 1. The decomposition times needed for complete consumption of the drugs under irradiation conditions were varied greatly. Since ε values of the compounds are very similar, we expected that a compound having a λ_{max} value close to the wavelength of irradiation light ($\lambda = 365$ nm) would show enhanced photoreactivity; however, any clear correlation was not found between λ_{max} and decomposition time. Though we also tried to isolate photoproducts from the reaction mixture to confirm the reaction course, no isolable products were obtained.[7]

Next, we carried out DNA cleaving assays of photo-drugs **1a–h**. Drugs **1a–h** were incubated with ΦX174R-1 plasmid DNA in a phosphate buffer solution (pH 7.8, containing 20% of DMSO) and the mixture was irradiated ($\lambda > 365$ nm) through a Pyrex™ filter for 1 h. The resultant DNA fragments were separated by electrophoresis on agarose gel and visualized by ethidium bromide staining: the results are summarized in Table 2. All compounds except for FLQ **1a** and **1c**, showed moderate to excellent DNA photo-cleaving activity but FLQ **1a** and **1c** exhibited only weak photo-bioactivity (entries 1 and 3). To exclude the possibility that the DNA photo-damage is caused by active oxygen species that were formed by photosensitization, we carried out the same experiments in the presence of NaN_3 (75 mM) or D-mannitol (75 mM). The addition of D-mannitol had no effect on the DNA-cleaving ability of photo-drugs **1a–h**, indicating that a hydroxyl radical is not involved in the DNA photocleavage by our FLQs. On the other hand, in the presence of NaN_3, decreases of about 10% in % cleavage values were observed for all FLQs examined. Although this observed decrement of % cleavage by NaN_3 implies participation of singlet oxygen in DNA photocleavage, we now consider that the major active species responsible for DNA damage must be carbene because the decrease of % cleavage values should reach to 80–90%, not 10%, if singlet oxygen is the major active species.[3g]

Table 1. Photochemical properties of FLQ **1a–h**

Entry	Compounds	λ_{max}/nm	log ε	Time/h[a]
1	**1a**	295	4.6	>48
2	**1b**	293	4.6	4
3	**1c**	287	4.5	6
4	**1d**	292	4.6	9
5	**1e**	293	4.6	6
6	**1f**	301	4.6	15
7	**1g**	307	4.6	6
8	**1h**	301	4.5	6

FLQs were dissolved in the solution of $CHCl_3$–EtOH = 1:1 and λ and ε were measured.

[a] FLQs were dissolved in $CHCl_3$–EtOH and photo-irradiated through a Pyrex™ filter using VL-30L (VILBER LOURMAT, 1820 μW cm^{-2}, $\lambda > 365$ nm) for the indicated times.

Table 2. Photo DNA-damaging ability of **1a**–**h**

Entry	Compounds	% Cleavage[a]
1	**1a**	9 ± 3
2	**1b**	56 ± 5
3	**1c**	9 ± 3
4	**1d**	67 ± 5
5	**1e**	66 ± 5
6	**1f**	88 ± 2
7	**1g**	42 ± 2
8	**1h**	46 ± 5

ΦX174R-1 DNA (0.46 μg, WAKO Pure Chemical Industries, Ltd.) in 20 μL phosphate buffer solution (pH 7.8, containing 20% DMSO) with a drug (100 μM) was incubated and photo-irradiated using a VL-30L (VILBER LOURMAT, 1820 μW cm^{-2}, $\lambda > 365$ nm) through a Pyrex™ filter for 1 h at 25 °C. Immediately, 15 μL samples were loaded into 1% agarose gel. The running buffer was 20 mM TAE (pH 7.8). Electrophoresis was carried out at 50 V for 8 h. After electrophoresis, the gel was stained for 1 h in ethidium bromide (1 μg/mL) and de-stained for 5 min in water. Relative amounts of DNA in form I and form II were determined by densitometry.

[a] Each value presented is mean value ± SD of three runs. A control reaction mixture without the addition of any drug was incubated and photo-irradiated. The mean value of three runs was used as the background to be subtracted from the obtained values.

It is reasonable to expect that a compound that is labile to light would react faster to give a bioactive species causing DNA damage. However, our results indicate that photo-bioactivity of FLQ **1a**–**h** is correlated with neither λ_{max} nor decomposition time as shown in Table 1. In these results, DNA photo-cleaving abilities of amide **1c**–**e** and imide **1f** seemed to depend on the hydrogen-bonding ability of carbonyl moiety attached to the quinolone ring. Although amides **1d** and **1e**, which have an amide proton that is capable of forming intramolecular hydrogen bonds, showed a high level of photo DNA-damaging activity, dimethylamide **1c**, which has no amide proton, showed only poor photo-bioactivity. Furthermore, imide **1f**, bearing a more acidic imide proton, showed excellent DNA-cleaving activity. The time needed for photo-decomposition of FLQ **1c**–**f** seemed not to be related to the hydrogen-bonding ability of the attached amide or imide moiety, implying that the hydrogen bonding affects the reactivity of the photo-generated carbenes but not the photodefluorination step. These results can be explained as follows by considering the reactivity of the carbene which is essentially an aromatic cation flanked by two adjacent nitrogen atoms as shown in Figure 3.

Electron-donating abilities of the two nitrogen atoms is very important to modulate the reactivity and stability of the carbene. As two carbonyl groups become more electron-attractive, they would withdraw electron density from the two nitrogen atoms to reduce their electron-donating ability, which is indispensable for stabilizing the aromatic cation in a carbene form. In FLQ **1f**, hydrogen bonding between imide proton and carbonyl oxygen would reduce the electron-donating ability of two nitrogen atoms to cause destabilization of the carbene and as a result, the reactivity of the carbene would be enhanced (see Fig. 4).

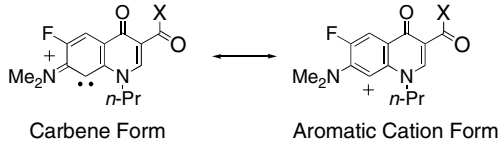


Figure 3. Resonance between the carbene and the aromatic cation.

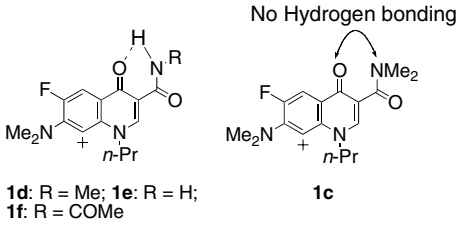


Figure 4. Hydrogen bonding interaction in the FLQs.

Based on the consideration mentioned above, it is reasonable that amide **1d** and **1e**, which have an amide proton capable of hydrogen bonding, showed a higher level of DNA-damaging activity than that of dimethylamide **1c** having no acidic proton and acid **1a** which exists in the form of carboxylate anion in phosphate buffer solution (pH 7.8), so acid **1a** is also not capable of forming a hydrogen bond. FLQ **1b**, **1g** and **1h**, whose carbonyl groups are sufficiently electron- attractive to show enhanced reactivity compared to acid **1a**, exhibited moderate to good DNA-cleaving activity, though they have no acidic proton available for hydrogen bonding.

In conclusion, we synthesized some FLQ analogues and the synthesized FLQ analogues showed moderate to excellent DNA photo-cleaving activity. DNA photo-damaging activity is affected by the character of the carbonyl groups attached to the quinolone ring, and intramolecular hydrogen bonding is thought to play an important role in regulation of the reactivity of photo-generated carbene species.

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7. Isolation of photo-decomposed products have been reported by other groups. See Refs. 3a and 4a.