PHOTOCHEMICAL TRANSFORMATION OF FLOUROQUINOLONE ANTIBIOTIC DRUG-BESIFLOXACIN

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**ABSTRACT:** In the present study, the photochemistry of flouroquinolone antibiotic drug-besifloxacin (I) was investigated under mild conditions similar to those encountered in biological systems namely aerobic as well as an anaerobic condition with UV-A light. When besifloxacin (I) was irradiated with a high-pressure mercury lamp under aerobic conditions it afforded 7-(2-Amino-5-oxopentylamino)-8-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid as photoproduct (2) and under anaerobic condition it afforded 7-(3-Amino-azepan-1-yl)-1-cyclopropyl-6-fluoro-8-hydroxy-4-oxo-1,4dihydro-quinoline-3-carboxylic acid as photoproduct (3). The formation of photoproduct was explained by the type-I and type II mechanism of Photosensitization.

**INTRODUCTION:** The Interest in the topic of drug photochemistry has amplified markedly due to the awareness among the scientific community of an increase in the UV portion of the sun spectrum reaching the earth. In the last few years majority of the work done on the mechanisms of both drug photodegradation and photosensitization, has appeared in recent reviews. The mechanism of Photosensitization induced by drugs are generally considered as belonging to either type I (radical mediated) or type II (singlet oxygen mediated). The interaction of many currently used drugs with UVA and UVB radiations is usually responsible for a series of photosensitizing effects, i.e. phototoxicity, photoallergy, photomutagenesis and photocarcinogenesis.

Thus to evaluate the photobiological risk associated with a drug, it is important to understand the molecular mechanism of drug photosensitization. Fluoroquinolone drugs are the most successful largely used class of antibiotics. They are considered to be well tolerated drugs with relatively limited adverse effects. In spite of this, they have been recognized to possess a remarkable phototoxic potential. In order to gain insight into the molecular mechanisms of the photoinduced biological damage, a great deal of attention was recently dedicated to the study of their photochemical degradation pathways. Besifloxacin is a topical ophthalmic flouroquinolone, most recently approved drug for the treatment of bacterial conjunctivitis caused by susceptible bacterial strains. Like other fluoroquinolones (FQs), besifloxacin also targets bacterial enzymes required for DNA replication such as DNA gyrase and topoisomerase IV. However, besifloxacin show more balanced activity against these enzymes than do other fluoroquinolone In addition, besifloxacin has been found to have greater in vitro activity than...
gatifloxacin and moxifloxacin against several drug-resistant organisms. In spite of this, they induce phototoxicity as a significant side effect so theoretical and experimental studies are needed in order to shed light on possible molecular mechanism of photosensitization to help in developing possible routes to avoid these complications.

In continuation of our interest in the photochemical reactions responsible for the phototoxicity of the photosensitizing drugs and their mechanisms, herein we have examined the photochemical behaviour of newly approved fluoroquinolone antibiotics besifloxacin under different reaction conditions (aerobic and anaerobic). The irradiation of besifloxacin with UV-A light under aerobic condition give one photoproduct (2) and under anaerobic conditions gives two different photoproducts (3 & 4) (Scheme 1). These photoproducts were identified from their spectral (IR, 1H-NMR, 13C-NMR, and mass spectra) properties.

**Experimental:**

**Apparatus and Chemicals:**
All chemicals used were of analytical grade. Pure besifloxacin was obtained from Swapnoop drugs & pharmaceuticals (India); IR spectra were recorded as KBr discs on a Perkin Elmer model spectrum RXI. 1H NMR and 13C NMR Spectra were recorded on a Bruker Avance –DRX -300 Spectrometer using TMS as internal standard and CDCl3 as solvent. High resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 e V ionization voltage. Merck silica gel 60 F254 plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (60-120mesh).

**Photoirradiation procedure:**
A solution of besifloxacin (in ethanol) was stirred and flushed with argon or oxygen (as desired) for 1 h before irradiation and was kept bubbling during the irradiations. The course of reaction was monitored by thin layer chromatography on precoated silica gel TLC plates using chloroform-acetone (9:1) mixture. After the completion of reaction (when desired conversions have reached) the solvent was removed in a rotary evaporator and products were purified by silica gel column chromatography.

**Irradiation of besifloxacin under aerobic condition:**
An ethanolic solution of 1 (238 mg) was irradiated for 5 h at 254 nm. After following the steps described in the photoirradiation procedure, 2 (110 mg) was obtained as the main photo product, as detected on TLC.

7-(2-Amino-5-oxo-pentylamino) - 8 - chloro-1-
cyclopropyl-6-fluoro-4- oxo - 1,4 - dihydro-
quinoline-3-carboxylic acid (2): Yield: 110 mg (46.21 %) HRMS calcd. For (M+) C19H19ClF3N4O4 395.10 Found 395.05; IR (KBr): 3400, 1735, 1495 cm−1; 1H-NMR (CD3OD, δ, ppm): 11.2 (s, 1H, COOH), 9.69 (t, 1H, CHO), 7.91 (s, 1H, H-2), 6.92 (s, 1H, H-5), 4.1 (t, 1H, NH), 3.23 (d, 2H, H-1 of pentylamo), 2.85 (m, 1H, H-2 of pentylamino), 2.38 (m, 2H, H-4 of pentylamino), 1.80 (m, 2H, H-3 of pentylamino); 13C NMR (CD3OD, δ, ppm): 198.8, 186.8, 168.5, 155.4, 140.5, 137.2, 135.2, 115.4, 110.9, 107.5, 58.5, 50.4, 42.5; MS: m/z: 395 (M+),377(M+ - 18), 359.5 (M2-35.5), 349 (M+ - 45).

**Irradiation of besifloxacin under anaerobic condition:**
An ethanolic solution of 1 (238 mg) was irradiated for 5 h at 254 nm. After following the steps described in the photoirradiation procedure, 3 (108 mg) was obtained as the main photo product, as detected on TLC.

7-(3-Amino-azepan-1-yl)-1-cyclopropyl-6-fluoro-
8-hydroxy-4-oxo-1,4-dihydro - quinoline - 3-
carboxylic acid (3):
Yield: 108 mg (45.37%) HRMS calcd. For (M+) C19H22FN3O4 375.08 Found 375.02 IR (KBr): 3400, 2650, 1720, 1605, 1495; 1H-NMR (CD3OD,δ, ppm): 11.2 (s, 1H, COOH), 7.92(s, 1H, H-2), 6.64 (s, 1H, H-5), 5.0 (s, 1H, OH), 3.52 (m, 2H, H-2 of azepan-1-yl), 3.32(m, 2H, H-7 of azepan-1-yl), 2.82 (m,1H, H-3 of azepan-1-yl), 2.0 (d, 2H, NH2), 1.50 (m, 4H, H-4 & H-6 of azepan-1-
yl), 1.27 (m, 2H, H-5 of azepan-1-yl) 13C NMR (CD3OD, δ, ppm): 187.4, 170.4, 156.4, 137.2, 128.3, 127.5, 125.1, 113.3, 111.2, 64.3, 56.2, 49.5,
36.1, 28.1, 22.3; MS: m/z: 375 (M⁺), 358 (M⁺-17), 330 (M⁺-45).

RESULTS AND DISCUSSION:
Irradiation of an ethanolic solution of besifloxacin under both aerobic and anaerobic conditions with corex filtered light followed by purification of crude product by silica gel column chromatography afforded two different photoproducts, which was identified by their spectral studies (Scheme-1). The photoproduct formation can be rationalized by the involvement of different mechanism under aerobic and anaerobic conditions. Photodegradation of drugs generally take place by two mechanism type-I and type-II mechanism. Type I mechanism involve free radical process and type II mechanism involve excited singlet oxygen.¹⁹

Under aerobic condition in presence of molecular oxygen drug besifloxacin first generated singlet oxygen by energy transfer as most of the fluoroquinolone generated singlet oxygen on irradiation. In the next step besifloxacin react with generated singlet oxygen to from a reactive intermediate which on hydrolysis produces photoproduct 2 thus under aerobic condition photoproduct is formed by singlet oxygen mediated photooxidation of drug. From the above discussion it is clear the under aerobic condition

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**Scheme-1**

**Photodegradation Products of Besifloxacin**
photodegradation of photosensitizing drug besifloxacin follow type II mechanism (Scheme 2).

The photoproduct was isolated and identified by their spectral properties as 7-(2-Amino-5-oxo-pentylamino)-8-chloro-1-cyclopropyl-6-fluoro - 4-oxo-1,4-dihydro-quinoline-3 carboxylic acid. The spectral features correlated to the assigned structure of the products and were done in comparison with the spectra of the starting drug. The $^1$H-NMR spectrum of the photoproduct (2) showed signals similar to those of besifloxacin, except for signals at $\delta$ 9.69 ppm which indicate the formation of CHO group. The $^{13}$C NMR spectrum of photoproduct (2) also showed signals similar to those of basifloxacin except for the carbon signals of azepan ring. A new signal that appeared at $\delta$ 198 ppm was assigned to the carbon of CHO group.

The IR absorption of spectra of Photoproduct 2 show the absorption band at 1735 cm$^{-1}$, 3400 cm$^{-1}$ which indicate the presence of aldehyde and primary amine group respectively. From the IR, NMR and $^{13}$C NMR spectra of Photoproduct 2 clearly indicate that during photochemical transformation of besifloxacin under aerobic condition the azepan ring of Starting drug is break and a new aldehyd and primary amine group is formed. As indicated in literature that the fluoroquinolone antibacterial drugs having F atom at either 6 or 8 positions undergoes defluorination process similarly under anaerobic condition the photoexicited drug molecule of our interest undergo defluorination. In principle, this reaction may involve unimolecular fragmentation of the C–F bond in the excited state or water addition followed by fluoride anion loss resulting the formation of Photoproduct 3.

The photoproduct was isolated and identified by their spectral properties as 7-(3-Amino-azepan-1-yl)-1-cyclopropyl-6-fluoro-8-hydroxy-4-oxo - 1,4-dihydro -quinoline-3-carboxylic acid. The spectral features correlated to the assigned structure of the products and were done in comparison with the spectra of the starting drug. The $^1$H-NMR spectrum of photoproduct (3) showed signals similar to those
of parent drug basifloxacin, except a new proton signals appear at $\delta$ 5 ppm which indicate the introduction of new hydroxyl group, this is also supported by the IR spectra of the photoproduct (3) in which new a absorption band at 3400 cm$^{-1}$ was appear, thus the spectral study of the photoproduct (3) also supported the purposed mechanism of the photochemical transformation which indicate that the photoproduct (3) is formed by the deflurenation Process.

On the basis of the results of this investigation we concluded that when besifloxacin was irradiated with UV light under different reaction condition the besifloxacin undergoes photochemical transformation. When besifloxacin was irradiated under aerobic condition it photochemically degraded and give the photoproduct (2) and the mechanism of this photochemical transformation shows that under aerobic condition drug undergo photodegradation via type-I mechanism and it is also revels’ that the probable phototoxicity of besifloxacin under aerobic condition is due to the formation of singlet oxygen and when the drug was irradiated under anaerobic condition drug undergo photodegradation and produces the photoproduct (2) the mechanism of this photochemical transformation indicate that the under anaerobic condition drug show deflurenation via highly reactive reaction intermediate and the possible phototoxicity of these drug under anaerobic condition is due to the formation of this reactive intermediate.

The combination of drugs and optical radiation has a great potential in cancer therapy and will find new applications in the near future (e.g. in the battle against resistant bacteria). Hence the investigation of photochemical properties of besifloxacin used in clinical medicines is of great relevance from photo biological as well as medical point of view.
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