A pH Dependent Photochemistry of Photosensitizing Drug Temafloxacin

Waseem Ahmad[1]*, Amit Parashar[2] & Harish Chandra Joshi[1]
[1]Department of Chemistry, Uttaranchal University, Dehradun
[2]Department of Chemistry, GL Bajaj Group of Institutions, Mathura.

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ABSTRACT *The UV-A irradiation of, Temafloxacin, a fluoroquinolone antibacterial under anaerobic condition in aqueous solution was studies by using different buffer solution (phosphate buffer pH 7.4 and borate buffer pH 9.0-9.5) leads to formation of two main photoproducts characterized by a decarboxylation process and an opening of the piperazinyl ring, respectively. The products were characterized on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. The formation of products was also explained. The free radical produced from different charged form at pH-7.4 and pH- 9-9.5 may be responsible to produced phototoxicity through photosensitization of Temafloxacin under UVA irradiation.*

Keywords: *Temafloxacin, UVA irradiation, Photosensitization,Phototoxicity*

Introduction

The topic of drug photochemistry has always received great attention but recently this interest has markedly intensified due to the increase in the UV portion of the sun's spectrum reaching the earth. Much of the work reported in the last few years is on the mechanisms of drug photodegradation and drug photosensitization. Many drugs belonging to different pharmacological classes are characterized by one or more protonation sites and, as a consequence of the relative prototropic equilibria, different forms can be present at different pH values. Due to this, markedly different photo-behavior and phototoxic response can be observed for such drugs depending upon the different prototropic form present [1-5]. However, not many papers have focused attention on the dominant role played by pH on the photochemical behaviour of drugs. 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. By both product studies and detection of transient intermediates, photoreactivity trends have been assessed for some representative derivatives of flouroquinolones, i.e. norfloxacin (NF), the 1,8 naphthyridinone analogue enoxacin (EN), lomefloxacin (LM), rufloxacin (RF), ciprofloxacin and ofloxacin. The investigations mainly focused on the zwitterionic form of these drugs, as the predominant structure in the physiologically relevant, neutral pH conditions [6]. Temafloxacin (TMF) (1) a new ,belongs to a large group of structurally related antibiotics, Fluoroquinolones. , is highly active in vitro against a broad spectrum of gram-positive and gramnegative aerobes and anaerobes, including those resistant to aminoglycosides and B-lactams [7] its activity is greater than those of ciprofloxacin and ofloxacin against gram-positive, anaerobic, and intracellular organisms also [8] it is also used in the treatment of many infections diseases caused by Mycobacterium tuberculosis [9], pneumococci [10] ,majority of organisms responsible for bacterial lower respiratory tract infections[11] genital [12] and urinary infections like prostatitis, and skin infections inspite of immense medicinal use Temafloxacin was associated with severe hemolytic–uremic syndrome [13] which was withdrawn from sale in the U.S. shortly after its approval in 1992 because of serious adverse reactions including allergic reactions and hemolytic anemia, resulting in three death . with known phototoxicity. Due to the presence of two different protonation sites, TMF is present in the, zwitterionic and different ionic forms [14] which undergo different photochemistry and may correlate to the phototoxicity response of this drug.

Within the context the photodegradation of temafloxacin (1) under anaerobic condition in aqueous solution was studies by using different buffer solution phosphate buffer(pH 7.4)and borate buffer (pH 9.0-9.5). Two major photodegradation products were obtained by decarboxylation and opening of the piprazinyl ring through deprotonation followed by photoinduced electron transfer and identified by IR, ^{1}H- NMR, ^{13}C-NMR and mass spectral studies as 2 and 3. The formation of 2 and 3 are rationalised in Scheme-1.

Experimental:

Apparatus and chemicals:

All chemical used were of analytical grade. Pure Temafloxacin was purchased from Addobt chemical laboratory , Mumbai (India) and recrytallized from methanol. IR spectra were recorded as KBr discs on Perkin Elmner model spectrum RX_1,^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX-

300 spectrometer using TMS as internal standard and $(CD_3)_2O$ as solvent high resolution mass spectra were determine with a VG-ZAB-BEQ-9 spectrometer at 70 ev ionization voltage. Merk silica gel 60(F_{254}) plates were used for analytical TLC; coloumn chromatography was performed on Merk silica gel -60 (70-230 mesh)

Irradiation procedure:

Two different set of experiment were carried out at differet pH 7.4 and 9-9.5. In first set of experiment the aqueous solution of (640 mg/250ml) temafloxacin using 10 mM phosphate buffer solution (pH 7.4) was deaerated by bubbling with nitrogen with continuous stirring and irradiated for about 3 hours in a raynot photochemical reactor (The Southern New England Ultraviolet Co Model PRP-208 equipped with four RUL 350 nm fluorescence lamp) for the complete conversion of reactant. The progress of reaction was monitored by thin layer chromatography (chloroform: methanol: 98:2). At the end of reaction formation of number of product was indicated on TLC. The one major photoproduct 2 were isolated by eluting with dichloromethane: ethyle ether (1:1, v/v) on a silica gel columns. In another set, the similar experiments were carried out by using 10 mM borate buffer (pH 9-9.5) with the same amount of temafloxacine. The progress of reaction was monitored on TLC using same solvents (chloroform: methanol: 98:2). At the end of reaction photoproduct (3) was indicated on TLC and isolated by eluting with the same solvents by using dichloromethane ethyle ether (1:1, v/v) on silica gel columns. The photoproducts were identified as 2 and 3 from various spectroscopic methods like UV NMR ^{13} C NMR, Mass spectrometry.

Characterization of products:

2- 1-(2,4-difluorophenyl)-6-fluoro-7-(3-methylpiperazin-1-yl)quinolin-4(1H)-one (2)

Yield: 80 mg; HRMS calcd. for M^+ -- $C_{20}H_{18}F_3N_3O$ 373.37, found 355.20 ;IR (KBr) :1625,1725; ^{1}H -NMR ($(CD_3)_2O$): δ 1.25 (d, 3H, CH_3), 2.95 (m,1H, NCH), 3.11(m, 2H, NCH_2), 3.48(m, 2H, $2NCH_2$)6.21(d,1H,H-8), 7.95 (m,1H, aromatic), 8.04 (d, 1H, H-5); ^{13}C- NMR ($(CD_3)_2O$): δ 38.7 (N^4-CH_3), 50.5 (piperazinyl ring), 56.6(piperazinyl ring),108.2(C-5)109.3(C-3), 129 (C-8), 119.4 (C-3 of 4-phenyle), 146.5(C-4), 139 (C-7), 159 (C-6), 174.3(C-4) MS: m/z 373(M+), 332 (M+- COOH).

3-1-(2,4-difluorophenyl)-6-fluoro-7-((2-formamidopropyl)(methyl)amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid(3)

Yield: 25 mg, HRMS calcd. For M^+ -- $C_{22}H_{22}F_3N_3O_4$ 449.42, found 449.12 ; IR (KBr):; 1695, 1560, 3300, 1395-1440, 1744, 1679, 1610 ^{1}H-NMR: δ1.26 (s,1H,HCONH) 3.35 (m, 2H, $CHCH_3$ NCH_3) , 3.50 (m,2H,$CHCH_3CH_2NCH_3$), 7.88(s,1H,HCON) ,7.96(d,1H,H-5),7.8(s,1H,H-2) 8.86 (s, IH, H-8) 10.50 (s, IH, COOH) ^{13}CNMR: 35.2(CH CH_3 CH_2 NCH_3) 40.9 (HCONH), 49.1 ($CHCH_3CH_2NCH_3$), 53.4 ($CHCH_3CH_2NCH_3$), 109.3 (C-5), 114.0 (C-3),152.1 (C-2), 159.9(C-6),165.4(HCON),171(COOH),177.8(C-4) MS (EI): m/z 405 [M-CO_2] (6.5), 346 [405 $HCONHCH_3$]$^+$ (38.7) 333 [405- [$HCONCH_3CH_2$·]$^+$ (100) ,318 [346-CH_2=CH_2]$^{·+}$ (6.3), 270 [313-CH_2=NCH_3]$^+$

Results and discussion

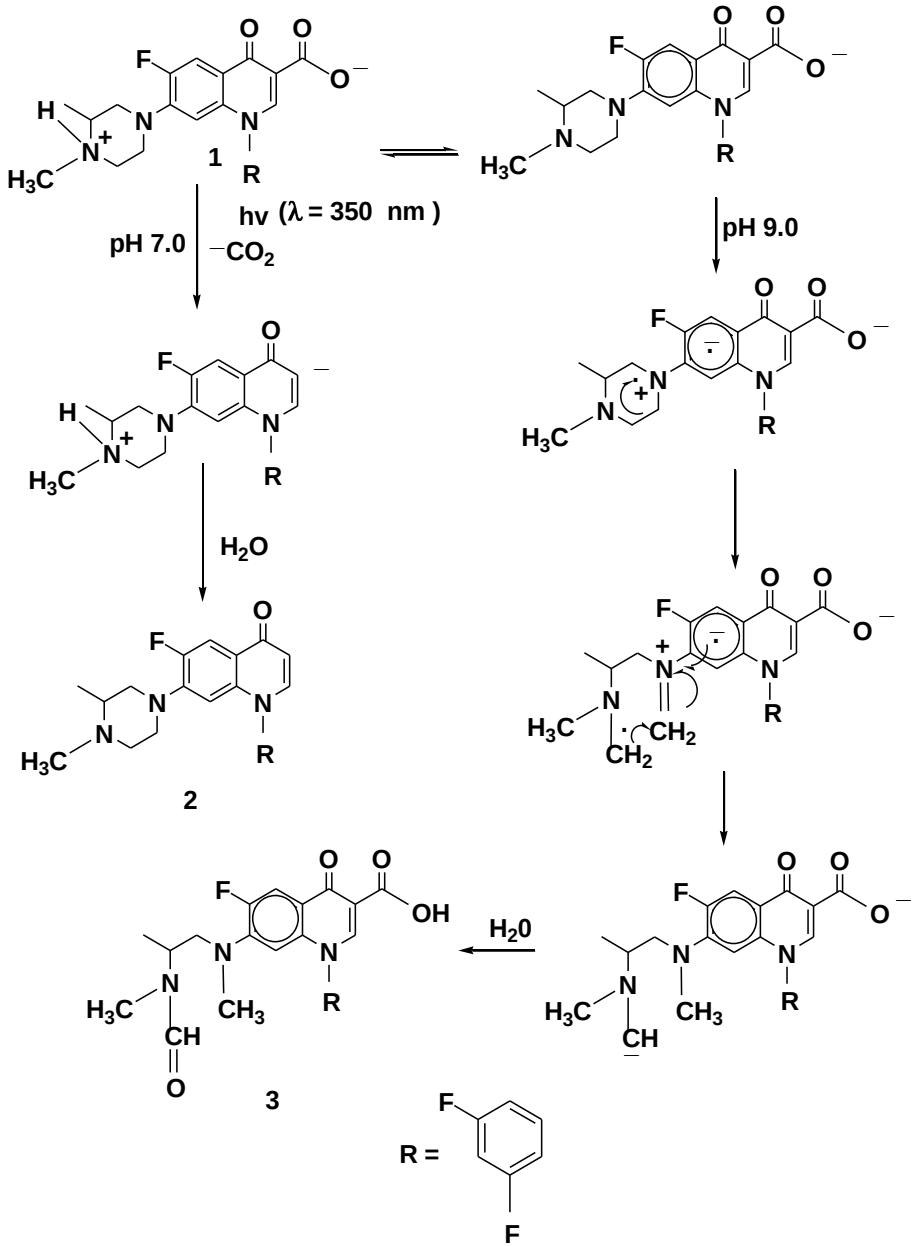
Irradiation of deaerated nitrogen saturated aqueous solution using phosphate buffer (pH 7.3) and borate buffer (pH 9-9.5) of Temafloxacin (TMF) (1) in a water-cooled immersion well type photo reactor equipped with medium pressure mercury vapours lamp and purification of the crude product by silica gel column chromatography afforded two compound as **2** and **3** in isolable yield (Scheme 1). The structures of photoproducts were characterized by the spectroscopic method.

Mechanistic Study:

The photoirradiation of temafloxacine at pH 7.3 which is relevant to physiological pH leads to the formation of photoproduct 2.The drug remains essentially in zwitterions at physiological pH. The product formation can be explained through the following mechanism at pH 7.3 shown in scheme 1. Irradiation of zwitterion species leads to a decarboxylation process followed by H- insertion in place of carboxylic group [15, 16]. The decarboxylated photoproduct is likely formed via protonation of carbanionic intermediate as in the quinolone derivative Nalidixic acid [17]. This seem the more reasonable hypothesis, otherwise a homolytic clevage with formation of free radicals would leads to their stabilization through hydrogen abstraction from solvent which is thermodynamically unfavoured process [18].When irradiation was performed at pH 9-9.5 (where the drug is present in anionic form) [14]. The photoproduct 3 was obtained in low yield.

Product formation can be explained by the following mechanism. The deprotonation of piperazinyle ring plays a key role in the formation of this photoproduct (3). A mechanism that could account for the formation of 3 could involve a fast photo induced electron transfer between N^1- of piperazinylring and the close chromohore [19] that could be formed by deprotonation, a subsequent hemolytic cleavage of the c-c

bond α to the radical cation followed by electron and H –transfer, in accordance with the behavior of several compound containing 1-2 etheroatom [20].



Scheme 1

Mechanistic Pathway of the Photodegradation of Temafloxacin

This process should lead, via the generation of carbene intermediate expected to highly reactive with DNA [21,22] from which photoproduct could be formed. These results are in good agreement with the mechanism of TMF photo degradation proposed in scheme 1. In fact, the main photodegradation process occurring under anaerobic condition at physiological pH, probably proceed via the carbanionic species this later compound is unable to attack, the membrane. Such damage could be attributed either to the radicals formed from the transfer of electron by the carbanionic intermediate species to the residual presence of oxygen after nitrogen bubbling in the Temafloxacin photo degradation and the radical formed in TMF photo degradation path begins with its basic form (ie anionic form) at pH (9-9.5). TMF – induced photosensitization that takes into account drug photo degradation and the transient species such as free radical produced from at pH 7.4 and 9-9.5 from different charged form may be responsible in producing photo toxicity via type 1 mechanisms.

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