Chapter 4

Photochemical Studies on Flutamide
Introduction

Flutamide (2-methyl-N-[4-nitro-3-( trifluoromethyl)phenyl] propanamide), 1, FM) is a non-steroidal androgen drug which blocks androgen receptor sites and is used in advanced prostate cancer.1-5 Recent reports have shown that use of this drug is associated with photosensitivity reaction and can induce adverse biological effects such as sun-exposed eruption in patients after drug treatment.6-10 The photochemistry of FM in homogeneous media has been characterized by low photoreactivity as well as by very low efficiency in photosensitizing singlet oxygen formation.11-13 The photoreactivity of free molecule has been rationalized on the basis of an intermolecular nitro to nitrite rearrangement followed by cleavage of nitrite intermediate leading to phenol derivative 2 as the main stable product. The out of plane geometry of the nitro group and the consequent overlap of the p-orbital of oxygen with the adjacent orbital of the aromatic ring is prerequisite to observe such type of intramolecular photorearrangements. The photolysis of FM in presence of biological mimic systems such as cyclodextrin, micelles and phospholipid bilayers has been shown to dramatically increase the photodecomposition and the different photodegradation pathways were observed.12,13 (Scheme 4.1)

Herein we have investigated:

[A] Photolysis of flutamide adsorbed on silica on the silica gel TLC plates.

[B] Photostability determination of commercially available flutamide oral dosage formulations.
Scheme 4.1
Photolysis of flutamide adsorbed on silica on the silica gel TLC plates.

Photochemical investigation of phototoxic drugs in biologically mimicking systems is becoming an active area in research, because real life processes occur at surfaces, interfaces and multiphase heterogeneous systems. Therefore, to present a model for a close correlation between phototoxic and photochemical behaviour of the drug in vivo, it is significant to investigate drug photobehaviour in biologically mimicking systems. Interface chemistry has played an important role in industrial and natural processes, a common feature of this chemistry is the existence of an interface that provides an extensive surface whose chemical and geometrical features lead to optimum reaction rates and the high chemical selectivity. With this interest, herein, we have investigated photolysis of flutamide adsorbed on silica on the silica gel TLC plates, a biologically mimic system, similar to situation in liposomes.

Experimental

Instrumentation

The photochemical irradiation was carried out by using a medium pressure mercury vapour lamp (125 W). The light was passed through a pyrex glass filter. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Bruker DRX-300 spectrometer using SiMe$_4$ as internal standard. FAB-mass spectra were recorded on a Jeol SX 102/DA-6000 spectrometer at 10 KV accelerating voltage using $m$-nitrobenzyl alcohol (NBA) matrix and argons as FAB gas. Merck silica gel 60 F$_{254}$ was used for preparing photoirradiation plates and column chromatography was performed on Merck silica gel 60 (70-230 mesh).
**Chemicals and reagents**

All chemicals used were of analytical grade. Flutamide was extracted from commercial medicinal product Cytomid-250 (Cipla Ltd., Mumbai, India) with a soxhlet extractor using benzene as a solvent and recrystallized from the same solvent. The purity of the crystallized drug was checked by TLC.

**Photoirradiation procedure**

Flutamide solution in methanol was misted on silica gel TLC plates by using a TLC reagent sprayer and then irradiation of these TLC plates was carried out with UV lamp under a stream of nitrogen. After the photolysis, the plates were scarped and adsorbate was extracted with methanol and filtered. The analysis of product was performed by the thin layer chromatography (TLC), on a precoated silica gel TLC plates using dichloromethane: cyclohexane 80:20 (vol/vol), which showed the photoproduct 3 (Rf = 0.30) and the starting compound (Rf = 0.20). The solvent was removed in a rotary evaporator at room temperature (30° C) and the crude product so obtained, was purified by silica gel column chromatography, which yielded 2-methyl-N-[4-nitroso-3-(trifluoromethyl) phenyl] propanamide (3) as product.

**Characterization of products**

2-Methyl-N-[4-nitroso-3-(trifluoromethyl) phenyl] propanamide (3): $^1$H-NMR (CD$_3$OD) δ 8.32 (d, J= 2.4 Hz, 1H, H-2), 7.94 (dd, J= 8.5 Hz, 1H, H-6), 6.28 (d, J=8.5 Hz, 1H, H-5), 2.58 (m, 1H, CH(CH$_3$)$_2$), 1.18 (6H, CH(CH$_3$)$_2$); $^{13}$C-NMR (CD$_3$OD) δ 181.0 (CONH), 162.2 (C-4), 138.5 (C-1), 131.9 (C-3), 124.0 (CF$_3$), 122.6 (C-6), 112.7 (C-2), 110.4 (C-5), 31.3 (CH(CH$_3$)$_2$), 18.6 (CH(CH$_3$)$_2$); FAB-MS: m/z (rel. int.): 261
Results and discussion

Irradiation of silica gel TLC plates misted with flutamide under UV light and a stream of nitrogen afforded the photoproduct 3 as the only major isolated product (Scheme 4.2). The structure of photoproduct 3 was confirmed on the basis of IR, NMR and mass spectral studies (experimental section). A similar photobehaviour of FM, after its incorporation to β-cyclodextrin cavities and in unilamellar phospholipid bilayer vesicles has been well characterized by Sortino et al.12 The photoreactivity of this drug in homogenous media is exclusively characterized by nitro to nitrite rearrangement leading to phenol derivative 2 as the main stable photoproduct (Scheme 4.1). The photolysis of FM after its incorporation into β-cyclodextrin cavities increases the photodecomposition with the photogeneration of photoproduct 2 along with 3 and 4, whereas in phospholipid bilayers photoproduct 3 was noticed as the sole stable photoproduct. In this study, for the photolysis of FM on silica gel we have also isolated photoproduct 3 as the only product. A possible hypothesis to explain the photogeneration of 3 and inhibition of 2 may be due to the structural changes of FM occurring upon its incorporation in silica gel cavities.

It is generally accepted that the structure of the surface of silica consists of interconnected network of silanol (SiOH) and siloxane (SiOSi) linkage.16 Silanol linkage are generally classified as (1) the isolated or free (non-hydrogen bonded silanols that are characteristic of crystalline silica) (2) the vicinal or hydrogen bonded or active
silanols that are characteristic of amorphous silica and (3) hydrated silanols that may be the result of water binding to either free or vicinal silanols (Figure 4.1). The active silanols are so positioned on the silica surface so as to permit hydrogen bonding. In this regard, perpendicular geometry of nitro group with respect to aromatic ring, which is necessary for the formation of 2, may be lost due to weak interactions involving active silanol and CF₃/NO₂ groups present in flutamide, and would account well for the obtained results.

Our results concerning to photobehaviour of FM on silica gel further support the fact that the drug photochemical behaviour in homogeneous media is the first step for understanding of molecular basis of the drug but alone can not be used to understand in vivo photochemistry. For a correlation between photobehaviour and phototoxicity of the drugs, its photoreactivity should also be investigated in heterogeneous systems of increasing complexity, specific interactions and steric constraints.
Scheme 4.2
Figure 4.1 The chemical composition of silica surface (a) Siloxane (b) Silanol (c) Isolated silanol (d) Vicinal silanol (e) Hydrated silanols.
Photostability determination of commercially available flutamide oral dosage formulations.

Some drugs on exposure to light undergo important chemical changes accompanied by alternation in their activities, and in some cases there may be a total loss of the therapeutic activity. Manufacturers of pharmaceutical products use light resistant coating/or packing to minimize their photodegradation. Long term exposure to sunlight or artificial light may result in photodamage, if pharmaceutical formulations are improperly stored, and thus poor storage conditions may potentially decrease clinical efficiency of these products. Differences in the degree of light protection may also exist between different formulation types. Consequently, if differences in photostability between these formulations are present, product substitution may not be warranted.

With this interest, herein, we have investigated the photostability of commercially available flutamide oral dosage formulations (tablets) available in India. We have also compared the photodegradation of FM powder and methanol solution of three commercially available FM oral dosage formulations.

Experimental

Chemicals and reagents

All chemicals used were of analytical or HPLC grade. Pure flutamide powder was extracted from commercial medicament Cytomid-250 (Cipla ltd., Mumbai, India) with a soxhlet extractor using benzene as a solvent and recrystallized from the same solvent. The purity of the crystallized drug was checked by TLC. Three FM tablets (250 mg)
[Cytomid-250 (Cipla Ltd.), Plutamide (Torrent Pharm. Ltd.), Prostamid (BDH Industries Ltd.)] obtained from local market in India were studied. 2-hydroxy-5-nitrobenzaldehyde was used as internal standard (IS).

The FM stock solution was prepared in methanol (10 mg/ml) and standard solutions were obtained by serial dilution. The Internal standard solution (IS) was prepared (40 μg/ml) in methanol. 1 ml of IS solution was mixed with 1 ml of the tested concentrations of FM (12.5–150 μg/ml) and 20 μl of the mixed solution was injected to HPLC.

**Irradiation test**

For artificial light irradiation, a 40 W tungsten lamp was used. FM samples were placed 50 cm from the lamp in a cabinet. Protected samples from extraneous light were placed in aluminum foils. Exposure to indirect sunlight was also used to compare the efficacy of artificial light and natural room daylight in photodecomposition of FM. Samples were irradiated from 0-12 weeks in artificial light and indirect sunlight. Samples were collected at 0, 1, 2, 4, 6, 8, 10 and 12 weeks intervals (n=3). FM powder samples (10 mg) were placed in 10 ml clean glass vials, irradiated from 0-12 days and samples were collected at 0, 1, 2, 3, 7, 10 and 12 days. Also a total of 11 × 1 ml methanolic FM solution samples (10 μg/ml) were placed in 5 ml clear glass vials and irradiated for a period of 0-360 min. Samples were taken at 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360 mins.
Sample preparation

Flutamide tablets: Five irradiated FM tablets were crushed into fine particles and a quantity equivalent to 10 mg of FM was placed in a centrifuge tube and 2 ml of chloroform was added. The mixture was vortexed for 30 s and centrifuged for 5 min. Twenty μl of supernatant was drawn by Hamiltonian syringe and added to a tube containing 100 μl of IS solution (40 μg/ml). This solution was evaporated to a dry residue under Nitrogen stream. Two hundred μl of mobile phase was added to the residue and vortexed for 10 s. Aliquots of 10 μl were injected to HPLC.

Flutamide powder samples: Each of the irradiated FM powder samples were diluted with 2 ml of chloroform and vortexed for 20 s. Twenty μl of this solution was mixed with 100 μl of IS solution (40 μg/ml), and dried over nitrogen stream, then 1 ml of mobile phase was added and vortexed for 10 s, Finally, 10 μl of this solution was injected to HPLC.

Flutamide methanolic solution: One hundred μl of IS was added to the vials containing 1 ml of FM (100 μg/ml) and vortexed, and then 10 μl of the solution was injected to HPLC.

Identification of the photoproduct

0.2 mg/ml of FM methanolic solution was exposed to indirect sunlight. At 5 min intervals 10 μl of the solution were injected to HPLC until no FM peak was observed in
chromatogram (45 min). The solution was then evaporated over nitrogen stream and subjected to mass spectrometry after purifying by using column chromatography.

**Chromatography and instrumentation**

FAB-mass spectra were recorded on a Jeol SX 102/DA-6000 spectrometer at 10 KV accelerating voltage using m-nitrobenzyl alcohol (NBA) matrix and argons as FAB gas. Merck silica gel 60 F$_{254}$ plates were used for photoirradiation and column chromatography was performed on Merck silica gel 60 (70-230 mesh). HPLC was performed on a Hewlett-Packard 1100 chromatograph equipped with online photodiode array detector. The analysis of FM and its photoproducts was achieved on a LiCharoCart RP-18 column (5 µm packing, 4× 250 mm) eluting with a linear gradient of CH$_3$CN at a flow rate of 1 ml/min with monitoring at 300 nm.

**Results and discussion**

In the present study photostability of FM formulations available in India was determined after exposure to indirect natural light and continuous artificial light. FM, its photoproduct 2 and IS were eluted at approximately 8.8, 6.5 and 4.9 min respectively. Resolution between FM and 2 was adequate and no buffer and pH adjustment was required with this chromatographic system. Mass spectra of photodecomposition product produced major fragments at 248 (M$^+$, 4%), 247 (M$^+$ 30%), 177 (M$^+$-(CH$_3$)$_2$CHCO, 100%), 157 (50%), 71 (20%) that confirmed the structure of photoproduct 2.
Figure 4.2 shows a typical chromatogram of (a) commercial 10 mg FM tablet irradiated for 12 weeks with indirect natural sunlight (b) a sample of FM methanolic solution irradiated with indirect natural sunlight for 6 hours and (c) a sample of FM methanolic solution irradiated with artificial light for 6 hours.

Figure 4.3a shows photodegradation plots of FM powder and figure 4.3b shows photodegradation of FM methanolic solution after artificial light irradiation. Photodegradation of FM powder, measured as percentage loss of FM powder exceeds 10% in 30 hours. Photodegradation of FM methanolic solution exceeds 10% in approximately 45 min. and was essentially complete within 7 hours. It was also observed that the efficacy of artificial light in photodecomposition of FM is more than natural indirect sunlight and it can be due to the higher intensity of artificial light than natural light. The percentage of FM content (w/w initial FM content) was also measured in all three tested formulations irradiated for up to 12 weeks by artificial and natural light.
Figure 4.2 chromatogram of (a) commercial 10 mg FM tablet irradiated for 12 weeks with indirect natural sunlight (b) a sample of FM methanolic solution irradiated with indirect natural sunlight for 6 hours and (c) a sample of FM methanolic solution irradiated with artificial light for 6 hours; 1=IS, 2= photoproduct 2, 3=FM.
Figure 4.3a Photodecomposition of FM powder by using artificial light
Figure 4.3b Photodecomposition of FM methanolic solution using artificial light
Table 1 summarizes photodegradation data for each formulation after 0, 5 and 12-week exposure to artificial light. The average percentage of photoproduct 2 found in the formulations was 1.05, 1.21, 1.49 for 0, 5, and 12 weeks, respectively. Results obtained in this study showed that differences between data obtained were not significant and none of the tested FM formulations underwent any appreciable decomposition (> 10%), even after 12 weeks irradiation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Percentage of photoproduct 2 (w/w initial FM content)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 week</td>
</tr>
<tr>
<td>Cytomid-250</td>
<td>1.0</td>
</tr>
<tr>
<td>Prostamid</td>
<td>1.0</td>
</tr>
<tr>
<td>Plutamide</td>
<td>1.15</td>
</tr>
</tbody>
</table>

*Table 1* Flutamide formulations photodegradation data
References


