Chapter 4

Photocheamistry of Antidiabetes Drug

Tolbutamide and Tolazamide
Introduction

Over the last decade there has been increasing interest in the photosensitization mechanism in the biological systems, in relation to both deleterious and therapeutic aspects of this phenomenon\(^1\). Photosensitivity reactions may be more specifically categorized as phototoxic or photoallergic in nature. Phototoxic disorders have a high incidence, whereas photoallergic reactions are much less frequent in human population\(^2\). The primary event in any photosensitization process is the absorption of a photon, and the following free radical and singlet oxygen generation by photoexcited drug molecules may seem to be the principal intermediate species in the phototoxic response\(^3\). Great interest has been recently shown in photodegradation and *in vitro* phototoxicity studies of drugs belonging to the pharmacological class of antibacterial sulphonamide derivatives\(^4\).

The sulfonamide group is considered as a chromophores which is present in a number of biologically active molecules, have been clinically used for many years and found to possess a large number of biological activities, including antimicrobial\(^5\)-\(^6\), carbonic anhydrase inhibitors\(^7\)-\(^9\), anti-cancer\(^10\), and as an anti-inflammatory agents\(^11\). Benzene sulphonamide is a derivative of sulphonamide family have attracted intense interest in recent years as they are important clinical agents, mainly are used in the treatment of gastro-intestinal– duodenal ulcers, neurological disorders, glaucoma, altitude sickness and for some forms of tumor\(^12\) and some benzene sulphonamide is also clinically used as a antidibetic agent has been associated in some patient with the appearance of phototoxic effect such as erythema, flaring and urticarial weal\(^13\). These compound have been recognized as a very good photo sensitizers in clinical test and in cell culture\(^14\). Although it is very useful but it can produced adverse photo biological
effect such as clinical photosensitization which occurs on the skin of patient 15. The primary reactions responsible for initiating phototoxicity can often be described in terms of basic photochemical reaction. It is therefore to obtain extensive information on the photoreactivity of benzenesulphonamide derivative drugs; we used a stepwise experimental approach involving photodegradation, and an *in vitro* phototoxicity determination by linoleic acid peroxidation assay in the following studies.

[A] **Photochemical behavior and Photobiological Action of Photosensitive Drug**

Tolbutamide

[B] **Photochemistry of Photodynamic biological Action of Photosensitizing Drug**

Tolazamide
Section [A]

*Photochemical behavior and Photobiological Action of Photosensitive Drug Tolbutamide*
Photochemical behavior and Photobiological Action of Photosensitive Drug

Tolbutamide

Tolbutamide (1), a benzene sulphonamide derivative\textsuperscript{16-18} is a first generation potassium channel blocker, oral hypoglycemic drug. It is structurally similar to acetoheaxamide, chlorpropamide and tolazamide. Tolbutamide (1) is twice as potent as the related second-generation agent glipizide. This drug may be used in the management of type II diabetes if diet alone is not effective. Tolbutamide (1) stimulates the secretion of insulin by the pancreas. Since the pancreas must synthesize insulin in order for this drug to work, it is not effective in the management of type I diabetes (condition in which the body does not produce insulin, and therefore, cannot control the amount of sugar in the blood). It may be effective in some patients who have become unresponsive to one or more of the other sulphonylurea drugs. It generally has a short duration of action due to its rapid metabolism, and is therefore safe for use in elderly diabetics\textsuperscript{19}. Tolbutamide (1) with sulfonamide chromospheres is expected to be photolabile and probable photo sensitizer of biological substrate and it is also known to exhibit phototoxic effect in patient treated with this drug\textsuperscript{20,21}. In continuation of our previous work we became interested in investigating the photochemical behavior of the tolbutamide (1) under aerobic condition in UV- B light for an appropriate correlation to its photo toxicity. Photolysis of tolbutamide (1) in presence of oxygen resultant in the formation of two degradation products identified as (2) and (3) from their spectral properties: (\textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR, IR and Mass spectra) is shown in scheme- 4A.1. Product (2) and (3) presumably produced by the cleavage of CO-N bond of tolbutamide (1).
Experimental

Apparatus

All chemicals used were of analytical and pharmaceutical grade. Tolbutamide (1) was extracted from commercial medicament Rastinone (Aventis Parma Limited, India). The purity of drug extracted was checked by TLC and comparing its melting point with the literature value. Rose bengal and reduced gluthione (GSH) were purchased from Sigma Aldrich (India).

Chemicals

Photochemical reactions were carried out in quartz fitted immersion well photochemical reactor equipped with 400W medium pressure mercury vapour lamp with continuous supply of water. UV spectra were recorded on a Shimadzu 160 A Instrument. IR spectra were recorded in KBr discs on a Perkin Elmer model spectrum RXI. \(^{1}\)H-N.M.R and \(^{13}\)C-N.M.R Spectra were recorded on a Bruker Avance DRX - 300 Spectrometer using SiMe\(_4\) as internal standard and CDCl\(_3\) as solvent. High resolution mass spectra were determined with a VG-ZAB-BEQ 9 spectrometer at 70 eV ionization voltages. Column chromatography was performed on silica gel 60 (70-230mesh); thin layer chromatography (TLC) was carried on Merck silica gel60 F\(_{254}\) (0.2 mm thick plates).

Irradiation of tolbutamide in methanol

Tolbutamide (1) 285 mg (1.06 mM) was dissolved in 400 ml methanol and irradiated at room temperature in the Rayonet photochemical reactor. Progress of the reaction was monitored by thin layer chromatography (chloroform-methanol, 98:2). After the irradiation of mixture for 71 hr the solvent was removed in a rotary evaporator and
crude product was subjected to silica gel column chromatography elution with
CH$_3$CN/H$_2$O (30:70, v/v) on silica gel column gave 2 and 3 as photoproducts.

**Butanamine-1 (2):**
Yield: 60mg (21.8 %); UV $\lambda_{\text{max}}$(MeOH) 210 nm ; HRMS calcd. for (M$^+$) C$_4$H$_{11}$N 73.33, found 73.20, IR(KBr): 3350, 2967, 2880, 1090, 800 cm$^{-1}$, $^1$H-NMR(CDCl$_3$, $\delta$, ppm) 
2.7 (t, J=7.2 Hz, H-1), 1.43 (m, 4H, H-3 & H-4), 1.16 (s, 2H, NH$_2$), 0.92 (t, J=7 Hz, 3H, H-4) ; $^{13}$C-NMR(CDCl$_3$, $\delta$, ppm) 41.8 (C-1), 35.0 (C-2), 19.9 (C-3), 13.8 (C-4), MS: m/z: 73 (M$^+$), 30(M$^+$-CH$_2$=NH$_2$).

**N-tosylformamide (3):**
Yield: 95mg(34.5 %); UV $\lambda_{\text{max}}$(MeOH) 261 nm and 210 nm ; HRMS calcd. for (M$^+$) C$_8$H$_9$NO$_3$S 199.2270, found 199.2268;IR(KBr): 3359, 3325 cm$^{-1}$(NH), 1340, 1160 cm$^{-1}$(SO$_2$); $^1$H-NMR (CDCl$_3$, $\delta$, ppm) 8.75(s, 1H, CHO) 7.82 (d, J=7.9 Hz, 2H, tolyl H-3 and H-5) 7.32 (d, J=7.9 Hz, 2H, tolyl H-2 and H-6), 2.38 (s, 3H, tolyl,CH$_3$), 2.0(s, 1H, NH), $^{13}$C-NMR (CDCl$_3$, $\delta$, ppm) $\delta$ 175.01 (CO, CHO), 142.6, 136.9, 129.8 128.3, 23.4, (C-1, C-4, C-2 and C-6, C-3 and C-5,CH$_3$ of the toluene moiety). MS: m/z: 199(M$^+$), 185(M$^+$-CH$_3$), 170(M$^+$-HCO), 155(M$^+$-HCONH), 92(M$^+$-HCONHSO$_2$).

To observe a possible quenching effect of the tolbutamide (1) on singlet oxygen ($^1$O$_2$), tolbutamide (1) was also irradiated in presence of photosensitizer rose bengal and maintaining all other experimental conditions the same.

**Photosensitized per oxidation of linoleic acid**
Phosphate buffered solution of Linoleic acid (1×10$^{-3}$ M) was irradiated in the presence of compound (1) and also in a pre-irradiated solution of 1 (1×10$^{-5}$). The formation of dienic hydroperoxides was monitored by UV–spectrophotometer, by the appearance and progressive increases of a new band at $\lambda$=233nm$^2$ (fig-4A.1). The lipid
peroxidation test was repeated in the presence of reduced glutathione (GSH) as radical scavenger. This test was also carried out under argon atmosphere.

**Results and discussion**

Irradiation of methanolic solution of (1) under oxygen atmosphere with a medium pressure mercury vapor lamp in an immersion well type photo reactor gave two hemolytic cleavage photoproducts: butanamine-1(2) and N-tosylformamide (3) as shown in scheme-4A.1. The assigned structures to these products well correspond to their observed spectral properties.

No degradation of tolbutamide (1) was observed when irradiation was carried out in the presence of rose bengal maintaining all other experimental conditions the same. This indicated that quenching of singlet oxygen by tolbutamide (1) was negligible in the presence of rose bengal, which is a well known efficient singlet oxygen generator. Hence, it is proposed that tolbutamide (1) undergoes homolytic cleavage of CO-NH bond resulting in the generation of two intermediates radicals (2a) and (3a), as presented in scheme-4A.2

Hydrogen abstraction by intermediate radicals generated in tolbutamide (1) photodegradation leads to lipid peroxidation (Scheme-4A.3). The efficient inhibition of lipid peroxidation by well-established radical scavengers GSH confirmed the involvement of type I mechanism.
Scheme-4A.1
Tolbutamide (1)

\[ \text{hv} \quad \text{homolytic cleavage} \]

\[
\begin{align*}
\text{hv} & \quad \text{homolytic cleavage} \\
\text{hv} & \quad \text{hydrogen abstraction} \\
\end{align*}
\]

Scheme-4A.2
Fig-4A.1-Photoperoxidation of linoleic acid (L, $10^3$M) sensitized by tolbutamide (I)
Scheme-4A.3
Lipid peroxidations certainly correlate with damage produced in the cell membranes. The phototoxicity mechanism for tolbutamide (1) most probably involves reaction of free radical intermediates and photoproducts with cellular components. The insignificant decrease in lipid peroxidation test under argon atmosphere indicates that tolbutamide is capable of photosensitizing lipids through a process where oxygen does not play principal role.

The studies of phototoxicity carried out in this work may help to explain the damage produced in protein and organs. The observations in this work may contribute to elucidate the observed accumulations and damaging activity of oxidized proteins during aging and in pathologies such as diabetes, atherosclerosis and neurodegenerative diseases. Lipid photoperoxidation certainly correlates the damage produced in the cell membranes. The phototoxicity mechanism for tolbutamide (1) most probably involves reaction of free radical species than singlet oxygen, super oxide anion or photoproducts with cellular components. The result obtained may be very useful from medical point of view to perform the appropriate screening of phototoxicity in vitro before introducing drugs and chemicals in to chemical therapy.
Section B

Photochemistry of Photodynamic

biological Action of Photosensitizing

Drug Tolazamide
[B] Photochemistry of Photodynamic biological Action of Photosensitizing Drug

Tolazamide

Tolazamide (4, \(N\)-[(azepan-1-ylamino) carbonyl]-4-methylbenzenesulfonamide) is a member of a class of oral hypoglycemic agents\(^{23}\) and chemically known as sulfonylureas which includes other compounds such as tolbutamide, Chlorpromamide, glyburide and glipizide\(^{24}\). All are aryl sulfonylureas containing substitution on the urea and benzene groups. They are effective in the treatment of Type II diabetes mellitus which usually occurs in later life\(^{25}\). Tolazamide (4) is approximately five times more potent than tolbutamide in the human diabetic\(^{26}\). The typical individuals who would benefit from tolazamide therapy would be one whose type II diabetes developed after the age of forty. Tolazamide, as well as other sulfonylureas, is ineffective in the treatment of type I diabetes mellitus which usually occurs in children and young adults\(^{27}\). The mechanism of action of tolazamide, typical of other sulfonylureas, is its ability to stimulate the secretion of insulin from functioning \(\beta\) cells of pancreatic islet tissue\(^{28}\). In addition tolazamide, as well as some other sulfonylureas, has been shown to be effective in lowering blood glucose levels of non insulin dependent diabetics whose disease dose not respond to diet caloric restriction or physical activity\(^{29}\).

Tolazamide (4) with sulfonamide chromophores is expected to be photolabile and probable photosensitizer of biological substrate. Interest in the photochemistry of tolazamide arises from the clinical and pharmacological reports of phototoxic effects which is associated with the use of this drug\(^{30}\). In this study, our goal was to characterize the photochemical properties of tolazamide (4), in order to understand and rationalize the basic photochemical reaction involved in the phototoxicity.
Photolysis of tolazamide (4) in the presence of oxygen resulted in the formation of two photodegradation products, identified as (5) and (6) from their spectral (IR, $^1$H-NMR, $^{13}$C-NMR, mass spectra) properties (Scheme-4B.1). Product (5) and (6) presumably produced by the rupture of CO-N bond of tolazamide (4).

**Experimental**

**Chemicals**

All chemicals used were of analytical and pharmaceutical grade. Tolazamide (4) was extracted from the commercial medicament Tolinase (Pfizer, India). The purity of drug, extracted was checked by thin layer chromatography (TLC) and comparing its melting point with the literature value. Rose bengal and reduced glutathione (GSH) were purchased from Sigma Aldrich (India).

**Apparatus**

Photochemical reactions were carried out in quartz fitted immersion well photochemical reactor equipped with 400W medium pressure mercury vapour lamp with continuous supply of water. UV spectra were recorded on a Shimadzu 160 A Instrument. IR spectra were recorded in KBr discs on a Perkin Elmer model spectrum RXI. $^1$H-NMR and $^{13}$C-NMR Spectra were recorded on a Bruker Avance DRX-300 Spectrometer using SiMe$_4$ as internal standard and CDCl$_3$ as solvent. High resolution mass spectra were determined with a VG-ZAB-BEQ9 spectrometer at 70 eV ionization voltage. Column chromatography was performed on silica gel 60 (70-230 mesh); thin layer chromatography (TLC) was carried on Merck silica gel 60 F$_{254}$ (0.2 mm thick plates).
**Irradiation of tolazamide in methanol**

Tolazamide (4) 275 mg (0.8 mM) was dissolved in 400 ml methanol and irradiated at room temperature in the Rayonet photochemical reactor. Progress of the reaction was monitored by thin layer chromatography (chloroform-methanol, 98:2). After the irradiation of mixture for 70 hr the solvent was removed in a rotary evaporator and crude product was subjected to silica gel column chromatography elution with CH$_3$CN/H$_2$O (30:70, v/v) on silica column gave (5) and (6) as products.

**Azepan-1-amine (5):**

Yield: 65 mg (23.6 %); UV $\lambda_{max}$ (MeOH) 238 nm and 242 nm; HRMS calcd. for (M$^+$) C$_6$H$_{14}$N$_2$ 114.1888, found 114.1757; IR (KBr): 3250 (NH$_2$), 3140, 2967, 2880 cm$^{-1}$; $^1$HNMR (CDCl$_3$, $\delta$, ppm): 2.57 (t, J=7.1 Hz, 4H, H-2 & H-7), 2.0 (s, 2H, NH$_2$), 1.42 (m, 4 H, H-3 & H-6), 1.28 (m, 4H, H-4 & H-5); $^{13}$C-NMR (CDCl$_3$, $\delta$, ppm): 61.1 (C-2 & C-7), 26.5 (C-4 & C-5), 25.5 (C-3 & C-6); MS: m/z: 114 (M$^+$), 98 (M$^+$- NH$_2$).

**N-tosylformamide (6):**

Yield: 98 mg (35.6 %); UV $\lambda_{max}$ (MeOH) 261 nm and 210 nm; HRMS calcd. for (M$^+$) C$_8$H$_9$NO$_3$S 199.2270 found 199.2268; IR (KBr): 3359, 3325 (NH), 1655 (CONH), 1340, 1160 cm$^{-1}$ (SO$_2$); $^1$H-NMR (CDCl$_3$, $\delta$, ppm): 8.75 (s, 1H, CHO), 7.82 (d, J=7.9 Hz , 2H, tolyl H-3 and H-5), 7.32 (d, J=7.9 Hz, 2H, toyl H-2 and H-6), 2.38 (s, 3H, toyl CH$_3$), 2.0 (s, 1H, NH); $^{13}$C-NMR (CDCl$_3$, $\delta$, ppm): 175.01 ( CO, CHO), 142.6, 136.9, 129.8 128.3, 23.4, (C-1, C-4, C-2 and C-6, C-3 and C-5, CH$_3$of the toluene moiety); MS: m/z: 199 (M$^+$), 185 (M$^+$- CH$_3$), 170 (M$^+$- HCO), 155 (M$^+$- HCONH), 92 (M$^+$- HCONHSO$_2$).
To observe a possible quencher effect of the tolazamide on singlet oxygen (\( ^1\text{O}_2 \)), tolazamide (4) was also irradiated in presence of photosensitizer rose bengal and maintaining all other experimental conditions the same.

\textit{Photosensitized per oxidation of linoleic acid}

Phosphate buffered solution of Linoleic acid (1×10^{-3} M) was irradiated in the presence of compound (4) and also in a pre-irradiated solution of (4) (1×10^{-5}). The formation of dience hydroperoxides was monitored by UV spectrophotometry, by the appearance and progressive increases of a new band at \( \lambda = 233 \) nm (fig-3B.1). The lipid peroxidation test was repeated in the presence of reduced glutathione (GSH, a radical scavenger). This test was also carried under argon atmosphere.

\textbf{Results and discussion}

Irradiation of methanolic solution of tolazamide (4) under oxygen atmosphere with a medium pressure mercury vapour lamp in an immersion well type photo reactor gave Azepan-1-amine (5), N-tosylformamide (6) as photoproducts (Scheme-4B.1) which were characterized from their spectral studies. No degradation of tolazamide was observed when irradiation was carried out in the presence of rose bengal. Therefore, the interaction with or quenching of singlet oxygen (\( ^1\text{O}_2 \)), by tolazamide was negligible.

Tolazamide (4) undergoes homolytic cleavage (rupture of) CO-NH bond resulting in the generation of two intermediate radicals (5a) and (6a) (Scheme-4B.2). Hydrogen abstraction by intermediate radicals generated in tolazamide photodegradation leads to lipid peroxidation (Scheme4B.3). The efficient inhibition of lipid peroxidation by well-established radical scavengers GSH confirmed the involvement of type I mechanism.
Scheme 4B.1
Scheme-4B.2

Tolazamide

hv homolytic cleavage

N\text{\texttextunderscore NH} + O\text{\texttextunderscore S}\text{\texttextunderscore CH}_{3}

hydrogen abstraction

N\text{\texttextunderscore NH}_{2} + O\text{\texttextunderscore S}\text{\texttextunderscore CH}_{3}

(5a) \quad (6a)

(5) \quad (6)
Fig-4A.1 Photoperoxidation of linoleic acid (L, 10^{-3}M) sensitized by tolazamide (4)
Scheme-4B.3
The phototoxicity mechanism for tolazamide most probably involves reaction of free radical intermediates and photoproducts with cellular components. The insignificant decrease in lipid peroxidation test under argon atmosphere indicates that tolazamide is capable of photosensitizing lipids through a process where oxygen does not play a principal role.

The outcome of the studies of phototoxicity carried out in this work may help to explain the damage produced in cellular component. The observations in this work may contribute to elucidate the observed accumulations and damaging activity of oxidized proteins during aging and in pathologies such as atherosclerosis, diabetes and neurodegenerative diseases. Lipid photoperoxidation certainly correlates the damage produced in the cell membranes. On the basis of the obtained photochemical results, the phototoxicity of tolazamide can be attributed to radical formation. We hope that our results will contribute to a better comprehension and prevention of the harmful side reactions produced by the antidiabetic drug tolazamide (4).
References


