

# Thermally Generated Triplet Excited Ketones Mediated Photooxidation of Penciclovir

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## ABSTRACT

**Objective:** The objective of the present study to investigate photooxidation of PC in presence of thermally generated triplet excited ketones and in presence of photosensitizers rose bengal and riboflavin.

**Methods:** The Photooxidation of antiviral drug penciclovir was studied under different reaction conditions. First we treated the penciclovir (PC 1) with triplet excited ketones, which have been generated in thermal decomposition of 3-(hydroxymethyl)-3, 4, 4-trimethyl-1, 2-dioxetane (HTMD), in the dark. Photosensitized oxidation of penciclovir was also carried out in presence of photosensitizers riboflavin and rose Bengal to compare the yield of photoproducts under both conditions.

**Results:** Three major oxidation products were detected by means of spectroscopic measurements. The products were 4-hydroxy(3-Hydroxymethyl) butyl spiroiminodihydantoin (2), 4-[4-hydroxy- (3-hydroxymethyl)-butylamino]-2-imino-1,2-dihydroimidazol-5-one(3), 2, 2-diamino-4[4-hydroxy-(3-hydroxymethyl)butylamino]oxazol-5-one(4).

**Conclusion:** The result of our present investigation reveals that triplet excite ketone generated by thermal decomposition of triplet excited ketone oxidize PC efficiently to the Sp, by a type II photooxidation mechanism and to the Ox and Im by a type I mechanism.

**Keywords;** Penciclovir, photooxidation, 1,2dioxetane, triplet excited ketone.

## INTRODUCTION

Indeed, despite their excellent therapeutic activity, many pharmacologically important chemicals such as antibacterials, antimicrotics and non-steroidal anti-inflammatory drugs (NSAIDs) can induce phototoxic, photo allergic and photo mutagenic phenomena strictly related to the drug photochemical reactivity [1]. Recently the interest in the adverse photosensitization mechanism of various pharmacologically important chemical has been markedly intensified. The pharmacologically active drug shows photosensitization by two mechanisms either by type I mechanism called radical mediated or type II mechanism or singlet oxygen mediated. Many of the endogenous (e.g. flavins, tetrapyrrols, protoporphyrins etc.) or exogenous photo sensitizers can elicit phototoxic or photo allergic responses [2, 3]. Likewise there are many sensitizing dyes e.g. rose Bengal, benzophenone, riboflavin etc. that are activated by light.

Triplet-excited ketones, important classes of photo oxidative sensitizer [4, 5] are of biological interest since they may be generated in cellular systems upon exposure of endogenous chromophores to the UV irradiation or by dark reactions (e.g. lipid per oxidation and enzymatic oxidation) [6]. Triplet excited ketones may be produced either by thermal decomposition of 1, 2-Dioxetanes or by their conventional photochemical generations [7]. These thermally generated triplet excited ketones are analogous to those produced photo chemically and operate as type I or type II photo oxidants [8]. 1, 2-Dioxetanes are unique class of four membered ring peroxide and are of biological interest, since they have been implicated as labile intermediates in oxidative stress [9].

Penciclovir is a nucleoside analogue that inhibits HSV1 and HSV2 similar to the acyclovir but has the advantage of prolonged half life in infected cells [10]. Penciclovir 1% has been introduced as an effective topical treatment of herpetic eruptions [11] independent of the stage of development of lesions. Penciclovir has similar in vitro efficacy and mechanism of action to acyclovir [12, 13].

In our recent study for the singlet oxygen mediated photooxidation of PC in aqueous solution, we have isolated spiroiminodihydantoin (sp, 2), imidazolone (Im, 3) and oxazolone (Ox,4) as the three major products [14]. Here in we have investigated photooxidation of PC in presence of thermally generated triplet excited ketones and in presence of photosensitizer's rose bengal and riboflavin. The distribution of products were compared with those PC photooxidation by type I sensitizer, riboflavin and a type II sensitizer rose Bengal [15].

## EXPERIMENTAL

### Chemicals and Reagents

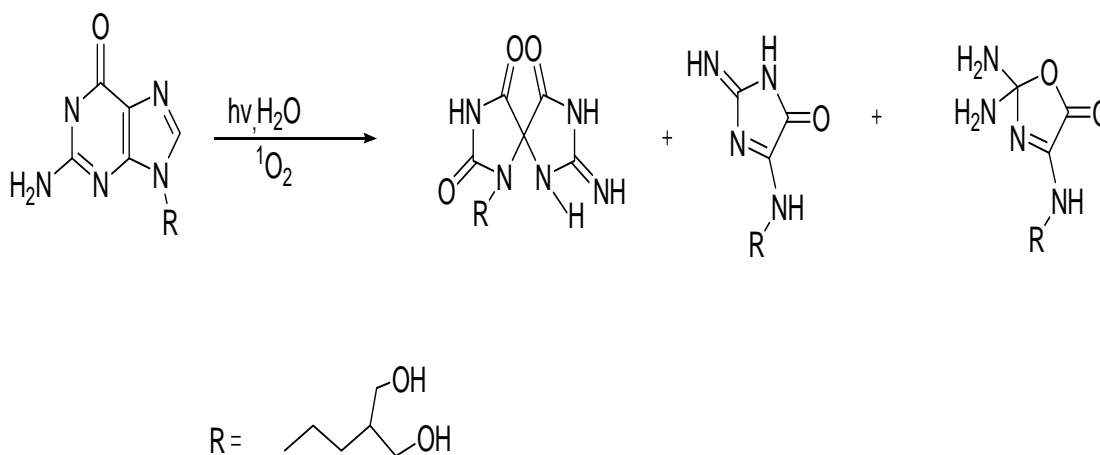
All chemicals used were of analytical grade. Penciclovir was extracted from the commercial medicament denavir (dev chem., Mumbai, India) with a soxhlet extractor, purified by TLC and recrystallized from the same solvent. Melting point,  $^1\text{H-NMR}$  and Co-TLC with authentic pure sample determined the purity of penciclovir. HTMD was synthesized according to the literature procedure. Standard samples of spiroiminodihydantoin, imidazolone and oxazolone were prepared by rose Bengal photosensitized oxidation of Penciclovir.

### OXIDATION PROCEDURE

For the dioxetane mediated oxidation of Penciclovir (PC), a 0.5 mM solution of PC in 10 mM sodium cacodylate buffer (pH 7.0) and 10 vol% dioxetane solutions in acetonitrile was kept at  $50^\circ\text{C}$  for several hour in absence of light. The photosensitized oxidation of phosphate buffered solution of PC (0.5mM) was carried out in the presence of photosensitizers riboflavin and rose Bengal. A 150-W sodium lamp was used for carrying out photosensitized oxidation of PC with riboflavin and rose Bengal. The lamp was placed at 15cm below the bottom of the ice filled beaker, in which a round bottom flask having reaction mixture was placed. HTMD-induced photooxidation was carried out in dark for 18 hr at  $50^\circ\text{C}$  by using acetonitrile (10% by vol) as co solvent while the photooxidation by riboflavin and rose Bengal was carried out for 2.5 hr. A number of products were indicated on TLC at the end of reaction, from which the only three major photoproducts **2**, **3** and **4** were obtained in isolable yields. The amount of photoproducts formed and un-oxidized PC was assessed by isolation and purification of the photolyst using silica gel column chromatography. Effects of  $\text{D}_2\text{O}$  on the yield of Sp in HTMD-induced photooxidation were also observed to confirm the involvement of singlet oxygen.

### RESULT AND DISCUSSION

The thermal HTMD mediated Photooxidation of PC at  $50^\circ\text{C}$  Produces three major products (3-Hydroxymethyl) butyl spiroiminodihydantoin (**2**), 4-[4-hydroxy-(3-hydroxy methyl) butyl amino]-2-imino-1,2-dihydroimidazol-5-one(**3**), 2, 2-diamino-4 [4-hydroxy-(3-hydroxymethyl) butyl amino] oxazol-5-one (**4**) (Scheme-1) . All the photoproducts were identified by comparing their spectral data and TLC with those of authentic pure samples of spiroiminodihydantoin (Sp), imidazolone (Im) and oxazolone (Ox) generated in the rose Bengal mediated photooxidation of PC. The spectral data of all the three products were found to be in a close agreement with well established singlet oxygen mediated PC photooxidation products.



Scheme-1 Photoproducts of Penciclovir

#### 4- hydroxyl-(3-Hydroxymethyl) butyl spiroiminodihydantoin (**2**)

IR(KBr): 3490, 3465, 3360, 3320, 1800, 1765, 1735, 1700, 1150  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$ (DMSO, d, ppm): 8.59, 8.47, 8.46, 8.20 (4H, NH), 3.49 (d, 2H), 3.16 (t, 2H);  $^{13}\text{C-NMR}$  (DMSO, d, ppm): 165.9 (C-8), 170.7 (C-4), 173.3 (C-6), 157.2 (C-2), 99.8 (C-5), 63.9, 41.9, 38.3, 23.9; FAB-MS  $m/z$  301  $[\text{C}_{11}\text{H}_{18}\text{N}_5\text{O}_5+\text{H}]^+$ , 198  $[\text{C}_6\text{H}_7\text{O}_3\text{N}_5+\text{H}]^+$

#### 4-[4-hydroxy-(3-hydroxymethyl) butyl amino]-2-imino-1, 2-dihydroimidazol-5-one (**3**)

IR (KBr), 3240,1735, 1642, 1523, 1153 $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (DMSO, d, ppm): 9.07 (2-NH), 8.43 (3-NH), 8.9 (5-NH), 3.49 (d, 2H), 2.65 (t, 2H), 1.65 (t, 2H), 1.51 (t, 2H);  $^{13}\text{C-NMR}$  (DMSO, d, ppm): 184.6 (C-2), 176.7 (C-4), 166.5 (C-5), 63.9, 42.0, 37.5, 27.2; FAB-MS  $m/z$  215  $[\text{C}_8\text{H}_{14}\text{N}_4\text{O}_3+\text{H}]^+$ , 112  $[\text{C}_3\text{H}_3\text{N}_4\text{O}+\text{H}]^+$ .

#### 2, 2-diamino-4-[4-hydroxy-(3-hydroxymethyl) butyl amino] oxazol-5-one (**4**)

IR(KBr): 3350, 2945, 1780, 1735, 1659, 1490, 1160  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$ (DMSO, d, ppm): 7.58 (NH2-4H), 8.20 (4-NH), 3.49 (d, 2H), 2.65 (t, 2H), 1.65 (t, 2H), 1.51(d, 2H);  $^{13}\text{C-NMR}$  (DMSO,d, ppm): 166.3(C-2), 160.2(C-5),

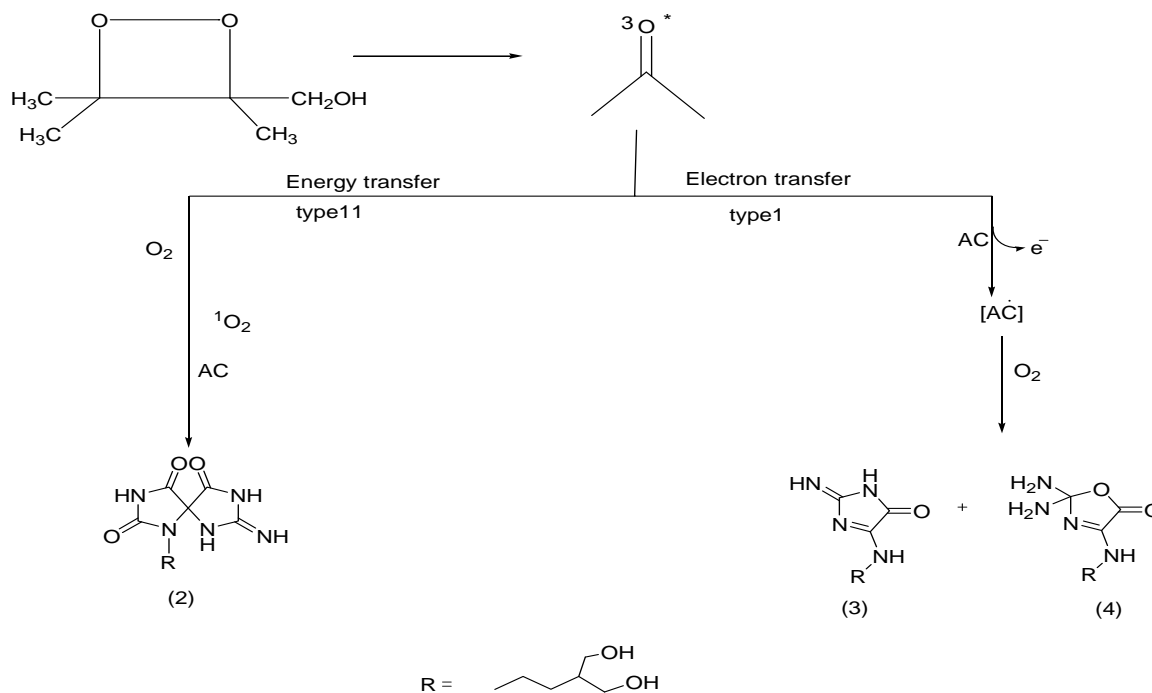
156.0 (C-4), 63.9, 42.0, 38.2, 27.6; FAB-MS m/z 233 [C<sub>8</sub>H<sub>16</sub>N<sub>4</sub>O<sub>4</sub>+H]<sup>+</sup>, 130 [C<sub>3</sub>H<sub>5</sub>N<sub>4</sub>O<sub>2</sub>+H]<sup>+</sup>, 189 [C<sub>8</sub>H<sub>16</sub>N<sub>4</sub>O<sub>4</sub>-CO<sub>2</sub>+H]<sup>+</sup>, 86 [C<sub>3</sub>H<sub>5</sub>N<sub>4</sub>O<sub>2</sub>CO<sub>2</sub>+H]<sup>+</sup>.

Similarly when PC was exposed in aerated aqueous solution of photo excited riboflavin and rose Bengal formation of photoproduct follow same pattern but the yields of photoproducts obtained were different depending upon the photo sensitizer, which was used. In the type II sensitized photooxidation by rose Bengal, spiroiminodihydroxyacetone was detected as the major product where as the type I photo sensitizer riboflavin gave imidazolone and oxazolone as major product. During HTMD sensitized photooxidation both type I and type II products were obtained (Scheme-2). The percentage yield of HTMD mediated oxidation photoproducts lies between the type I and type II process. Furthermore the relative yield of **Sp** in HTMD-mediated oxidation is 24% which is significantly higher (12%) than that of riboflavin sensitized oxidation and smaller (12%) than that in the rose Bengal sensitization. On other hand the yields of the type I products in HTMD-induced oxidation (23%) are comparable with those of the type I sensitizer (24%) and significantly higher than those in type II photooxidation (12%). This difference in product distribution clearly indicate that rose Bengal produces singlet oxygen in large amount by a type II mechanism where as riboflavin, which act mainly by type I photosensitized oxidation do not produce significant amount of singlet oxygen. On the other hand HTMD is not a typical type I or type II photo oxidant it show both photooxidation modes quite efficiently (Table-1).

Table-1 Product distribution of penciclovir oxidation by dioxetane HTMD and by riboflavin and rose Bengal photosensitizer.

S.NO.	Oxidant	Product Yield (%)			
		Type II	Type-I		
		SP	IM	OX	Total
1	HTMD/50 <sup>0</sup> C	24	9	14	23
2	Riboflavin	12	7	17	24
3	Rose bengal	40	6	6	12

In order to confirm the role of singlet oxygen in HTMD mediated photooxidation of penciclovir we cannot use the single Singlet oxygen quenchers such as DABCO, sodium azide etc. because they react with dioxetane [16]. The involvement of singlet oxygen in HTMD-mediated oxidation was confirmed by the substantial increase in the yield sp when we use D<sub>2</sub>O in place of H<sub>2</sub>O.



Scheme-2 Triplet excited ketone mediated oxidation of penciclovir

The result of our present investigation reveals that triplet excited ketone generated by thermal decomposition of triplet excited ketone oxidize PC efficiently to the Sp, by a type II photooxidation mechanism and to the Ox and Im by a type I mechanism. In addition the riboflavin sensitized type-I photooxidation of penciclovir produces OX and IM as major photooxidation product where as riboflavin sensitized type II photooxidation of gave SP as major photooxidation product.

The Study of oxidative photodegradation of a compounds used in clinical medicines is of great relevance from photo biological as well as photochemical point of view. Since singlet oxygen formation and the singlet oxygen mediated photooxidation of the drug and biomolecules is one of the main routes for the drug phototoxicity, the present findings may have an implication to understand the phototoxic effect of the drug.

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