CHAPTER 3

UPTAKE AND PHOTOTOXICITY OF

\( C_{p6} \)-HISTAMINE CONJUGATE IN

CANCER CELLS
Oral squamous cell carcinoma (OSCC) is the most common type of cancer in India and other south East Asian countries [247]. Currently, PDT using Foscan (meta-tetra(hydroxyphenyl)chlorin or m-THPC) has been clinically approved for the treatment of head and neck squamous cell carcinoma (HNSCC) in European countries [11]. Several clinical studies on OSCC have demonstrated that PDT can help retain normal tissue function such as speech, swallowing and voice with no scarring [248]. So far, there exist no report on use of conjugated PS for PDT of oral cancer except studies by Soukos et al where Chlorin e6 (Ce6), conjugated to epidermal growth factor receptor monoclonal antibody (EGFR-MAB) has been investigated for PDT of oral pre-cancer in hamster [249]. In this chapter, the cellular uptake and phototoxicity of Cp6-his and free Cp6 has been studied in two human oral cancer cell lines 4451 and Nt8e. Among the two cell lines, 4451 which is derived from a recurrent tumor in the lower jaw is a p-53 mutant cell line for which resistant to radiotherapy has been reported previously [250]. The other cell line NT8e is derived from tumor specimen of the upper aerodigestive tract (pyriform Fossa) and has wild type p-53 [251]. Since the status of H2 receptor is not known in oral cancer, we have first confirmed the presence of histamine receptors in the both cell lines by western blot. Similar studies on the cellular uptake and phototoxicity of Cp6-his and free Cp6 has also been investigated in human breast carcinoma cell line MCF-7, in which the expression of histamine receptors is well documented [236, 238]. Also, the receptor mediated uptake of the conjugate in these cell lines was explored by studying the effects of low temperature, agonist and antagonist on cellular uptake of Cp6-his or Cp6. In addition to this, mode of cell death induced by Cp6-his or Cp6 was assessed by estimation of
percentage of necrotic and apoptotic cells after PDT using Hoechst 33342-propidium iodide staining.

3.1 Results

3.1.1 Characterization of the conjugate:

The purity of Cp6-his was checked by thin layer chromatography (TLC) on preparative silica gel plate using 95% methanol as mobile phase. The results of TLC show that Cp6-his separated as a single spot on silica gel plates with retardation factor (Rf) of ~0.9 and in comparison the Rf for Cp6 was ~0.1 (Fig.3.1a). The mass spectrum of the conjugate gave anticipated molecular ion peak at 719.8 (calculated mass 719.76 for disodium salt C38H39N7Na2O5) (Fig. 3.1b). The chemical structures of Cp6 and its conjugate are shown in Fig. 3.1c.

Fig. 3.2 shows the absorption spectra of free Cp6 and Cp6-his dissolved in Ethanol:PEG(400):buffer system. Attachment of histamine to Cp6 did not cause any major change in its absorption peak positions in visible region except that the q band position was slightly red shifted to 666 nm from 663 nm. The molar absorption coefficient of Cp6-his was estimated to be 42,314 M⁻¹ cm⁻¹ and 12,750 M⁻¹ cm⁻¹ at soret and q band position which is ~1/2 of the molar absorption coefficient of Cp6 at the same wavelengths. The 400 nm excited fluorescence of Cp6 and Cp6-his are shown in inset.
Figure 3.1. Photograph of TLC plate showing mobility of $Cp_6$ and $Cp_6$-his after chromatography using 90% methanol as mobile phase (a), Mass spectrum of $Cp_6$-his showing heaviest molecular ion peak at 719.8 (b), Chemical structure of $Cp_6$ and $Cp_6$-his (c).
Figure 3.2 Absorption spectra of $Cp_6$ and $Cp_6$-his in Ethanol: PEG: Water system. Respective fluorescence emission spectra are shown as Inset.
3.1.2 Intracellular uptake of Cp6 and Cp6-his:

The kinetics of intracellular uptake of Cp6 (5 μM) and Cp6-his (5 μM) in 4451 and Nt8e cells is shown in Fig. 3.3(a). In both the cell lines the uptake of Cp6 was seen to increase up to 1 hr and saturated thereafter. In case of Cp6-his cellular uptake showed an initial rise till 1 hr followed by a slower phase of increase up to 5 h. The intracellular concentration of Cp6-his was noticeable higher as compared to Cp6 by a factor of 5 and 10 at 1 h and 3 h incubation, respectively. The cellular uptake of Cp6-his in MCF-7 cells also followed similar trend with the increase in intracellular content of Cp6-his by a factor of 2 and 3 at 1 h and 3 h incubation, respectively when compared to free Cp6 (Fig. 3.3b).

The effects of histamine (1 mM and 5 mM), ranitidine (100 μM) and pheniramine (100 μM) on the cellular uptake of Cp6-his and Cp6 are shown in Fig. 3.4 and table 1. Cells were incubated with each photosensitizer (5 μM) alone and in combination with above test compounds for 3 h. While, in the presence of histamine in case of both oral cancer cell lines, a significant increase (p < 0.01) in cellular uptake of Cp6-his was observed (Fig. 3.4a). In contrast with this slight but not significant (p < 0.01) decrease in cellular uptake of Cp6-his was found in MCF-7 cells (Table 1). However, addition of histamine H2 receptor antagonist ranitidine led to ~30% reduction (p value < 0.01) in the cellular uptake of Cp6-his whereas pheniramine, a histamine H1 receptor antagonist showed less inhibition (15-20%, p value < 0.05) (Fig. 3.4b and table 1) in all three cell lines. For Cp6, no
significant change in the cellular uptake was observed in the presence of any of these compounds (Fig. 3.4a-b and table 1).

Figure 3.5 shows the effect of lower temperature on the cellular uptake of \( Cp_6 \)-his and \( Cp_6 \) in the presence or absence of 10% serum in the culture medium. The cellular uptake of both \( Cp_6 \)-his and \( Cp_6 \) was found to decrease due to incubation of cells at lower temperature and in the presence of serum in the medium (fig. 3.5a). The percent inhibition was slightly higher for \( Cp_6 \)-his (50-60%) as compared to \( Cp_6 \) (30-40%). When serum is omitted from the culture medium, the inhibition in cellular uptake due to lower temperature was found to almost diminish in case of \( Cp_6 \) (7-10%) whereas for \( Cp_6 \)-his, it remained nearly same (40%) (Fig.3.5b). Similar results were found in case of MCF-7 cells (Table 1).
Figure 3.3. Time dependent cellular uptake of $Cp_6$ and $Cp_6$-his Conjugate in (a) 4451 and Nt8e cells and (b) MCF-7 cells. Cells were incubated with 5 μM of $Cp_6$ and $Cp_6$-his each for different time periods (0.5-7 h). Each data point represents the average ± SD of values obtained from three independent experiments.
Figure 3.4. The effect of histamine (a) and histamine receptor antagonist (b) on the cellular uptake of \( C_p_6 \) and \( C_p_6 \)-his conjugate. Cells were incubated with 5 \( \mu \)M \( C_p_6 \) and \( C_p_6 \)-his alone or with histamine (1 mM and 5 mM), ranitidine (100 \( \mu \)M) and pheniramine (100 \( \mu \)M) for 3 hr. Each data point represents the average ± SD values obtained from three independent experiments. [** \( p \) value < 0.01, * \( p \) value < 0.05]
Figure 3.5. The effect of temperature on the cellular uptake of $C_{P_6}$ and $C_{P_6}$-his conjugate. Cells were incubated with 5 μM $C_{P_6}$ and $C_{P_6}$-his at 37 °C or 15 °C in culture medium containing 10% serum (a) or without serum (b). Each data point represents the average ± SD values obtained from three independent experiments. [*p value < 0.05, ** p value < 0.01].
Table 3.1. The effect of histamine receptor antagonist ranitidine, pheniramine, low temperature and histamine on the cellular uptake of $Cp_6$ and $Cp_6$-his conjugate. MCF-7 Cells were incubated with 5 μM $Cp_6$ and $Cp_6$-his alone or with ranitidine (100 μM), pheniramine (100 μM), histamine (1 mM), and low temperature (15 °C) for 3 hr. Each data point represents the average ± SD values obtained from three independent experiments. [** p value < 0.01]

<table>
<thead>
<tr>
<th>Effect of agonist, antagonist and low temperature on cellular uptake of PS</th>
<th>Photosensitizer uptake (nm/μg protein)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>$Cp_6$-his</td>
</tr>
<tr>
<td>Ranitidine (100 μM)</td>
<td>1.18±0.17</td>
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<td></td>
<td></td>
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<tr>
<td>Pheniramine (100 μM)</td>
<td>1.18±0.17</td>
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<tr>
<td>Low temperature (15°C)(without serum)</td>
<td>4.68±0.29</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine (1 mM)</td>
<td>4.68±0.29</td>
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3.1.3 Detection of histamine receptor in cells:

In order to find out the presence of histamine receptor in two oral and in a breast cancer cell lines western blot was performed. Immunoblotting with H2 receptor antibody revealed four bands with molecular masses of approximately 30 kDa, 60 kDa, 80 kDa and 100 kDa (Fig. 3.6). The presence of four bands for H2 receptor is in agreement with the previous studies and suggests presence of oligomeric form of H2 receptors with actual molecular masses of 31.5 kDa, [252].

Figure 3.6. Image of nitrocellulose membrane showing presence of H2 receptor in 4451, Nt8e and MCF-7 cells after Western blot of the cellular protein using polyclonal rabbit anti-H2 receptor antibody and HRP conjugated Goat Anti-Rabbit IgG and detection by use of enhanced chemiluminescence reagents.
3.1.4 Intracellular Localization:

In Fig. 3.7 the bright field (left panel) and fluorescence images (right panel) of 4451, Nt8e and MCF-7 cells showing cell morphology and intracellular localization of \(Cp_6\) and \(Cp_6\)-his are displayed. In all three cell lines the fluorescence of \(Cp_6\) was observed in punctuated granular structures indicating its localization at multiple sites inside the cells Fig. 3.7b, f & j. The intracellular localization of \(Cp_6\)-his was noticeably different from \(Cp_6\) (Fig. 3.7 d, h & l). The fluorescence of \(Cp_6\)-his in 4451 and MCF-7 cells is observed in discrete vesicle type structures around the perinuclear region of the cytoplasm (Fig. 3.7 d & l). While Nt8e cells displayed the fluorescence of \(Cp_6\)-his within granular structures as diffused patch near the nucleus (Fig. 3.7 h). In all the cell lines, the fluorescence labeling of the cell membrane by \(Cp_6\)-his is also clearly visible (Fig. 3.7 d, h & l). Moreover, the fluorescence of \(Cp_6\)-conjugate was much more intense as compared to the fluorescence of \(Cp_6\) due to higher uptake. The brightness and contrast of the images shown in Fig. 3.7 were adjusted for proper visualization of the intracellular localization of \(Cp_6\) and \(Cp_6\)-his.
Figure 3.7. Microphotographs of 4451 (a-d), Ntse (e-h), MCF-7 (i-l) cells incubated with 5.0 μM Cp6 or Cp6-his in growth medium. Left panel – Bright field images of the cells, Right panel – corresponding fluorescence images showing localization of Cp6 (b & f) and Cp6-his (d, h). Magnification 100X, Bar ~20 μM.
3.1.5 Phototoxicity

The phototoxicity of Cp6-his was determined by subjecting the cells to photodynamic treatment using different concentrations of the conjugate and a fixed red light irradiation dose at 28 kJ/m². Percent phototoxicity was measured with respect to a control sample that received no drug and no light exposure and these results are presented in Fig. 3.8. The percent phototoxicity can be seen to increases in a concentration dependent manner in 4451, Nt8e and MCF-7 cell lines. The concentration of Cp6-his required to obtain 95% phototoxicity at the light dose of 28 kJ/m² was found to be 5 μM (Fig. 3.8a & c). To compare the effectiveness of Cp6-his with Cp6, all three cell lines were subjected to photodynamic treatment using same concentration (5 μM) and variable light dose (0-38 kJ/m²). The results presented in Fig. 3.8b & d show that for a given light dose the phototoxicity was much higher with Cp6-his than Cp6. The light dose required to achieve 50-60% cell killing was ~12 kJ/m² and 32 kJ/m² for Cp6-his and Cp6 respectively. At 28 kJ/m² light dose, the phototoxicity induced by Cp6-his was ~95% and in comparison, Cp6 led to ~50% phototoxicity. These data clearly show that Cp6 conjugate is more effective than Cp6. No dark toxicity was noticed for either Cp6-his or Cp6 at the concentration used.
Figure. 3.8. Percent phototoxicity induced by $C_{p6}$-his at varying concentration from 1-5 µM and fixed light dose at 28 kJ/m² (a-c) and both $C_{p6}$ and $C_{p6}$-his conjugate at fixed concentration 5 µM with varying light dose from 0-38 kJ/m² (b-d) in 4451, Nt8e, and MCF-7 cells. Cells were incubated for 3 h with photosensitizer in growth medium and irradiated with respective light dose. Phototoxicity was calculated as percent decrease in MTT reduction with respect to a control sample, which received no photosensitizer and no light. The zero dose point shows phototoxicity in cell sample incubated with photosensitizer but not exposed to light. Each data point represents the average ± SD values obtained from three independent experiments.
3.1.6 **Mode of cell death induced by C\textsubscript{p6}-his**

To identify the mode of cell death in the cells subjected to photodynamic treatment with C\textsubscript{p6}-conjugate, parameters such as cellular and nuclear morphology, and DNA fragmentation were studied. The cellular morphology of the untreated cell and cells subjected to photodynamic treatment is shown in Fig. 3.9. Photodynamic treatment of 4451 cells led to rupture of cell membrane and release of cytoplasm indicating necrotic cell death (Fig. 3.9b). In contrast, the cell morphology of Nt8e and MCF-7 cells (Fig. 3.9 c & e) after photodynamic treatment (Fig. 3.9d & f) shows formation of plasma membrane blebs and cellular shrinkage, hallmark of apoptosis in nearly 50% cells. In 20-30% cells formation of membrane bubbles and release of content of the cytoplasm typical of necrotic death was observed in case of Nt8e and MCF-7 cells.

To further confirm the apoptotic DNA fragmentation, DNA isolated from cells was subjected to gel electrophoresis and the results are shown in Fig.10. It can be seen that the DNA of 4451 cells after PDT show smeared patterned DNA fragmentation whereas, in Nt8e and MCF-7 cells laddered DNA fragmentation typical of apoptosis is clearly visible (Fig. 3.10). Together, these results suggest that in 4451 cells PDT with C\textsubscript{p6}-histamine conjugate led to cell death via necrosis while apoptosis was predominant in Nt8e and MCF-7 cells.
A comparison of the relative magnitude of necrosis or apoptotic cell death in both oral cell lines treated with \( C_{P6} \) or \( C_{P6-\text{his}} \) is shown in fig.3.11. There was no major difference between the PDT treatment by \( C_{P6} \) and \( C_{P6-\text{his}} \) with respect to the percentage of apoptotic or necrotic cells in both the cell lines. Also in both the cases, the cell line 4451 showed higher percentage of necrotic cells as compared to Nt8e cells for which the percentage of apoptotic cells in turn was more. Similarly, in case of MCF-7 cells both apoptosis and necrosis contributes equally following PDT with \( C_{P6-\text{his}} \) (table 2).
Figure 3.9: Microphotographs of 4451 (a, b), Nt8c (c, d) and MCF-7 cells (e, f) showing changes in the cellular morphology after PDT with Cpσ-his. Untreated cells (a, c, e) cells 18 hr after PDT (b, d, f). Cells were incubated with 5.0 µM Cpσ-his for 3 h in growth medium and then irradiated with red light at ~ 28 kJ/m². Magnification 40X, Bar -50 µM.
Figure 3.10: DNA gel electrophoresis. Lane 1- untreated 4451 cells, 2- 4451 cells subjected to PDT, 3- untreated Nt8e cells, 4- Nt8e cells subjected to PDT, 5- untreated MCF-7 cells, 6- MCF-7 cells subjected to PDT. Cells were incubated with 5.0 µM Cp6-his for 3 h in growth medium and then irradiated with red light at ~ 22 kJ/m². DNA was isolated 18 h after PDT.
Figure 3.11. Percentage of apoptotic and necrotic cells in 4451 and Nt8e cells after 18 hr of photodynamic treatment with C₇₆ or C₇₆-his. C₇₆ was used at 10.0 μM with light dose 38 kJ/m² and for C₇₆-his, 5.0 μM concentration and ~28 kJ/m² was used to obtain ~95% phototoxicity in both the cases. Fluorescence microscopy after staining the cells with Hoechst and propidium iodide was used to recognize the apoptotic, necrotic and live cells. Each data point represents the average ± SD values obtained from three independent experiments.
Table 3.2. Percentage of apoptotic and necrotic cells in MCF-7 cells after 18 hr of photodynamic treatment with \( Cp_6 \)-his. \( Cp_6 \)-his was used at 10.0 \( \mu \)M with light dose 38 kJ/m\(^2\) and for \( Cp_6 \)-his, 5.0 \( \mu \)M concentration and \( \sim 28 \) kJ/m\(^2\) was used to obtain \( \sim 95\% \) phototoxicity in both the cases. Each data point represents the average \( \pm \) SD values obtained from three independent experiments.

<table>
<thead>
<tr>
<th>Mode of cell death</th>
<th>Number of cells (%) average ( \pm ) SD</th>
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<tbody>
<tr>
<td>Apoptosis</td>
<td>40.2( \pm )6.3</td>
</tr>
<tr>
<td>Necrosis</td>
<td>55.3( \pm )8.8</td>
</tr>
<tr>
<td>Live</td>
<td>4.2( \pm )5.6</td>
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3.2 Discussion

The motivation for the present study was to investigate the use of histamine, a biogenic amine to enhance the uptake and tumor selectivity of Cp6 by exploiting histamine receptors for delivery of photosensitizer in cancer cells. The results of cellular uptake studies presented in fig. 3.3a-b show that the Cp6-his is taken up more efficiently by the cells than free Cp6. To check if the uptake occurred via histamine receptors we also measured the cellular uptake of Cp6-his in the presence of histamine. Instead of the expected inhibition, histamine led to slight increase in the uptake of Cp6-his in case of both oral cancer cell lines (fig 3.4 a). The reason for this effect is presently not clear. Based on the fact that the receptor affinity of some agonist/antagonist is higher than histamine [253] one can assume Cp6-his to have stronger receptor affinity which prevented histamine to compete efficiently for the binding site. Moreover since exogenous histamine has been shown to up-regulate expression of histamine receptors [212,236,254], this would also be considered as possible reason for observed increase in uptake of Cp6-his. However, in MCF-7 an expected decrease in uptake of Cp6-his was observed. Furthermore to confirm the involvement of histamine receptors, cellular uptake of the conjugate was measured in presence of pheneramine and ranitidine which are known antagonist for H1 and H2 histamine receptors, respectively. In Nt8e, 4451 and MCF-7 cell lines these antagonists at 100 μM led to significant inhibition in the cellular uptake of Cp6-his and the inhibition was more pronounced in the presence of ranitidine, a potent H2 receptor antagonists suggesting that at least a part of cellular uptake or binding of the conjugate occurred via H2 receptors. Using western blot we found that H2R receptor is expressed in all three cell lines.
However, since higher concentration of the antagonist did not lead to further inhibition in the uptake of the Cp6-his, the possibility that receptor independent mechanism also contribute to its intracellular uptake cannot be ruled out. Indeed some histamine agonist, antagonist and BODIPY FL histamine, a fluorophore used to label histamine receptors, have also been shown to be internalized and sequestered in cells by a receptor-independent mechanism [255]. Therefore to further confirm that the uptake is receptor-mediated, the effect of low temperature on cellular uptake of both Cp6-his and Cp6 was studied. Interestingly, incubation at 15 °C led to inhibition of cellular uptake of both the photosensitizers (figure 3.5a). Although the magnitude of inhibition for Cp6 was slightly lower than Cp6-his, it was not unexpected due to the fact that hydrophilic Cp6 via interaction with serum LDLs can also be taken up by receptor mediated endocytosis [256]. To check this possibility, we omitted serum from the culture media during the incubation period. Results show that the inhibition of cellular uptake due to lower temperature is persistent for Cp6-his but in case of Cp6 it is almost diminished (figure 3.5b and table 1). These results confirmed that the uptake of Cp6-his is indeed receptor mediated. Apart from histamine receptors, there also exists membrane associated organic cation transporters (OCTs) which function to remove excess amount of histamine from the extra-cellular space by its re-uptake and transport into the cytoplasm to a yet unidentified site where it is metabolized into inactive metabolite N²-methylhistamine [254]. However, studies in murine hematotopoecite progenitor cells and basophiles have shown that the uptake of histamine by OCT is not affected by the presence of H1 or H2 receptor antagonist [258,259]. Since we found significant inhibition in uptake of Cp6-his by histamine antagonist the possibility of role of OCTs in its cellular uptake is less likely. Since
attachment of \( C_{P6} \) to histamine can lead to alterations in its physico-chemical properties such as relative hydrophobicity, molecular charge and amphiphilicity, one would expect this also to contribute to the improved cellular uptake of \( C_{P6}\)-his in a manner similar to reported for N-aspartyl ce6 (MACE, LS11) a conjugate of chlorin e6 with aspartic acid [260].

Our results on intracellular localization show that in all three cell lines \( C_{P6}\)-his localizes on the cell membrane and also inside the cells in the form of vesicles near the perinuclear region. This is similar to the intracellular localization of histamine reported earlier in rat immune cells [261]. In contrast, the intracellular localization of \( C_{P6} \) was distinctly different and occurred in the form of punctuated granular structures inside cytoplasm indicating its localization at multiple sites such as endoplasmic reticulum, Golgi apparatus and lysosomes. This is consistent with our previous studies [262]. The uptake of \( C_{P6}\)-his via histamine H2 receptor would lead to its accumulation in endosome/lysosome pool. This is due to the fact that histamine H2 receptor is G protein-coupled receptor (GPCRs) which when binds to agonist or antagonist undergoes internalization through the process of endocytosis resulting in its accumulation in the perinuclear endosomal pool and subsequent trafficking to the lysosomes [263]. The receptor is either recycled back to the plasma membrane or undergoes proteolytic degradation for down regulation [264]. For GPCRs which utilize endocytosis machinery for receptor regulation, it is generally believed that the receptor and ligand are internalized together [242]. It is therefore likely that the vesicles in the perinuclear region where \( C_{P6}\)-his is localized represents the endosome/lysosome compartments. Intracellular binding site of histamine to microsomal
cytochrome P450 and nucleus have also been identified through which histamine is believed to regulate cell growth and homeostasis [266]. These binding sites designated as H1C are not specific because it can interact with several other compounds also such as imidazoles (including HA, l-histidine, histidinol), polyamines (putrescine, spermidine, spermine) and hormones (androgens, estrogens, progestins and, to a lesser extent, adrenal steroids) etc [266]. However, we did not find localization of C9-his in the cell nucleus. The identification of the exact site of C9-his localization needs further investigations.

The results presented in fig. 3.8 show that the phototoxicity induced by C9-his was ~4 times higher as compared to C9 whereas, the magnitude of increase in the uptake of the conjugate was ~ 10 times higher than free C9. One important factor that can contribute to this observation is that the absorption coefficient of the conjugate was ~1/2 than C9 at 660 nm. Since the mode of PDT-induced cell death is determined by the intracellular localization of the photosensitizer [267] and significant differences were observed in the intercellular localization of C9 and C9-his, we also investigated the cell death response induced by the two photosensitizers. The results on cell morphology(fig 3.9) and DNA electrophoresis (fig 3.10) suggest that while cell death in 4451 is mostly by necrosis, for NT8e and MCF-7 cells both apoptotic and necrotic cell death is induced by photodynamic treatment with C9-his. Measurements on the percentage of apoptotic and necrotic cells after PDT with C9 and C9-his (fig 3.11) showed no difference except that in case of C9-his slight increase in necrotic cells was observed which can be attributed to the observed localization of C9-his on the cell membrane. The reasons for the differences in the PDT-induced cell death response observed between the two cell lines may be because of their
p53 status. While the 4451 cells are reported to be a p53 mutant [250], the cell line Nt8e contains wild type p53 [254]. The tumor suppressor gene p53 is known for its ability to induce apoptosis by activating downstream cell death effectors including bax, Puma, and Noxa [268,269]. In a study on PDT with haematoporphyrin derivative (HpD) similar results have been reported in 4451 cells and in cell line BMG-1 having wild type p53 [248]. Moreover, in case of MCF-7 cells, percentage of apoptotic and necrotic cells was found to be same, showing that both the mechanism contributes equally to the cell death following PDT with $C_{P_6}$-his (table 2).

3.3 Conclusion

To conclude, the results of our study show that conjugating $C_{P_6}$ to histamine improves its cellular uptake and hence the PDT efficacy in both oral and breast cancer cell lines. The observations that the cellular uptake of $C_{P_6}$-his is significantly inhibited by ranitidine and lower temperature, suggest that part of its uptake occurred via histamine receptors. Similarly, in human breast carcinoma cell line MCF-7 in which the expression of histamine receptors is well documented, higher uptake of $C_{P_6}$-his and the expected enhancement in phototoxicity was observed. It is concluded that conjugating $C_{P_6}$ with histamine can help to improve the effectiveness of PDT in oral and breast cancer cells by enhancing its intracellular delivery.