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Photodynamic Therapy and Nuclear Imaging Activities of Zinc Phthalocyanine Integrated TiO_2 Nanoparticles in Breast and Cervical Tumors

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In recent years, phthalocyanines (Pcs) have been widely used as photosensitizer in photodynamic therapy applications. Because of their strong absorptions in the near-infrared region (640-700 nm). The integration of phthalocyanine derivatives to a nanoparticle is expected to be efficient way to improve the activity of the photosensitizer on the targeted tissue. It is known that the integrated molecules not only show better accumulation on tumor tissue but also reduce toxicity in healthy tissues.

In this study, the ZnPc molecule was synthesized and integrated to the TiO_2 nanoparticle, to investigate the potential of PDT and its cytotoxicity. Additionally, ZnPc and ZnPc-TiO_2 molecules were labelled with ^{131}I and it was aimed to put forth the nuclear imaging/therapy potentials of ^{131}I labeled ZnPc/ZnPc-TiO_2 by determining *in vitro* uptakes in mouse mammary carcinoma (EMT6), human cervical adenocarcinoma (HeLa).

In result of our study, it was observed that the radiolabeling yields of the synthesized ZnPc and ZnPc-TiO_2 with ^{131}I were quite high. *In vitro* uptake studies shown that ^{131}I-ZnPc-TiO_2 could be a potential agent for nuclear imaging/treatment of breast and cervical cancers. According to PDT results ZnPc-TiO_2 might have as to be a potential PDT agent in the treatment of ovary tumor. ZnPc and ZnPc-TiO_2 might be used as theranostic agents.

Keywords: Photodynamic therapy, zinc phthalocyanine, nuclear imaging, titanium nanoparticle, cell culture.

1 | INTRODUCTION

Nowadays, the rate of cancer is increasing, and methods such as surgery, chemotherapy and radiotherapy are widely used in cancer treatment. The fact that these methods have many side effects which has led to the search for more effective and less side-effect treatments. Among

these new therapies, photodynamic therapy (PDT) is known to be the most promising treatment method for cancer treatment and has the least side effect.[1, 2]

PDT is an anti-tumor method that quickly destroys the tumor with a versatile mechanism. Preclinical and clinical studies have shown that PDT causes the tumor cell death by direct tumor-tissue death and indirectly via increasing anti-tumor immunity.[3] Photosensors (PSs) are used in PDT applications. When PSs are used in PDT should be absorbed at a long wavelength (far red or near infrared region) and have minimum toxic effects on normal tissue.[4] Phthalocyanines (Pcs), a type of photosensors, have excellent photochemical properties due to their high singlet oxygen quantum yield and high absorption characteristics at far red region wavelengths (600-850 nm).[5] It produces cytotoxic reactive oxygen that destroys tissue and tumor cells with the multifunction mechanism. A various Pcs derivatives have been widely used in PDT applications. The second generation Pcs exhibit the highest absorption at 600-800 nm and the accumulation in deep tissue is greater than their first generation derivatives.

In the last few years, nanoparticles for PDT have emerged as an alternative to efficiently performing conventional PDT on the target cancer cell. Nanoparticle reduces toxicity of Pc during PDT and provides high accumulation of Pc in the target tissue. In many studies, systems such as lipids, nanoparticles (gold, silica, titanium etc.), and monoclonal antibodies have been used to enhance the performance of the PDT agent and to selectively transport the agent to the tumor tissue. ZnPc@TiO_2 hybrid nanostructures modified with folic acid were evaluated as photodynamic agents by Flak and researcher group. They found that ZnPc has rather reasonable effect on the photodynamic activity of TiO_2 nanostructures, which is more significant in combination with FA attachment. According to in vitro studies that prepared hybrid nanostructures are able to selectively target HeLa cancer cells.[6] As a result of these

studies, it has been suggested that multifunctional nanoparticles, which can be adapted to the clinic in this way, may be good agents in effectively PDT and in-situ tumors.[7]

TiO_2 nanoparticles are preferred in many medical applications because of their properties like low toxicity, chemical stability, good biocompatibility and high photoreactivity. TiO_2 nanoparticles can be used for drug delivery, cell imaging, and biosensors studies. They also use as a carrier system to enhance the bioavailability and biostability of PS. Therefore it is expected that TiO_2 nanoparticles will increase the accumulation of compound in tumor tissue. They reach a deeper tumor tissue because of the absorption of the Pc in the visible region thus increase the PDT yield.[5,7,8] Lopez et al. reported on their study TiO_2 nanoparticles mainly played a role as phthalocyanine carrier, and were tested to enhance its cellular uptake on various cancer cells. Furthermore, sensitized TiO_2 with ZnPc is an excellent candidate as sensitizer in PDT against these cancer cells. These properties are thought that, TiO_2 nanoparticles are one of the promising biological application for PDT.[5]

In 2015, Pan et al. synthesized nitrogen doped TiO_2 nanoparticles (N–TiO_2) conjugated aluminum phthalocyanine chloride tetra sulfonate ($AlPcS_4$) by a two-step surface modification method. Then they evaluated the cytotoxicity, cellular uptake, intracellular distribution studies and the photokilling effect of the nanoparticles on different cancer cell lines. According their results, the cellular uptake of Pc is largely improved by its carrier N–TiO_2. The photokilling efficiency of N–TiO_2–Pc was over ten times higher than that of Pc. Their results showed that N–TiO_2–Pc is a promising candidate as a photosensitizer in PDT.

In our study, the ZnPc molecule was synthesized and this molecule was integrated into the TiO_2 nanoparticle (ZnPc-TiO_2) and *in vitro* PDT potencies of ZnPc/ZnPc-TiO_2 were examined in various tumor cells (HeLa and EMT6). Besides to investigate of ZnPc/ZnPc-

TiO_2 of nuclear imaging, ZnPc-TiO_2 was labeled with ^{131}I radioisotope and ^{131}I-ZnPc-TiO_2 uptake was determined in the cell lines.

2 | METHODS AND MATERIALS

2.1. | Materials

Thin-layer chromatography-cellulose gel (ITLC-F plastic sheets 20×20) was supplied from Merck. Iodogen was purchased from Sigma-Aldrich. Thin Layer Radio Chromatography sheets were analyzed using a Bioscan AR2000 TLC Scanner. All reagent used in *in vitro* studies were supplied from Biological Industries; all other chemicals were provided from Merck. Cell culture studies were performed in a Thermo MSC Advantage 1.2 laminar air flow cabinet. An Olympus Japan inverted light microscope was used for counting cells. A Thermo Multimode microplate reader was used to determine the IC_{50} values of cell cultures. Other chemicals were purchased from Aldrich. Field emission-scanning electron microscopy (FE-SEM) image was obtained using a Zeiss/Supra 55 FE-SEM.

2.2 | Synthesis

Zinc (II) 9,10,16,17,23,24-hexa(4'-tert-butylphenoxy)-2-[2´-(4´´-carboxyphenyl) ethynyl] phthalocyaninato (2-)-N^{29}, N^{30}, N^{31},N^{32} (ZnPc) and ZnPc integrated TiO_2 nanoparticles (ZnPc-TiO_2**)** were synthesized according to the previously reported method (Fig. 1).[8] Representative FE-SEM image of ZnPc-TiO_2 is shown in Fig. 2.

2.3 | Cell Culture

In vitro studies were performed using EMT6 (mouse breast carcinoma cell line), HeLa (cervical adenocarcinoma cell line). They were cultured in 75 cm^2 flasks with high glucose

Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C in a 5% CO_2, 95% air incubator.

2.4. Radiolabeling and Radiochemical Purity Analysis

Briefly, the radiolabeling of ZnPc/ZnPc-TiO_2 was performed at optimum conditions (pH 7, 1.00 mg iodogen and 45 minutes of reaction time for ZnPc; pH 5, 1.00 mg iodogen and 60 minutes of reaction time for ZnPc-TiO_2), by described Yurt et al. 2017.[8] The stock solution of the compounds was prepared ZnPc/ZnPc-TiO_2 (1mg) dissolved in 300 µL DMSO. Then 20 µL ZnPc/ZnPc-TiO_2 stock solutions (50 nmol) were diluted with 500 µL distillated water and the solutions were put into iodogen coated tube. Then 9.25-18.5 MBq ^{131}I was added into the tube and incubated. After the incubation ^{131}I-ZnPc compound was dropped to cellulose-coated plastic (ITLC-cellulose) plates (1×10 cm; Merck) and the plates were dipped into different mobile phases. The cellulose sheets were scanned on a TLC-scanner (BioScan AR-2000 Washington DC, USA) for determining the amount of radiolabeling yield and the R_f values of molecules. Three different mobile phases used in radiolabeling studies [mobile phase 1: n-butanol-water-acetic acid (4-2-1) and mobile phase 2: chloroform-acetic acid (9-1)].[8]

2.5. Intracellular Uptakes of ^{131}I-ZnPc/ZnPc-TiO_2

The *in vitro* cellular uptake of ^{131}I labeled ZnPc was performed using the EMT-6 and HeLa cancer cell lines. The cells were seeded on a 24-well cell culture plate (Corning Life Sciences) at 5×10^5 cells/well. The plates were incubated at 37 °C throughout two days for the cells to adhere to the plates. Two days later, the medium on the cells was removed and the wells were washed two times with 0.9 % NaCl solution and the radiolabeled ZnPc (20 µM, activity: 0.5 MBq) which was diluted with MEM (without FBS) was added on the cells. The intracellular uptake of Na^{131}I as the control group was assayed on the given conditions. At

this stage it was checked whether the uptake was caused by free iodine or radioiodinated compound. After different time period incubation (1, 2, 4, 6 and 24 h), the wells were counted by Cd (Te) RAD 501 signal channel analyzer. Then, the radioactive medium on the cells was removed. The cells were washed once and 0.9 % NaCl solution was added in each well. After the wells were counted again, the data were analyzed and the percentage of intracellular uptake was calculated.

2.6. Cytotoxicity and Photodynamic Therapy Experiments

In our study, cell proliferation assay was performed to determine the number of cells/mL to be used in the XCELLigence system of EMT6, and HeLa cell lines for 48 hours. 100 mM DMSO stock solutions were prepared for the cytotoxicity assays of ZnPc and ZnPc-TiO_2 and consecutive concentrations of ZnPc and ZnPc-TiO_2 of 1.57, 3.13, 6.25, 12.50, 25.00 and 50.00 μM were treated to the cells. Because DMSO was more diluted than 1/100, it was not added to the untreated control the cells. The IC_{50} value was determined by calculating the percentage cell viability according to the untreated control group. IC_{50} values were determined by sigmoidal dose-response analysis.

The white LED light source (ILX Lightwave OMH-6703B) was used in the photodynamic therapy. The power of the light source was designed at 10 mW/cm^2 and the distance to the cell was set at 7 cm. This distance has been used in photodynamic therapy studies. EMT6 and HeLa cell lines were cultured in EMEM with 1% non-essential amino acid and DMEM medium supplemented with 10% fetal bovine serum and the cells were incubated in a cell culture incubator at 37 °C, 95 % humidity, and 5 % CO_2 until adequate reproduction was achieved. The cells were seeded three times (triplicate) in a 96-well E-plate at the indicated cell/ml density and the cells were incubated overnight. The ZnPc and ZnPc-TiO_2 were added at varying concentrations (1.57, 3.13 and 6.25 μM) and then allowed to incubate for 3 hours

in the dark. The cells were exposed to the light at doses of 30, 60 and 90 J/cm^2. After photodynamic therapy, the cells were replaced with fresh medium and the cells incubated at 37°C in an incubator containing 5% CO_2 and 95% humidity for 24 h. The E-plate impedance was recorded every 15 minutes for 48 hours. The cytotoxicity and photodynamic therapy efficacy were achieved via the normalized cell index using the xCELLIGENCE software and the control group was generated from the untreated cells and the cells without light. Phototoxicity analysis was performed by one-way analysis of variance for each dose, compared to the control group without photosensitizer.

PDT studies were performed to determine the phototoxicity of ZnPc, ZnPc-TiO_2 and TiO_2 in EMT6 and HeLa cell lines. The photodynamic therapy efficacies of ZnPc ZnPc-TiO_2 and TiO_2 have been evaluated in control group that non-cytotoxicity at concentrations of 1.57, 3.13 and 6.25 μM without phototherapy (PT-). The phototoxicity of ZnPc, ZnPc-TiO_2 and TiO_2 was determined according to the untreated control group in which the substance was not applied and the light was applied.[8,10-12]

2.7. Fluorescence Microscopic Imaging

The intracellular distributions of ZnPc, ZnPc-TiO_2 and TiO_2 were studied by fluorescence microscopy. HeLa, EMT6 and WI-38 cells (2×10^3 cells/well) were plated on cell culture slides (SPL Life Sciences) and grown overnight in 37^0C. Afterwards, the cells were treated with IC_{50} concentration of each nanoparticle for 3 h at 37^0C. After the incubation cells were washed with PBS and were stained with DAPI counterstaining. The fluorescent images were captured and analyzed with a fluorescence microscope with DP2-BSW software v.2.2 (BX51, Olympus).

2.8. Statistical analysis

Each study was performed in triplicate and mean and standard deviation were calculated. The comparison of phototherapy was done by *student's t* test. All statistical analyses were performed using GraphPad Prism 6.0. A p value of < 0.05 was considered statistically significant.

3 | RESULTS AND DISCUSSION

3.1| Radiolabeling and Optimization conditions of ZnPc and ZnPc-TiO_2

The quality control of radiolabeling yields of ZnPc and ZnPc-TiO_2 was performed with TLRC method. R_f (relative front) values of $Na^{131}I$, oxidized ^{131}I, ^{131}I-ZnPc and ^{131}I-ZnPc-TiO_2 were determined as 0.45, 0.93, 0.95 and 0.97 for mobile phase1; 0.05, 0.05, 0.93 and 0.96 for mobile phase 2, respectively. According to the TLRC results, radiolabeling yields of ZnPc and ZnPc-TiO_2 were defined as 91.9±3.1 and 91.2±4.3%, respectively.[8]

3.1.1| Toxicity of ZnPc, ZnPc-TiO_2 and TiO_2

The IC_{50} values of ZnPc, ZnPc-TiO_2 and TiO_2 in EMT6 cell line were found as 4.70 μM, 7.06 μM and 764.54 μM, respectively. The result showed that TiO_2 nanoparticles were found to be non-cytotoxic for breast cancer cells. Furthermore, lower toxicity of ZnPc-TiO_2 was found and ZnPc integrated TiO_2 nanoparticles asserted to reduce the cytotoxicity of ZnPc. Although similar results had been found for WI-38 cell line (IC_{50} values for ZnPc: 5.60 μM, for ZnPc-TiO_2: 20.19 μM and for TiO_2: 739.62 μM), we observed that TiO_2 nanoparticles increased the cytotoxicity of ZnPc in HeLa cell line. The IC_{50} values of ZnPc, ZnPc-TiO_2 and TiO_2 in HeLa cell line were determined as 21.44 μM, 8.26 μM and 114.78 μM, respectively.

3.1.2 Intracellular Uptake Efficiencies of ^{131}I-ZnPc and ^{131}I-ZnPc-TiO_2

The intracellular uptake of ^{131}I-ZnPc and ^{131}I-ZnPc-TiO_2 in EMT6 and HeLa was determined as percentage. The intracellular uptake studies were performed with 2 μM concentration below the IC_{50} values. Additionally, the intracellular uptake of $Na^{131}I$ was examined as control in all cell lines.

Intracellular uptake efficiencies of ^{131}I-ZnPc were found 10 times higher than uptake of $Na^{131}I$ in EMT6 cell line, 8 times higher in HeLa cell line. Furthermore, it was shown that uptake of ^{131}I-ZnPc is 5 times higher than $Na^{131}I$ in WI-38 cell line.[8] While uptake of ^{131}I-ZnPc was 10.95±1.92% at 1 hour. The maximum uptake of ^{131}I-ZnPc reached 12.4±1.2% at 2 hours in the EMT6 cell line. On the other hand the maximum uptake of ^{131}I-ZnPc was observed as 10.2±1.3% at 1 hour in HeLa cell line (Figure 3). Additionally, in previous study, 5.8±1.0% uptake was observed in WI-38 cell line at 1 hour.[8]

In the intracellular uptake results, the highest uptake of ^{131}I-ZnPc-TiO_2 was determined at 1 hour as 5.0±0.7% in EMT6 cell line (Figure 3). While the uptake of ^{131}I-ZnPc-TiO_2 was 4.34 ±0.49% at 1 hour, the maximum uptake of the radiolabeled compound was observed at 6 hours (5.4±0.5%) in cervical cancer cell line (Figure 3). However in WI-38 cell line, the highest intracellular uptake was defined as 1.5±0.3% at 1 hour in the study of Yurt et al., 2017.[8] Additionally, the uptake of $Na^{131}I$ was found to be quite low in all cell lines.

According to the cell uptake results, the intracellular uptake of ^{131}I-ZnPc is found to be higher than ^{131}I-ZnPc-TiO_2 in the cell lines. The similar results were determined in study of Lopez et al.[5] In the study, cell localization of ZnPc were found to be higher than ZnPc-TiO_2 in mammalian cells and the localization of ZnPc and ZnPc-TiO_2 was observed in mitochondrial molecules. It was known that compounds with high lipophilicity have higher cell affinity.[13] Lipophilic photosensitizers are transported by lipoproteins. Thus, they are taken directly by

tumor cells and localized in the membrane structures.[14, 15] In previous study of our group, found that ^{131}I-ZnPc (10.1±1.9) is more lipophilic than ^{131}I-ZnPc-TiO_2 (8.1±2.3).[8] Furthermore, the uptake of lipophilic photosensitizers in the cancer cells is higher than healthy cells.[16] In our study we observed that the intracellular uptake in W-I38 cells was lower than tumor cell lines.[8] It was demonstrated in the study of Flak et al., fluorescence of ZnPc-TiO_2 nanostructures were detected in HeLa cell cytoplasm by confocal laser microscopy. However, cellular uptake of ZnPc-TiO_2 nanostructures in normal human fibroblasts (MSU-1.1) cells were less efficient.[6]

The uptake of ^{131}I-ZnPc and ^{131}I-ZnPc-TiO_2 in EMT6 cancer cell /healthy cell (WI-38) ratios were 2.24 at 2 hour for ^{131}I-ZnPc and 3.44 at 1 hour for ^{131}I-ZnPc-TiO_2. Additionally the ratios in HeLa cancer cell /healthy cell (WI-38) were 1.76 at 1 hour for ^{131}I-ZnPc and 3.69 at 6 hour for ^{131}I-ZnPc-TiO_2. The cancer cell lines/healthy cell line tissue ratio in imaging studies should be at least 2.[17] The cancer cells/healthy cell ratio for ^{131}I-ZnPc-TiO_2 is higher than ^{131}I-ZnPc. In this case it was determined that the ^{131}I-ZnPc-TiO_2 nanoparticle was more specific to the breast and cervical tumor cells than ^{131}I-ZnPc. This result might be explained by the dependence of cell uptake on surface charge for ZnPc and ZnPc-TiO_2 nanoparticle.[18]

3.1.3| Photodynamic Therapy Efficiencies of ZnPc, ZnPc-TiO_2 and TiO_2

In the PDT studies of ZnPc in the cell lines, we observed that phototoxic effect increased to depend on the dose of light (λ=684 nm UV-vis absorption) (Figure 4).

Maximum phototoxic effects were determined at 6.25 μM concentration of ZnPc and 90 J/cm^2 light dose (60.93% for EMT6 cells and 70.36% for HeLa cells.). At the same time, maximum phototoxic effect of ZnPc-TiO_2 was observed at 90 J/cm^2 light dose (69.01% for 1.57 μM, 65.70% for 3.13 μM and 73.39% for 6.25 μM). ZnPc-TiO_2 caused significant

phototoxicity at 1.57 and 3.13 μM with 30J/cm^2 and 6.25 μM with 60 J/cm^2 in EMT6 cell line ($p<0.001$). ZnPc-TiO2 also caused significant phototoxicity at 6.25 μM with 60 J/cm^2 but ZnPc was more phototoxic at 3.13 μM with 90 J/cm^2 in EMT6 cell line ($p<0.001$). PDT results of ZnPc-TiO_2 were seen in Figure 4. Similar in PDT results of ZnPc, increased phototoxic effect related with the dose of light was detected in all cells. The highest phototoxicities were determined as 82.05% in EMT6 cell line, 80.00% in HeLa cell line. No cytotoxicity of TiO_2 was found in two-cell lines. When the PDT effect of TiO_2 was examined, the results showed that TiO_2 has a proliferative effect at PDT(-) and 30 J/cm^2 light dose in EMT6 and HeLa cell lines in Figure 4. In addition, the phototoxic effects of ZnPc and ZnPc-TiO_2 were observed as to be 91.97% and 97.94% respectively in WI-38 cell line at a light dose of 90 J/cm^2 in the previous study performed by us.[8] When compared to WI-38 phototoxic effect, the high phototoxic effect was determined in WI-38 cells, the phototoxic effect occurs only in the presence of light, and the application of light only to the target tissues. At the same time, ^{131}I-ZnPc and ^{131}I-ZnPc-TiO_2 were showed less affinity in healthy cells than cancer cells.[8] Low affinity of the compound and absence of light protect the healthy cells during photodynamic therapy.

According to recent studies, it has been reported that reactive oxygen species (ROS) - mediated cancer therapy, called photodynamic therapy (PDT), is a potential alternative treatment for the minimally invasive and improved localized effect of the locus.[19] Zhang H et al. have compared TiO_2 and ZnO nanoparticles as photosensitizer for photodynamic therapy against cancer. They showed that TiO_2 and ZnO nanoparticles could form ROS in tumor cells after irradiation and attack cancer cells in their work. Also, as a therapeutic effect, they observed that there was no difference between TiO_2 and ZnO nanoparticles for PDT.[20]

According to the PDT results of present study, ZnPc-TiO_2 was found more phototoxic than ZnPc in mammalian and cervical cancer cell lines. In the study of Lopez et al., ZnPc-TiO_2

nanoparticles were found to be phototoxic for tumor and non-tumor mammalian cells but the phototoxicity was less than the pure ZnPc.[5] In another study, significant phototoxic effect of ZnPc@TiO_2 nanostructures were determined for HeLa cell line.[6] Lei Liu et al. have demonstrated the photodynamic killing effect of the Pt/TiO_2 nanocomposite on cancer cells in their Pt/TiO_2 nanocomposite study with human cervical tumor cells (HeLa).[21] Titanium dioxide nanoparticles are not toxic to mammalian cells as they are chemically ineffective and stable under physiological conditions.[22, 23] Yin M et al. show that in cytotoxicity experiments combined treatment mediates the highest mortality rate in breast cancer cells when compared to single chemotherapy or PDT.[24]

3.1.4| Fluorescence Microscopic Imaging

Cellular localization of the ZnPc and ZnPc-TiO_2 was found especially in cytoplasm but not nuclei of three cell lines (EMT6, HeLa, and WI-38). It was observed that TiO_2 had a reduce fluorescence intensity as in untreated control cells (Figure 5). Flak D et al demonstrate that ZnPc@TiO_2 nanostructures were also localized within the cell cytoplasm.[6]

4| CONCLUSION

In summary ZnPc and ZnPc-TiO_2 were prepared and employed as photodynamic agents for the photodynamic therapy as well as nuclear imaging agent. It was found that ZnPc has effect on PDT. However, ZnPc attachment has rather effect on photodynamic activity of TiO_2 nanostructure in mouse mammary carcinoma (EMT6), human cervical adenocarcinoma (HeLa). It was demonstrated that ZnPc and ZnPc-TiO_2 were localized within the cells cytoplasm by fluorescence microscopy. HeLa, EMT6 and WI-38 cells (In addition

ZnPc/ZnPc-TiO_2 were labeled with ^{131}I with high yield and it was determined that ^{131}I labeled ZnPc-TiO_2 has high uptake in breast and cervical tumor cell lines with intracellular uptake studies and might be used as nuclear imaging agent in breast and cervical tumors. Therefore, it suggested that ZnPc-TiO_2 can be a potential theranostic agent for PDT and nuclear imaging on breast and cervical tumors.

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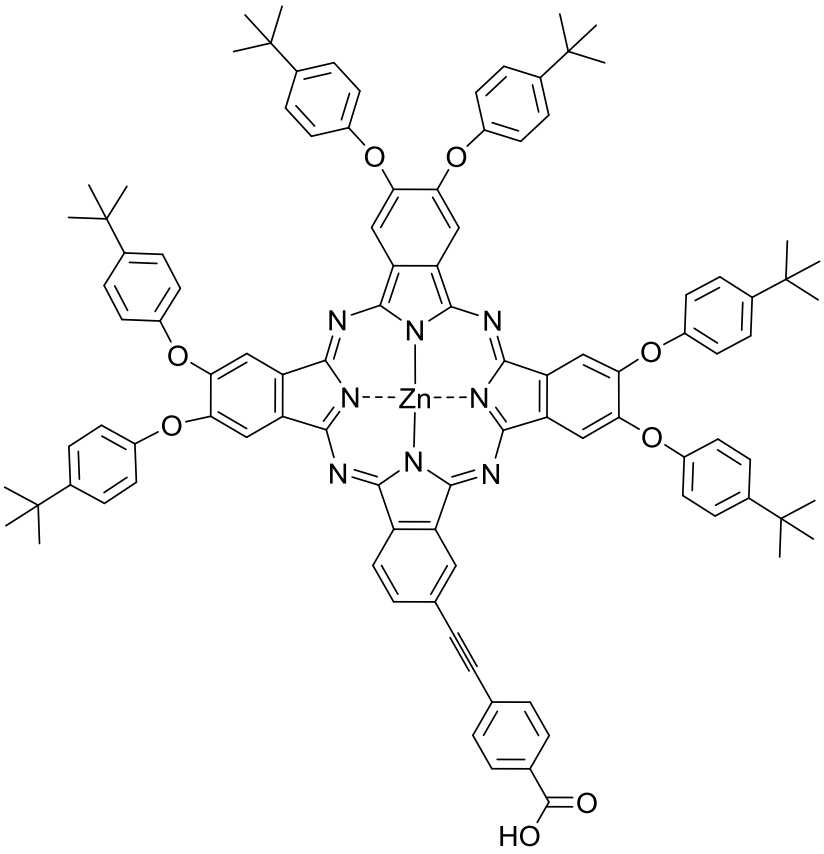


FIGURE 1. Molecular structure of ZnPc.

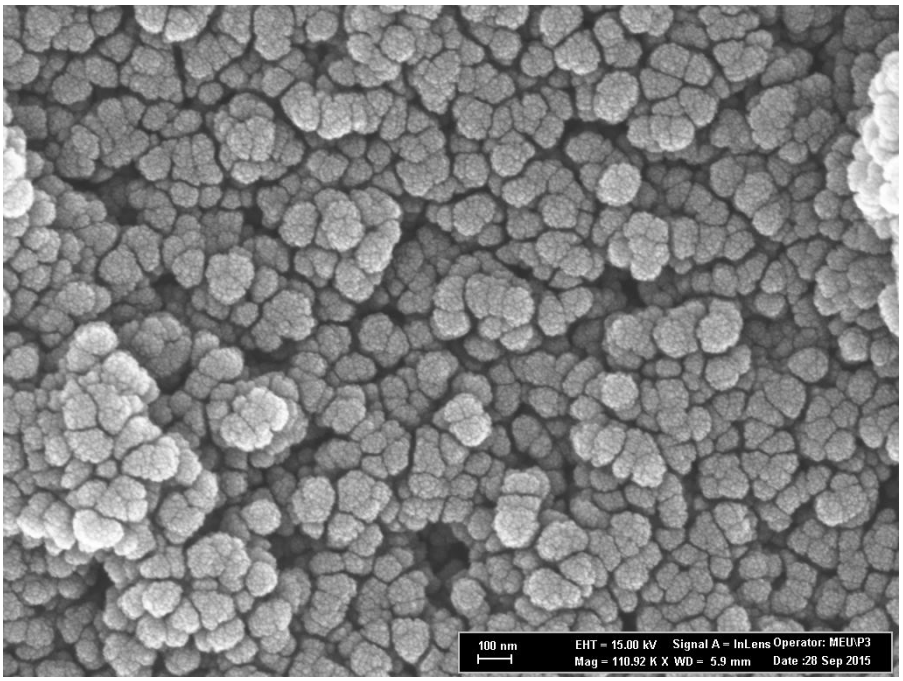


FIGURE 2. Representative SEM image of the ZnPc-TiO_2 nanoparticles.

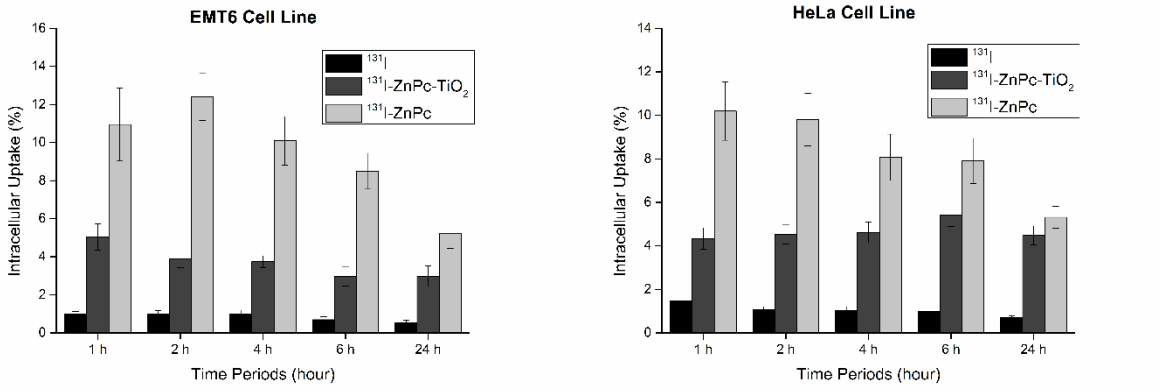


FIGURE 3. Intracellular uptake of ^{131}I-ZnPc and ^{131}I-ZnPc-TiO_2 in EMT6 and HeLa cell lines.

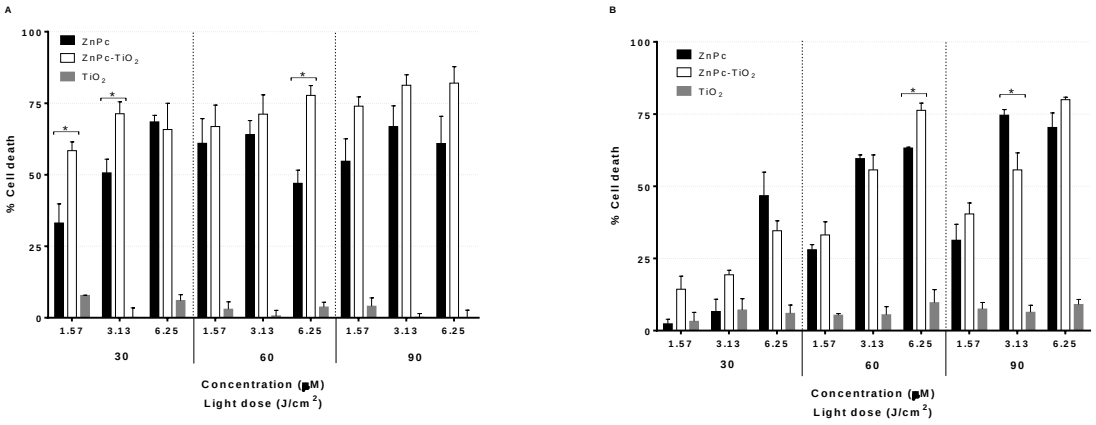
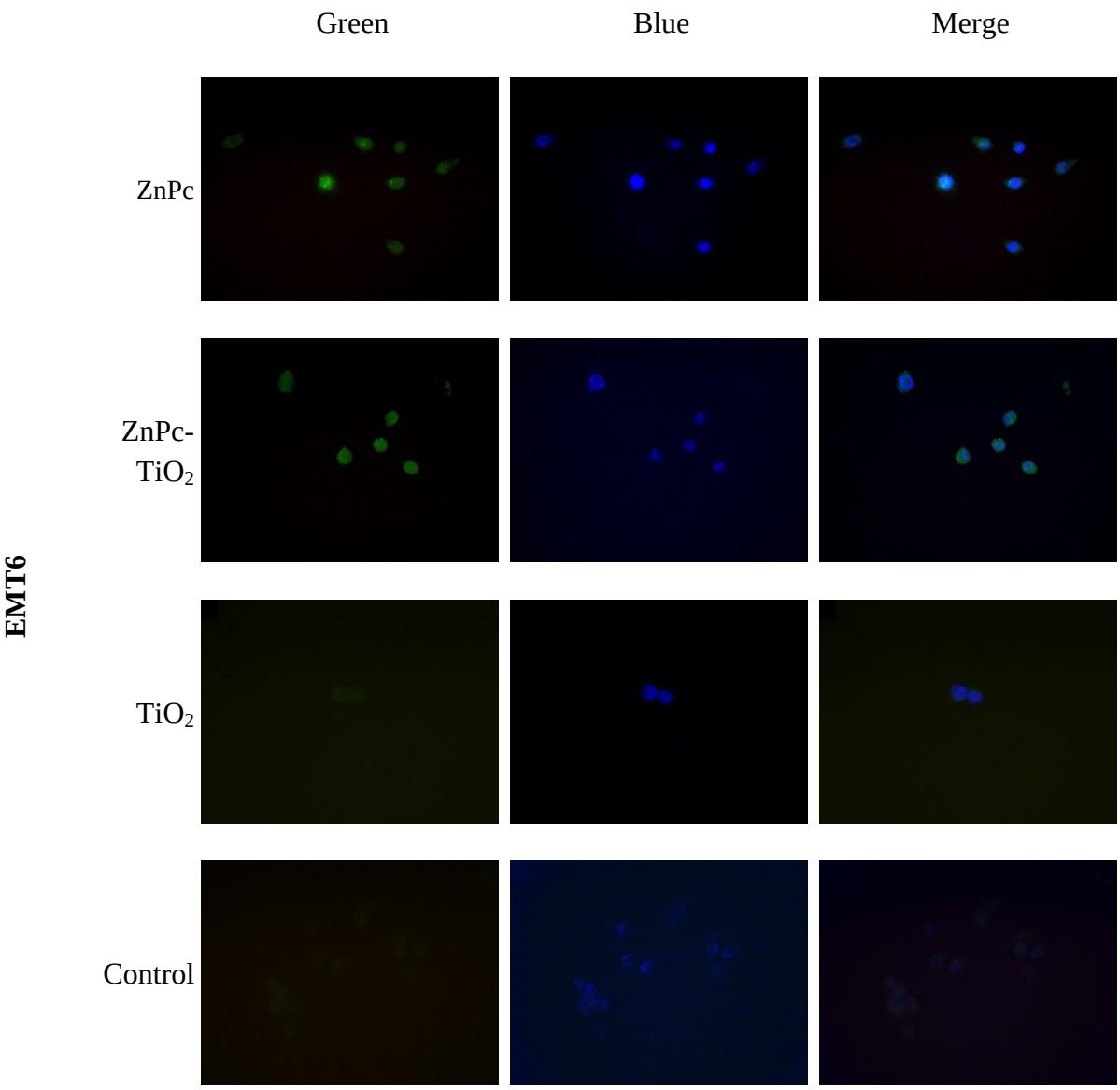
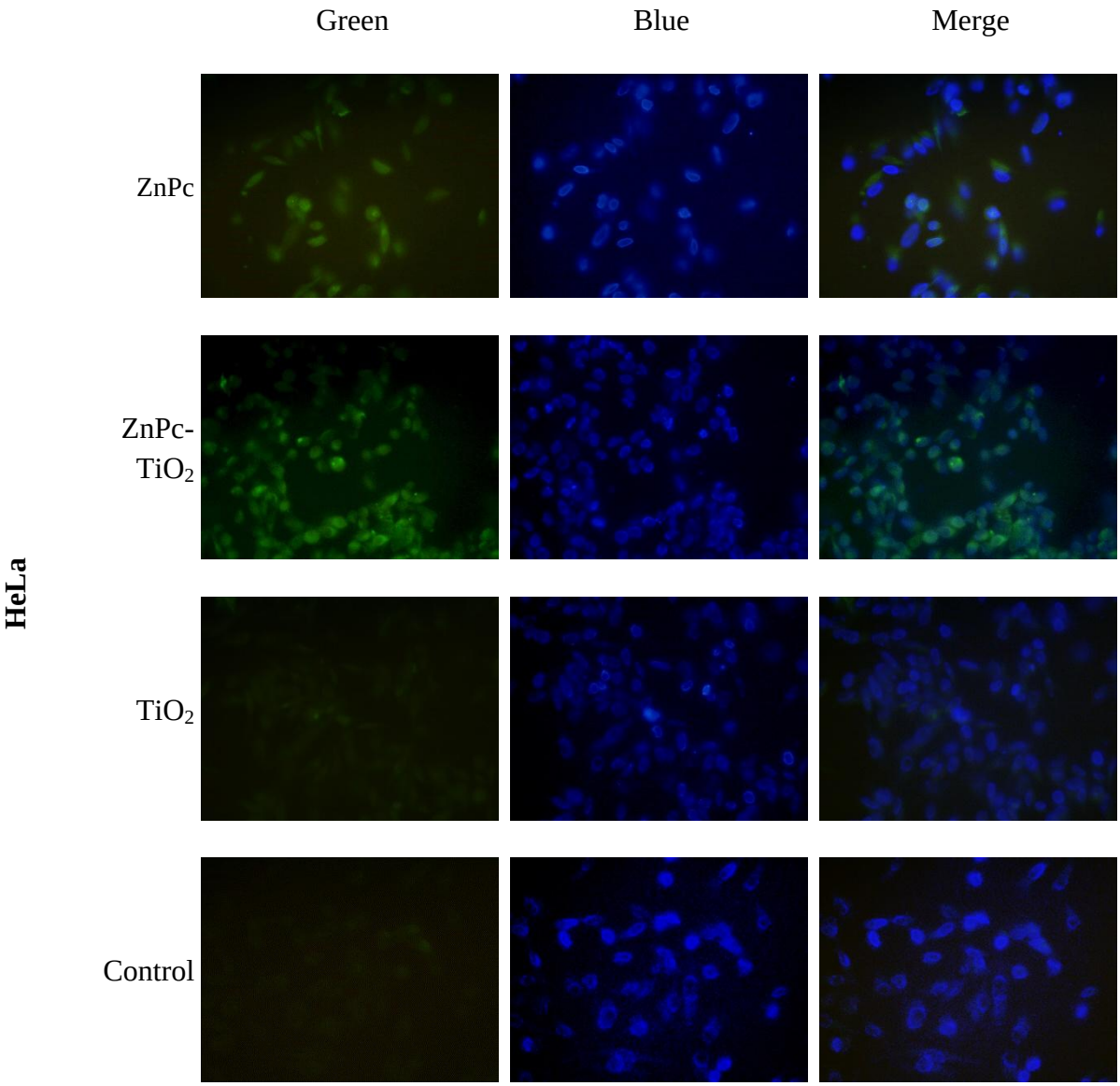


FIGURE 4. Photodynamic therapy efficiency of ZnPc, ZnPc-TiO_2 and TiO_2 in EMT6 and HeLa cell lines. PDT assays were performed with ZnPc, ZnPc-TiO_2 and TiO_2 prepared at concentrations of 1.57 μM, 3.13 μM and 6.25 μM using 30, 60 and 90 J/cm^2 light doses on EMT6 and HeLa cell lines. (A) ZnPc-TiO_2 caused significant phototoxicity at 1.57 and 3.13 μM with 30 J/cm^2 and 6.25 μM at 60 J/cm^2 in EMT6 cell lines ($p<0.001$). (B) ZnPc-TiO_2 shown significant phototoxicity at 6.25 μM with 60 J/cm^2 but ZnPc was more phototoxic at 3.13 μM with 90 J/cm^2 in HeLa cell lines ($p<0.001$).





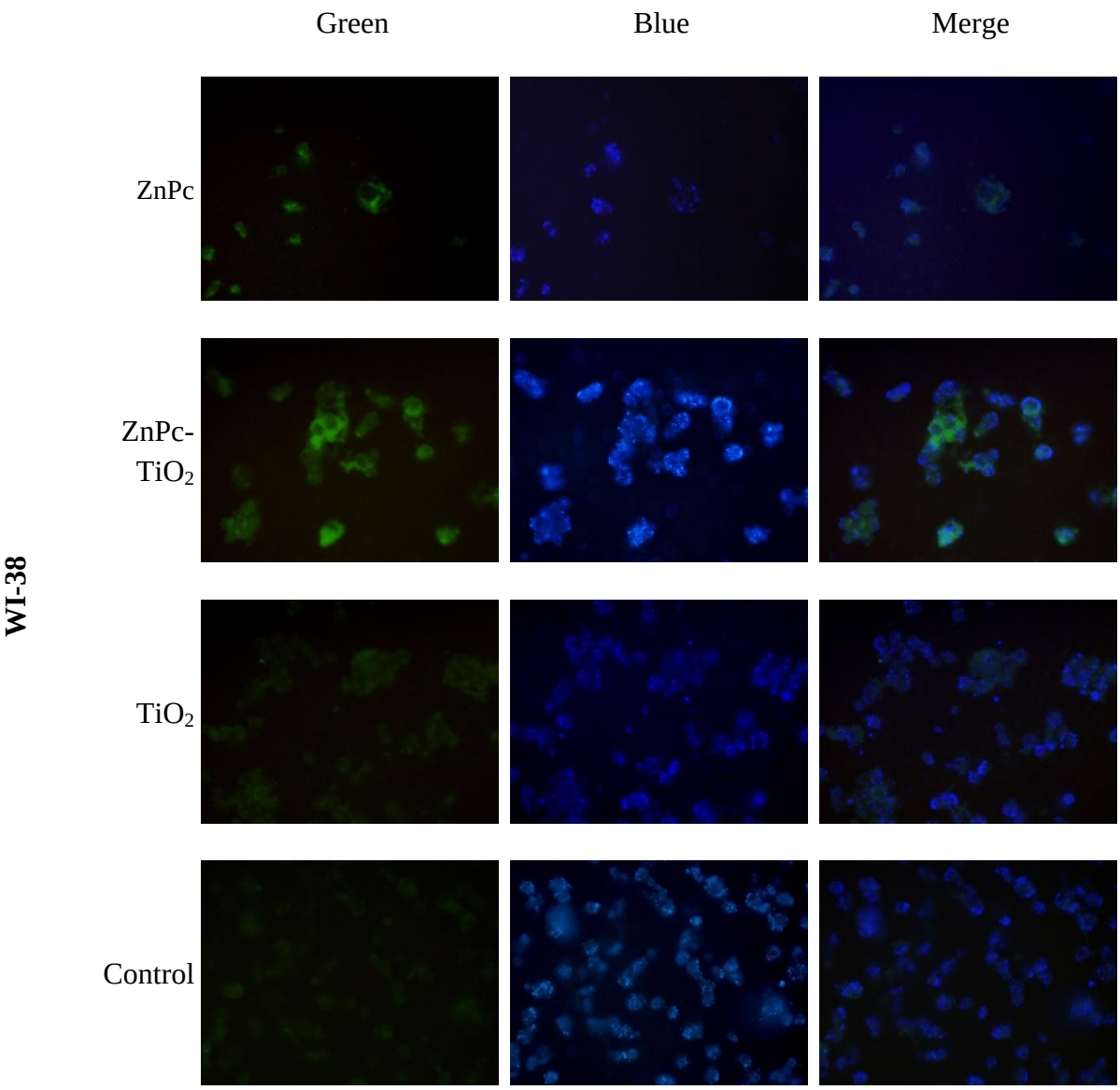


Figure 5. Fluorescence images and cellular localization of Pcs in EMT6, HeLa and WI-38 cell lines. ZnPc and ZnPc-TiO_2 is located especially in cytoplasm but not nuclei of three cell lines. TiO_2 had a reduced fluorescence intensity as in untreated control cells