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Research Paper

A Comparative Study of Photobiological and Photophysical Characteristic of Meso-Tetraphenylporphyrin and Meso-Tetraphenylchlorin as Photosensitizers for Photodynamic Therapy

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Abstract
Photosensitizer modifications are needed to improve the performance of Photodynamic Therapy (PDT) for the treatment of cancer. This work appraised the photophysical and photobiological characteristics of tetrapyrrolic macrocycles. Meso-tetraphenylporphyrin (m-TPP) and meso-tetraphenylchlorin (m-TPC) represent non-reduced and reduced pyrrole rings tetrapyrrolic macrocycles, respectively. The absorption spectra of m-TPP and m-TPC were performed. The relative quantum yield of singlet oxygen (Φ_Δ) was measured at two different pH (6.3 and 7.4) relative to chlorine e_6. Dark toxicity was monitored using the MTT assay on breast cancer T47D and NIH 3T3 cell lines. In addition, the stability of the compounds under indirect sunlight was also observed. The absorption maxima of the Q_1 band of both compounds are at a longer wavelength. The Q_1 peak of a reduced pyrrole ring is more intense than the non-reduced pyrrole ring with the lowest energy transition undergoing a redshift. The singlet oxygen generations are higher at pH 7.4 for both compounds with them-TPC showed a higher relative singlet oxygen quantum yield. In addition, the photosensitizers are stable under indirect sunlight radiation. m-TPC has higher cellular uptake than m-TPP in both cell lines. The dark toxicity of m-TPC is higher than m-TPP in T47D cells. However, the opposite occurred in observing dark toxicity in the NIH 3T3 cell line. Our study concludes that the cellular uptake and Φ_Δ can be enhanced by reducing the pyrrole ring of the tetrapyrrole macrocycle. Therefore, m-TPC proved to be a better photosensitizer than m-TPP.

Keywords
Meso-Tetraphenylporphyrin, Meso-Tetraphenylchlorin, Photodynamic Therapy, Photosensitizer, Photophysical Properties, Photobiological Properties

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1. INTRODUCTION

PDT is a method for treating cancer cells and tumors involving selective uptake of photosensitizer by the malignant cells following illumination of the light of a suitable wavelength. PDT can initiate photochemical reactions leading to the release of reactive oxygen species (ROS), primarily singlet oxygen ($^1O_2$), which ultimately results in malignant cell death (Acedo et al., 2014; Bonnett, 1995). In other ways, the destruction of cancer cells occurs after exposure to light. The mechanism results in lower side effects and toxicity compared to chemotherapy or radiotherapy (Gunaydin et al., 2021). Today, tetrapyrrolic macrocycles are receiving considerable interest as photosensitizing drugs for PDT. Several photosensitizers have received clinical approval from U.S. FDA (Food and Drug Administration) for cancer treatment. The drug includes motexafin lutetium (Lutrin® and Lutex®; Pharmacyclics Inc), temoporfin (Foscan®; Biolitec AG), palladium bacteriopheophorbide (Tookad®; Negma-Lerads), purpurins (Purlytin®), and verteporfin (Visudyne®; Novartis). These photosensitizers have a basic structure of porphyrin, chlorine, or cyanine of tetrapyrrole macrocycles (Baskaran et al., 2018). Even though a variety of tetrapyrrole macrocycles have been reported, there are only a few reports concerning the effects of reduced pyrrole ring in tetrapyrrole macrocycles (porphyrin versus chlorine) on the photophysical and photobiological properties.

A tetrapyrrolic macrocycle is an organic molecule with four pyrrole-type rings linked in a cyclic array via an atom bridge at the pyrrole rings α-positions. Some photosensitizers have strong absorption bands in the red region of the visible spectrum and, therefore, deeper penetration into the tissues (Acedo

et al., 2014). For this reason, PDT is suitable for treating deep-seated tumors. Moreover, photosensitizers (fluorophores) with stronger intense fluorescence emissions in the red region can be imaged deep within the body, showing the potential for photodynamic detection (PDD) (Fukuhara et al., 2021; Kim and Wilson, 2020; Owari et al., 2021). Therefore, it is considered necessary to explore the photophysical and photobiological effects of non-reduced pyrrole rings compared to reduced pyrrole rings of tetrapyrrolic macrocycles. An m-TPP (a non-reduced form of tetrapyrrolic macrocycle) and m-TPC (a reduced form of tetrapyrrolic macrocycle) represent a porphyrin and chlorine compound, respectively (Figure 1). The antitumor effect of m-TPP has been successfully demonstrated in human malignant cell lines (Canete et al., 1998; Ježek et al., 2003; Lovčinskỳ et al., 1999).

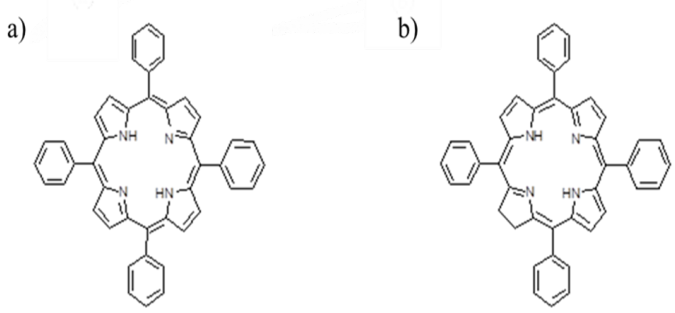

Figure 1. Chemical Structure of (a) Meso-Tetraphenylporphyrin, (b) Meso-Tetraphenylchlorin

Based on the reasons given above, the present research was conducted to obtain the correlation between reduced/non-reduced tetrapyrrolic macrocycles and photophysical/photobiological properties of photosensitizers. Here, we compared absorption spectra, relative quantum yields of singlet oxygen generation (Φ_Δ), dark toxicities, and cellular uptake of m-TPP and m-TPC. The stability of the compounds under indirect sunlight for 5 hours was also observed.

2. EXPERIMENTAL SECTION

2.1 Materials

m-TPP and m-TPC were obtained from Frontier Scientific Porphyrin Products. Absorption spectra and stability assay were measured using Shimadzu UV-2700 spectrophotometer. Singlet oxygen quantum yields were observed relative to chlorine e_6 (MedKoo) in aqueous media at pH 6.3 and 7.4 (in phosphate buffer) based on the decolorizing reaction of p-nitroso-N, N'-dimethylaniline (RNO, Sigma-Aldrich) with $^1O_2$ in the existence of imidazole (Sigma-Aldrich). T47D and NIH 3T3 cells were gained from the Laboratory of Gene Function in Animals, Nara Institute of Science and Technology, Japan. The cellular uptake of m-TPP and m-TPC were fluorometrically determined by a fluorescence spectrophotometer (Hitachi F7000). Dark toxicity was measured by observing cell viability after interacting with photosensitizer without exposure to light. The cell viability was determined using the MTT method by monitoring the absorbance using an ELISA reader (Biorad). Dulbecco's modified eagle medium (DMEM), penicillin-streptomycin, trypsin-EDTA 0.025%, fetal bovine serum (FBS), fungizone 0.5%, and fetal calf serum (FCS) were purchased from Gibco (Invitrogen, USA). Sodium dodecyl sulfate (SDS), phosphate-buffered saline (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich. All other chemicals and solvents were of analytical grade. All other solvents and chemicals were analytical grade.

2.2 Methods

2.2.1 Absorption Spectra

Several dilutions of m-TPP and m-TPC with chloroform were prepared and the absorbance values at the peak, λmax, were determined. These absorbance values should be between 0.1 and 1.0. Further, the spectra were examined to determine the extinction coefficient for each peak of the solutions. Photosensitizers were protected from light before absorbance measurement.

2.2.2 Relative Quantum Yield of Singlet Oxygen Generation (Φ_Δ)

Photosensitizer m-TPP and m-TPC induced singlet oxygen release was measured using Kraljic and El-Mohsni methods (Bose and Dube, 2008; Kraljić and Mohsni, 1978). The process indirectly evaluated singlet oxygen quantum yield by comparing singlet oxygen generated by the test compounds with the singlet oxygen generated by the standard chlorine e_6, which Φ_Δ value has already been known. In brief, a solution containing 10μM photosensitizer in 25mM Na_2HPO_4 (pH 6.3 or 7.4), RNO (A440≈0.8), and 10mM imidazole, was irradiated with visible light for different periods and at the end of each irradiation, the absorbance of the sample was evaluated at 440 nm. Analysis was conducted in two different pH values (6.3 and 7.4) (Bose and Dube, 2008).

2.2.3 Stability Measurement Under Indirect Sunlight Radiation

Stability test to indirect sunlight was conducted in chloroform as the solvent. The solutions were prepared in a dark room and then left under indirect sunlight in room conditions for 5 hours. The solutions avoid solvent evaporation. The photosensitizer absorbance was measured using a UV-Vis spectrophotometer in 30 minutes intervals.

2.2.4 Cell Culture

T47D human breast cancer cells lines were sustained in DMEM supplemented with 1% penicillin-streptomycin, 10% heat-inactivated FBS, and 0.5% fungizone. NIH 3T3 normal cells were maintained in DMEM medium containing 10% FCS. The cells were incubated in a humidified atmosphere containing 5% CO_2 at 37°C, and sub-cultured every 3-4 days with 0.025% trypsin–EDTA solution.

2.2.5 Cellular Uptake

T47D and NIH 3T3 cells ($1x10^6$ cells) were cultured in each well of 96-well plates and incubated for 24 hours. The culture medium in the plates was then substituted with a fresh culture medium. The cells were incubated with m-TPP/m-TPC (final concentration 1 μM). The solvent used was chloroform-DMSO with the final concentration of chloroform was below 0.01% and DMSO less than 1%. After the cells were washed with ice-cold PBS, they were scraped off to the bottom of the plates with 280 μL of DMSO with a cell scraper and were aspirated vigorously to obtain a single-cell suspension. Two wells of the same photosensitizer were combined and centrifuged for 20 minutes at 15,000 rpm. The amount of m-TPP/m-TPC was fluorometrically recorded (excitation wavelengths 418 nm, emission wavelength 650 nm).

2.2.6 Dark Toxicity

T47D and NIH 3T3 cells were cultured into 96-well plates (100μL/well) at densities of 10,000 cells/well and incubated for 24 hours. Subsequently, cells were washed with PBS and then 100 μL of medium containing m-TPP/m-TPC at a certain concentration, and chloroform-DMSO were added to each well, except the control wells. The cells were incubated for 24 hours.

Cell viability was conducted using the MTT assay. After incubation, the media was discarded and replaced with an MTT-containing medium (100μL) and incubated again for 4 hours at 37°C, 5% CO_2. The reaction was stopped with 10% SDS in 0.1 M HCl solution and then incubated overnight in a light-protected room to dissolve the formazan salt. The absorbance was observed with an ELISA reader at 595 nm. Cell viability was stated as the percentage of viable treated cells relative to untreated control cells.

3. RESULTS AND DISCUSSION

3.1 Absorption Spectra

m-TPP is soluble in ethyl acetate, chloroform, THF, and DMF. Moreover, m-TPC is soluble in chloroform and THF but only slightly soluble in ethyl acetate and DMF. Pyrrole ring reduction in m-TPC compound rendering it a little lipophilic when compared to m-TPP. Therefore, it was decided to study the absorption spectra of the photosensitizers in chloroform as the solvent. The absorption spectra are shown in Figure 2.

The absorption maximal of Q_1 bands, the long-wavelength band which was used to excite photosensitizers during PDT, are all at longer wavelengths (642, 649, 726 nm). The absorption spectrum of m-TPC is substantially different from m-TPP. The Q_1 band of m-TPC was more intense at 649 nm with absorptivity molar value ε= 4686 $M^{-1}cm^{-1}$. In addition, there is another peak at 724 nm.

The absorption spectra determined for m-TPP are in agreement with the results reported by Roeder et al. (1990). However, a different solvent was used, causing some shift in the UV-Vis band maxima. As clearly presented in Figure 2, the chlorine compounds, which are the reduced form of porphyrins, show

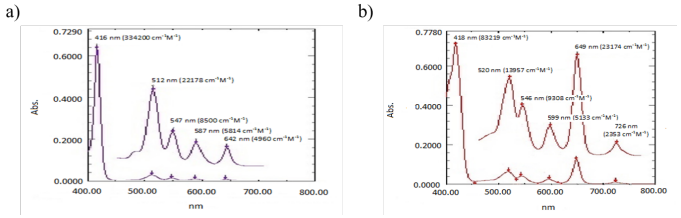

Figure 2. UV-Vis Band Maxima and Molar Absorptivities for (a) m-TPP (b) m-TPC in Chloroform, λmax nm (ε=M^{-1} cm^{-1}). The Inset in The Upper Shows an Expanded View of The Q-Band Region

better likely since they have an intensive absorption band at a relatively high wavelength where tissues are seen more transparent, indicating high phototoxicity. The high wavelength will impact the deeper tissue penetration, so it can be prospective to treat cancer in deeper tissues (e.g., large tumors). Meanwhile, photosensitizers with an intense absorption spectrum (significant extinction coefficient) can be used in small doses.

Concerning partition coefficient, the photosensitizer should be amphiphilic. The lipophilic characteristic will assist in entering the membrane, while the polar feature will aid the solubilization for injection. m-TPP and m-TPC tend to be lipophilic, so the photosensitizers need additional components to transport. Examples of such systems include cyclodextrins, cremophor EL, liposomes, and lipoproteins (Mojzisova et al., 2007).

3.2 Relative Quantum Yield of Singlet Oxygen Generation

The process of excitation of a photosensitizer in PDT will generate highly reactive and cytotoxic species of singlet oxygen resulting in cell death. Accordingly, the relative quantum yield of singlet oxygen generation is an important parameter of a photosensitizer's performance. To generate singlet oxygen, the photosensitizer is irradiated with a mercury lamp. There were three mercury lamps used (@160 watt, Philips) with total intensity of 5-6mW/cm^2. These lamps can emit light at 300-900 nm.

The relative quantum yield of singlet oxygen is indirectly determined compared with the known quantum yield comparative solution (chlorine e_6). This method is established for singlet oxygen in aqueous media (Kraljić and Mohsni, 1978). Due to the limited solubility of m-TPP and m-TPC in water, the first dissolution was carried out in tetrahydrofuran. The evaluation was performed in two different pH values. pH 7.4 represents a physiological solution, and pH 6.3 was chosen as the pH of cancer cells is a little acidic (Bose and Dube, 2008).

RNO absorbance data of the test compounds are made in graphics of the decrease in absorbance (–ln A) vs the time of irradiation (Figure 3). The concentration for the test solutions used was 1μM. In this concentration, the absorbance of the samples at 440 nm was less than 0.1 which will prevent the shielding effect (Bose and Dube, 2008). From the graphic of irradiation period vs –ln A, it was obtained the gradient values for pH 6.3 and 7.4, which were then used to calculate Φ_Δ as

seen in Table 1.

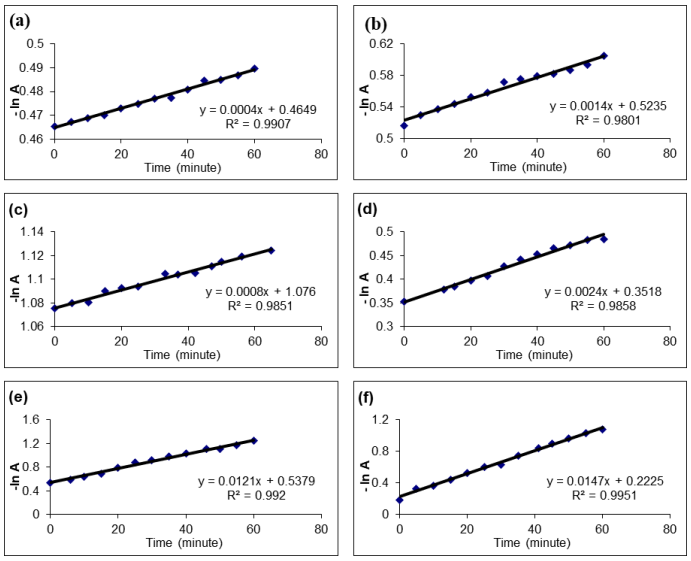

Figure 3. Influence of The Time of Irradiation on The Bleaching of p-Nitroso Dimethyl Aniline in The Imidazole-m-TPP /m-TPC/Chlorine e_6 System at 440 nm (a) m-TPP, pH 6.3, (b) m-TPP, pH 7.4, (c) m-TPC, pH 6.3, (d) m-TPC pH 7.4, (e) Chlorine e_6, pH 6.3, (f) Chlorine e_6, pH 7.4

m-TPP and m-TPC were able of producing singlet oxygens when irradiated with UV-A/ visible light in the existence of molecular oxygen. The relative quantum yield of singlet oxygen generation for chlorine e_6 Φ_Δ=0.5 and 0.6 for pH 6.3 and 7.4, respectively (Parkhats et al., 2009). These values may be used as standards to determine the quantum yield of singlet oxygen for m-TPP in an aqueous solution. Generally, the velocity of RNO bleaching both in m-TPP and m-TPC is slower than in chlorine e_6. The quantum yield of the singlet oxygen assay that was conducted in an aqueous medium probably has some contribution to the low value of Φ_Δ produced for both m-TPP and m-TPC compared to chlorine e_6. Chlorine e_6 solubility is higher than m-TPP/m-TPC in the aqueous medium. From the data obtained, m-TPC exhibits higher singlet oxygen quantum yield than m-TPP. These results indicate that reduction in pyrrole ring increases the relative quantum yield of singlet oxygen.

Our in silico previous study agreed with this singlet oxygen generation report (Djalil et al., 2016). The docking study showed that m-TPC interacted better at HSA (human serum albumin) and PBR (benzodiazepine receptor) receptors than m-TPP. Meanwhile, QSAR analysis observed that m-TPC was more potent as a photosensitizer on HCT116 human colon adenocarcinoma cells, as indicated by a lower IC50 value.

Further, singlet oxygen production was higher at higher pH (7.4) than at lower pH (6.3). Roeder et al. (1990) calculated the value of absolute singlet oxygen quantum yield of m-TPP in CCl_4 as 0.7. Other researchers reported the value for singlet oxygen quantum yield of m-TPP as 0.73 by using 1,3-diphenylisobenzofuran (DPBF) as quencher compounds (Spiller et al., 1998). These relatively high values may be due to the low solubility of m-TPP or m-TPC in aqueous solution and their tendency to remain slightly aggregated. Kraljic and Mohsni method is more proper for the detection of relative singlet oxygen in an aqueous solution. Therefore, it is decided to use THF and phosphate-buffered solution as the solvent to attain monomerization. Furthermore, this method can show the influence of pyrrole ring reduction on the capability of photosensitizers to produce singlet oxygen. The photophysical characteristics of the photosensitizers were further used to discuss antitumoral photoactivity.

The research group from Germany observed environmental acidity conditions on the performance of photosensitizers in producing singlet oxygen (Radunz et al., 2020). They found that the pKa value of the photosensitizer contributed to the generation of singlet oxygen and the acid-base equilibrium reaction in the cell. Photosensitizers with pKa values at slightly acidic cancer cell pH exhibit increased intersystem crossing (ISC) and hence on-switched singlet oxygen formation at lower pH.

3.3 Stability Measurement Under Indirect Sunlight Radiation

Stability assay was investigated under indirect sunlight for 5 hours (Figure 4). The experiment was conducted by observing the change in the absorption spectrum. The detection wavelength used for monitoring the stability was λ = 642 nm for m-TPP and 726 nm for m-TPC. The results showed that the photostability of m-TPC was higher than m-TPP. When a solution of m-TPC was exposed to indirect sunlight, it bleached faster than m-TPP solution. The absorbances of m-TPP started to decrease significantly at 120 minutes, while m-TPC at 30 minutes. After leaving this solution for 5 hours, the m-TPC and m-TPP decreased to 25.90% and 17.5%, respectively. In other words, tetrapyrrole macrocycles with non-reduced pyrrole ring bleach slower than tetrapyrrole macrocycles with reduced pyrrole ring.

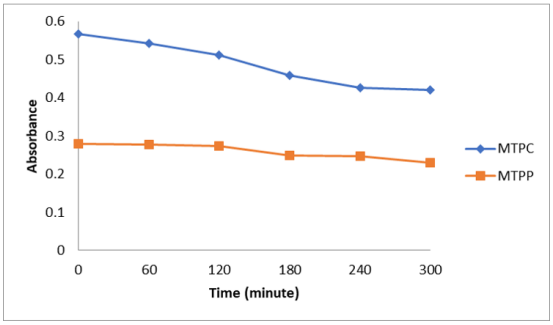

Figure 4. Stability Assay for m-TPP and m-TPC Under Indirect Sunlight for 5 Hours

3.4 Cellular Uptake

As previously shown in Figure 2, m-TPC showed a better absorption-type spectrum for PDT due to the possibility of

Table 1. Quantum Yield of Singlet Oxygen (Φ_Δ) of m-TPP and m-TPC (pH 6.3 and 7.4)

Compounds	pH 6.3 Gradient	Φ_Δ	pH 7.4 Gradient	Φ_Δ
Chlorine e_6	0.0098	0.50*	0.011	0.60*
m-TPP	0.0022±0.0002	0.1139	0.0032±0.0001	0.1781
m-TPC	0.0045±0.0002	0.2312	0.0052±0.0001	0.2872

*Parkhats et al. (2009)

light reaching deeper tumors. The PDT effect was obtained after irradiating the photosensitizer with a specific light because of a singlet oxygen generation. Since the short migration distance (less than 0.02 μm) and short lifetime (54-86 milliseconds) of singlet oxygen (Krumova and Cosa, 2016), a higher amount of cellular uptake is a crucial parameter before irradiation is applied.

Physicochemical properties of particles such as size, shape, charge, and surface chemistry are addressed as several factors that affect cellular uptake (Foroozandeh and Aziz, 2018). The current popular topic of cellular uptake mainly refers to the use of nanoparticles for drug delivery. In this research, we didn't observe the particle size of the photosensitizer. However, we proved that both photosensitizers could penetrate cells based on the data.

The photosensitizers studied are of the same size. The molecule's shape is slightly different due to the difference in reduction on the pyrrole ring. The difference in structure significantly affects the photosensitizer's ability to penetrate the cell membrane.

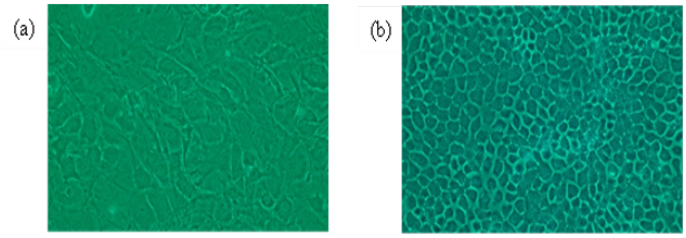

Figure 5. Phase Contrast Images of (a) NIH 3T3 Cells, (b) T47D Cell Lines (Under Green Light at 20-Fold Magnification)

In this study, we used T47D cancer cells and NIH 3T3 normal cells (Figure 5). m-TPP and m-TPC can enter cells well, as shown in Figure 6. The mechanisms of PDT in cells are complex and depend upon several factors. Photosensitizer uptake by tumor cells is vital for effective PDT. Osaki et al. (2006) had shown a negative correlation between cell survival and sensitizer uptake.

Cellular uptake assay (Table 2) showed that m-TPC has higher cellular uptake than m-TPP in the T47D cancer cells and NIH 3T3 normal cells. m-TPC had a stronger lipophilic character compared to m-TPP to enter the membrane. Polarity prediction using ECOSAR software showed that m-TPP dan

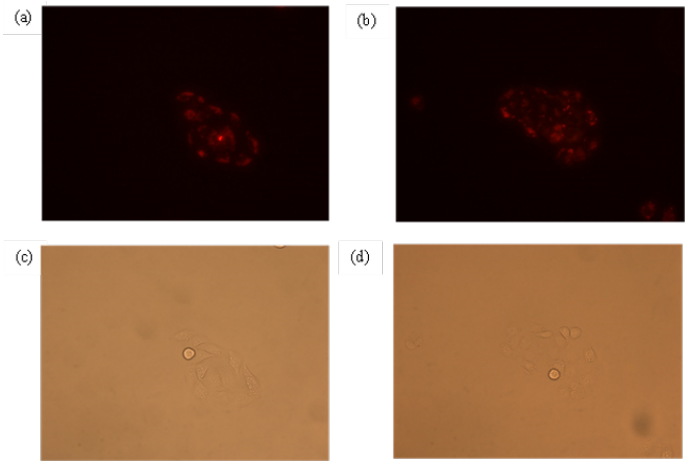

Figure 6. Fluorescence Microscopy Images of Intracellular Localization of (a) m-TPP, (b) m-TPC. Microscopy Images without Fluorescent Light of (c) m-TPP, (d) m-TPC at 1μM Concentration in Human Cancer Cell Lines of T47D

m-TPC have log Kow values of 11.46 and 11.68, respectively. Further, cellular uptake of photosensitizer was better in cancer cells than in normal cells.

Table 2. Photosensitizer Cellular Uptake Assay

Photosensitizer	Cell Lines	Cellular Uptake (%)
m-TPP	T47D	7.59
m-TPP	NIH 3T3	4.81
m-TPP	T47D	15.39
m-TPP	NIH 3T3	9.05

In addition to a photosensitizer, cellular uptake was also influenced by the cell size; the bigger the cell size, the more photosensitizers that could be taken (Osaki et al., 2006). Cellular uptake was also influenced by microenvironmental factors and cell characteristics, such as the state of proliferation and the density of lipoprotein receptors (Dougherty et al., 1998; Reddi, 1997). As reported in the previous research, higher peripheral benzodiazepine receptor accumulation in cancer cells causes more tetrapyrrole macrocycles compounds that could be accumulated when compared to normal cells (Chen et al., 2011).

3.5 Dark Toxicity

One of the desirable properties of photosensitizers is high phototoxicity with minimal or no dark toxicity (in the absence of lights). Further, tumor damage can be controlled by the light dose for a given drug dose. None of the photosensitizers, m-TPP, and m-TPC, displayed any significant dark toxicity at a concentration of <3μM (Figure 7). In contrast, doxorubicin showed significant dark toxicity with cell viability decrease up to 3.7% in 5 μM concentration in NIH 3T3 cells.

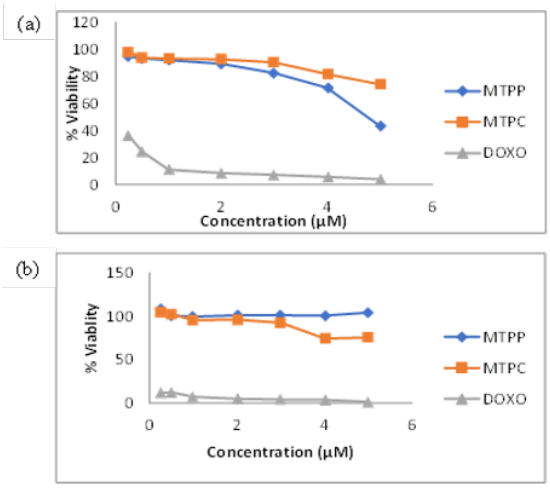

Figure 7. Dark Toxicity of m-TPP, m-TPC, and Doxorubicin to (a) NIH 3T3 Cells, (b) T47D Cancer Cells

m-TPP and m-TPC have similar values of dark toxicity. The dark toxicity of m-TPC is slightly higher than m-TPP in T47D cancer cells. The dark toxicity of m-TPP, on the other hand, is slightly higher than m-TPC in NIH 3T3 normal cells. The result of the dark toxicity test showed the correlation between dark toxicity and cellular uptake. In contrast, previous research showed that chlorine compounds had lower dark toxicities in T47D cells compared to porphyrin compounds (Djalil et al., 2012b). However, the compounds compared were not from homolog structures. Instead, in silico studies of compounds with two reduced pyrrole rings (pheophorbide a) showed higher toxicity to fish than compounds with one reduced pyrrole ring (bacteriopheophorbide a) (LC50 0.109 vs. 0.119 mg/L). This study concluded that reducing the pyrrole ring can decrease the compound's toxicity in fish under non-irradiated conditions (Djalil et al., 2012a).

The solvent used in the dark toxicity assay was a mixture of DMSO and $CHCl_3$, with the final concentration of DMSO less than 1% and chloroform below 0.01%. DMSO was relatively non-toxic to the cells compared to chloroform, but unfortunately, DMSO was not able to dissolve the test compounds completely. Chloroform, on the other hand, can well dissolve both m-TPP and m-TPC. However, chloroform is toxic to cells in a concentration of >0.10%. Accordingly, this mixture of solvents may increase the solubility of test compounds and be relatively non-toxic to the cells.

In the present study, doxorubicin was used as the positive control. Doxorubicin is a medication generally used in cancer chemotherapy. It is commonly used in the treatment of a wide range of cancers, including hematological malignancies, many types of carcinoma (solid tumors), and soft tissue sarcomas. Doxorubicin has higher cytotoxicity in T47D cells compared to NIH 3T3 cells (Figure 5). In T47D cells, the percent viability was reaching 11.8% for the lowest concentration (0.25μM), while for the highest concentration (5μM) the percent viability was only 1.3%.

4. CONCLUSIONS

Under experimental conditions, m-TPC showed a better photosensitizer than m-TPP. Advantageous characteristics as a result of its stronger absorptivity in the red spectrum area, higher yield of singlet oxygen quantum, higher cellular uptake, and lower dark toxicity in NIH 3T3 cells. This study concludes that photobiological/photophysical characteristics of photosensitizers may improve by reducing the pyrrole ring of tetrapyrrole macrocycles.

5. ACKNOWLEDGMENT

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REFERENCES

Acedo, P., J. Stockert, M. Cañete, and A. Villanueva (2014). Two Combined Photosensitizers: a Goal for More Effective Photodynamic Therapy of Cancer. *Cell Death & Disease*, **5**(3); 1–12

Baskaran, R., J. Lee, and S. G. Yang (2018). Clinical Development of Photodynamic Agents and Therapeutic Applications. *Biomaterials Research*, **22**(1); 1–8

Bonnett, R. (1995). Photosensitizers of The Porphyrin and Phthalocyanine Series for Photodynamic Therapy. *Chemical Society Reviews*, **24**(1); 19–33

Bose, B. and A. Dube (2008). Photodynamic Efficacy of Chlorine p_6: a pH Dependent Study in Aqueous and Lipid Environment. *Journal of Photochemistry and Photobiology B: Biology*, **93**(1); 32–35

Canete, M., A. Villanueva, V. Dominguez, S. Polo, A. Juarranz, and J. Stockert (1998). Meso-Tetraphenylporphyrin: Photosensitizing Properties and Cytotoxic Effects on Cultured Tumor Cells. *International Journal of Oncology*, **13**(3); 497–1001

Chen, Y., M. Sajjad, Y. Wang, C. Batt, H. A. Nabi, and R. K. Pandey (2011). TSPO 18 kDa (PBR) Targeted Photosensitizers for Cancer Imaging (PET) and PDT. *ACS Medicinal Chemistry Letters*, **2**(2); 136–141

Djalil, A., N. Nurulita, L. W. Limantara, S. Ibrahim, and D. H. Tjahjono (2012a). Biological Evaluations of Protoporphyrin

IX, Pheophorbide a, and its 1-Hydroxyethyl Derivativess for Aplication in Photodynamic Therapy. *International Journal of Pharmacy and Pharmaceutical Sciences*, **4**(3); 741–746

Djalil, A. D., N. Fadhilah, D. Saputri, S. Suparman, and A. Hamad (2016). Hubungan Kuantitatif Struktur-Aktifitas dan Doking Molekular Senyawa Meso-Tetraphenylporphyrin dan Meso-Tetraphenylchlorin sebagai Fotosensitizer untuk Terapi Fotodinamik. *Jurnal Pharmascience*, **3**(1); 19–31 (in Indonesia)

Djalil, A. D., R. E. Kartasasmita, I. Slamet, and H. T. Daryono (2012b). Toxicity Prediction of Photosensitizers Bearing Carboxylic Acid Groups by ECOSAR and Toxtree. *Journal of Pharmacology and Toxicology*, **7**(5); 219–230

Dougherty, T. J., C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, and Q. Peng (1998). Photodynamic Therapy. *Journal of The National Cancer Institute*, **90**(12); 889–905

Foroozandeh, P. and A. A. Aziz (2018). Insight Into Cellular Uptake and Intracellular Trafficking of Nanoparticles. *Nanoscale Research Letters*, **13**(1); 1–12

Fukuhara, H., S. Yamamoto, T. Karashima, and K. Inoue (2021). Photodynamic Diagnosis and Therapy for Urothelial Carcinoma and Prostate Cancer: New Imaging Technology and Therapy. *International Journal of Clinical Oncology*, **26**(1); 18–25

Gunaydin, G., M. E. Gedik, and S. Ayan (2021). Photodynamic Therapy for The Treatment and Diagnosis of Cancer-A Review of The Current Clinical Status. *Frontiers in Chemistry*, **9**; 608

Ježek, P., M. Nekvasil, E. Škobisová, E. Urbánková, M. Jirsa, M. Zadinová, P. Poučková, and I. Klepáček (2003). Experimental Photodynamic Therapy with Meso-Tetrakisphenylporphyrin (TPP) in Liposomes Leads to Disintegration of Human Amelanotic Melanoma Implanted to Nude Mice. *International Journal of Cancer*, **103**(5); 693–702

Kim, H. I. and B. C. Wilson (2020). Photodynamic Diagnosis and Therapy for Peritoneal Carcinomatosis from Gastrointestinal Cancers: Status, Opportunities, and Challenges. *Journal of Gastric Cancer*, **20**(4); 355

Kraljić, I. and S. E. Mohsni (1978). A New Method for The Detection of Singlet Oxygen in Aqueous Solutions. *Photochemistry and Photobiology*, **28**(4-5); 577–581

Krumova, K. and G. Cosa (2016). *Overview of Reactive Oxygen Species*, chapter Singlet Oxygen: Applications in Biosciences and Nanosciences, Volume 1. Royal Society of Chemistry

Lovčinskỳ, M., J. Boreckỳ, P. Kubát, and P. Ježek (1999). Meso-Tetraphenylporphyrin in Liposomes as a Suitable Photosenzitizer for Photodynamic Therapy of Tumors. *Gen Physiol Biophys*, **18**(2); 107–118

Mojzisova, H., S. Bonneau, and D. Brault (2007). Structural and Physico-Chemical Determinants of The Interactions of Macrocyclic Photosensitizers with Cells. *European Biophysics Journal*, **36**(8); 943–953

Osaki, T., S. Takagi, Y. Hoshino, M. Okumura, and T. Fujinaga (2006). Intracellular Localization and Concentration as well as Photodynamic Effects of Benzoporphyrin Derivative Monoacid Ring A in Four Types of Rodent Tumor Cells. *Cancer Letters*, **243**(2); 281–292

Owari, T., T. Iwamoto, S. Anai, M. Miyake, Y. Nakai, S. Hori, T. Hara, T. Ishii, U. Ota, and K. Torimoto (2021). The Sustaining of Fluorescence in Photodynamic Diagnosis after The Administration of 5-Aminolevulinic Acid in Carcinogen-Induced Bladder Cancer Orthotopic Rat Model and Urothelial Cancer Cell Lines. *Photodiagnosis and Photodynamic Therapy*, **34**; 102309

Parkhats, M., V. Galievsky, A. Stashevsky, T. Trukhacheva, and B. Dzhagarov (2009). Dynamics and Efficiency of The Photosensitized Singlet Oxygen Formation by Chlorine e_6: The Effects of The Solution pH and Polyvinylpyrrolidone. *Optics and Spectroscopy*, **107**(6); 974–980

Radunz, S., S. Wedepohl, M. Roohr, M. Calderon, H. R. Tschiche, and U. Resch-Genger (2020). pH-Activatable Singlet Oxygen-Generating Boron-dipyrromethenes (BODIPYs) for Photodynamic Therapy and Bioimaging. *Journal of Medicinal Chemistry*, **63**(4); 1699–1708

Reddi, E. (1997). Role of Delivery Vehicles for Photosensitizers in The Photodynamic Therapy of Tumours. *Journal of Photochemistry and Photobiology B: Biology*, **37**(3); 189–195

Roeder, B., D. Naether, T. Lewald, M. Braune, C. Nowak, and W. Freyer (1990). Photophysical Properties and Photodynamic Activity in Vivo of Some Tetrapyrroles. *Biophysical Chemistry*, **35**(2-3); 303–312

Spiller, W., H. Kliesch, D. Wöhrle, S. Hackbarth, B. Röder, and G. Schnurpfeil (1998). Singlet Oxygen Quantum Yields of Different Photosensitizers in Polar Solvents and Micellar Solutions. *Journal of Porphyrins and Phthalocyanines*, **02**(02); 145–158

