Synthesis and Photodynamic Activity of 5,10,15-Tris(*p*-chlorophenyl)-20-(2-hydroxy-3-methoxyphenyl)-21H,23H-porphyrin

Eder Arredondo-Espinoza, Susana López-Cortina, Isaías Balderas-Rentería*

Universidad Autónoma de Nuevo León, Laboratorio de Ingeniería Genética y Genómica, Facultad de Ciencias Químicas, Av. Universidad s/n, Cd. Universitaria, 66451 San Nicolás de los Garza, N.L., México. isaias.balderasrn@uanl.edu.mx.

Abstract: Photodynamic therapy (PDT) is an effective option for the treatment of solid neoplastic tumors, as well as non-malignant diseases such as dermatological, ophthalmological and benign pathologies. PDT destroys cancer cells by photochemical generation of reactive oxygen species upon absorption of visible light by a photosensitizing agent (PS). Here, we describe the synthesis and characterization of the unsymmetrical A_3B porphyrin 5,10,15-Tris(*p*-chlorophenyl)-20-(2-hydroxy-3-methoxyphenyl)-21H,23H-porphyrin, which presents different groups at the *meso* positions, *p*-chlorophenyl and 2-hydroxy-3-methoxyphenyl and is a novel target molecule in order to evaluate the synergistic effect of these hydrophobic and hydrophilic moieties distributed unsymmetrically on the porphyrin macrocycle. We demonstrated the *in vitro* cytotoxic effect of the synthesized porphyrin, by the WST-1 assay on a cultured human cervical adenocarcinoma cell line (HeLa ATCC: CCL-2), as well as the presence of apoptosis by T.U.N.E.L. assay. We compared the phototoxicity and intrinsic cytotoxicity of the PS and found a considerable difference in the IC_{50} between phototoxic activity (1.5 μM) and intrinsic cytotoxicity (7 μM). The results of the induction of apoptosis on HeLa cells treated with the unsymmetrical porphyrin at a final concentration of 80 μM showed 15% apoptotic cells, whereas the untreated HeLa cells had only 2.5% apoptotic cells.

Keywords: Photodynamic therapy, photosensitizer, unsymmetrical porphyrins, apoptosis, anti-cancer agents.

Resumen: La terapia fotodinámica (TFD) es una alternativa terapéutica efectiva en el tratamiento de tumores sólidos así como otras enfermedades benignas o de origen dermatológico u oftalmológico. La TFD elimina las células neoplásicas mediante la formación de especies reactivas de oxígeno generadas fotoquímicamente tras la absorción de luz visible por un agente fotosensibilizador. En el presente trabajo, se describe la síntesis y la caracterización de la 5,10,15-Tris(*p*-clorofenil)-20-(2-hidroxi-3-metoxifenil)-21H,23H-porfirina, porfirina no simétrica del tipo A_3B con diferentes grupos en las posiciones *meso*, *p*-clorofenilo y 2-hidroxi-3-metoxifenilo, obteniendo de esta forma una molécula estructuralmente novedosa para evaluar el efecto sinérgico de los grupos hidrofóbicos e hidrofílicos con una distribución no simétrica en el macrociclo de la porfirina. Se demuestra además, el efecto citotóxico *in vitro* mediante el ensayo de WST-1 en una línea de cáncer cervicouterino (HeLa ATCC: CCL-2), así como la inducción de apoptosis por medio del ensayo de T.U.N.E.L. Se comparó la fototoxicidad y citotoxicidad intrínseca de la porfirina no simétrica y se encontró una diferencia considerable en el IC_{50} entre la actividad fototóxica (1.5 μM) y la citotoxicidad intrínseca (7 μM). Los resultados de la inducción de la apoptosis en las células HeLa tratadas con la porfirina no simétrica a una concentración final de 80 μM, mostraron 15 % de células apoptóticas, mientras que las células HeLa no tratadas mostraron sólo 2.5% de apoptosis.

Palabras clave: Terapia fotodinámica, fotosensibilizadores, porfirinas no simétricas, apoptosis, agentes anticancerígenos.

Introduction

Photodynamic therapy (PDT) is based on the systemic administration of photosensitizing agents (PSs), followed by selective irradiation at an appropriate wavelength. Upon excitation of the PS, which interacts with molecular oxygen to produce singlet oxygen ($^1O_2$) and other reactive oxygen species, PDT is attractive as cancer treatment, because it has minimal side effects due to its preferential distribution of the PSs in tumors compared with normal tissues and to the delimited light irradiation around the tumor [1-3]. Porphyrins, one of the most popular PSs, are aromatic macrocycles formed by four pyrrole rings joined together by interpyrrolic methine bridges; the macrocycle contains a total of 22 π electrons with 18 π electrons in direct conjugation. Porphyrins are interesting molecules that play key roles in many biological processes (oxygen transport, electron transfer and oxidation catalysis) [4-6].

The singlet oxygen can directly destroy tumor cells by induction of necrosis and apoptosis and it can also cause the destruction of tumor microvasculature and produce a significant inflammatory response that attracts leukocytes such as neutrophils and macrophages [7-9]. Due to the short life time

of $^1O_2$ (<0.04 microseconds) and its limited range of action (<0.02 μm), the tissue damage is closely related to the location of the PSs. Most photosensitizers are selectively localized intracellularly in the lysosomes, membranes and mitochondria. In particular, injury to mitochondria is one of the most significant forms of damage in photodynamic therapy that may cause programmed cell death (apoptosis), allowing the release of cytochrome C and other mitochondrial factors into the cytosol that is also known to trigger apoptosis [10]. Changes induced in the plasma membrane and membranes of cellular organelles by photodynamic therapy can trigger far-reaching effects [11].

The mechanisms by which PSs are preferentially distributed to cancer cells are not fully understood. It is believed that the properties of tumor tissue can contribute to this selectivity, including the slightly acidic interstitial fluid due to the presence of lactic acid, the abnormal structure of tumor stroma characterized by a large interstitial space, cracking of the vascular system, lymphatic drainage being engaged, a large amount of collagen (which can bind PSs) and lipids [11, 12].

Since 1978, hematoporphyrin derivatives have been used as cancer therapy for skin tumors [13]; after 10 years, McCaughan [14, 15] extended its application to neoplasia in the

esophagus, head, neck, prostate and breast, among others. One decade later, Photofrin®, a combination of dihematoporphyrin esters, was approved in Canada to treat bladder cancer. Three years later, Photofrin® was approved in the United States to treat obstructive esophagus neoplasia. Disadvantages of this kind of PSs include its high skin photosensitivity, low selectivity and reduced phototoxic activity. Second-generation PSs have arisen with improved selectivity, higher cytotoxic effects and lower skin photosensitivity. Nowadays, it is known that localization and nature of substituents in the β and *meso* position on porphyrins are effective in their spectral, photophysical and biological characteristics [16]. In the present study, we describe the synthesis, characterization, phototoxic effects and intrinsic cytotoxicity of a unsymmetrical A₃B porphyrin, 5,10,15-Tris(*p*-chlorophenyl)-20-(2-hydroxy-3-methoxyphenyl)-21H,23H-porphyrin, as well as the induction of apoptosis after treatment with this second-generation PS. This kind of new PS exhibits less skin photosensitivity, chemically purity, exceptional stability and the ability of absorb light around 650 nm. The porphyrin structure synthesized in this work has different groups in the *meso* position, in order to evaluate the synergistic effect on the phototoxicity, cellular uptake and cellular localization of hydrophobic and hydrophilic groups in asymmetric position on the porphyrin macrocycle. Phototoxicity, intrinsic cytotoxicity and induction of apoptosis were determined in HeLa cells.

Results and Discussion

The compound 5,10,15-Tris(*p*-chlorophenyl)-20-(2-hydroxy-3-methoxyphenyl)-21H,23H-porphyrin was synthesized based on the assumption that *meso*-substituted porphyrins bearing different groups at the phenyls and a unsymmetric distribution of these groups would affect the hydrophobic/hydrophilic character of the PSs, a feature that is crucial for cell penetration and subcellular localization [17]. Previous report compared the cytotoxic activity of porphyrins substituted at *meso* position with hydroxyl and methoxyl groups, showing better results in hydroxy *meso*-substituted porphyrins, perhaps methoxy-substituted porphyrins also yielded good results [18]. Additionally, halogenated tetrapyrrolic compounds have been considered as potential photosensitizers and their structure also present other groups on *meso* positions allowing for an amphiphilic character [19, 20]. The structure of the synthesized porphyrin presents different groups at the *meso* position, *p*-chlorophenyl and 2-hydroxy-3-methoxyphenyl and is a novel target molecule to evaluate the synergistic effect of these hydrophobic and hydrophilic moieties distributed unsymmetrically on the porphyrin macrocycle.

The synthesis of the unsymmetrical porphyrin **1** (Scheme 1), was accomplished following the procedure described by Lindsey and co-workers [21] via acid-catalyzed condensation of pyrrole with two aromatic aldehydes (4-chlorobenzaldehyde and *o*-vainillin). After the first step, the porphyrinogen intermediate was formed and then oxidized to porphyrin **1**
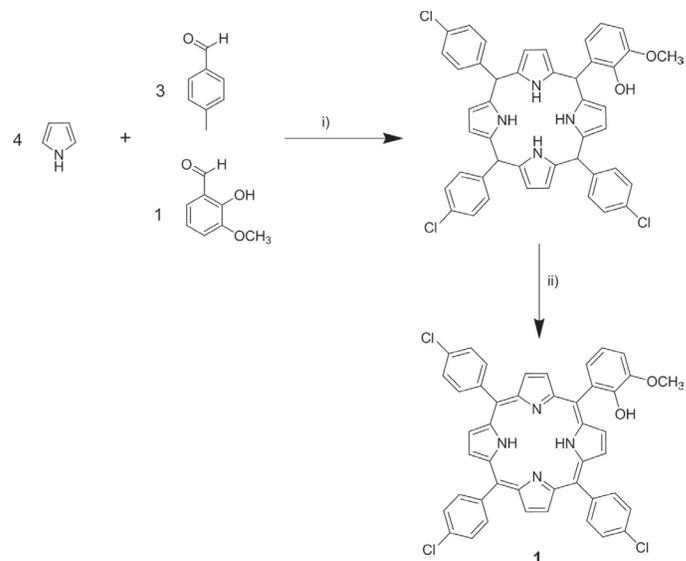
Scheme 1. Synthesis of unsymmetrical porphyrin. Reaction conditions: i) TFA/ BF₃•O(Et)₂, CH₂Cl₂, rt, 2 h; ii) DDQ, rt, 2 h.

with 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The purification of this reaction mixture by column chromatography allowed us to obtain a new unsymmetrical porphyrin **1** (17.8 % yield). Chemical yields reported for unsymmetrical porphyrins by the Lindsey method are typically about 20%; Whang *et al*. reported yields of 15% and 10% for a couple of tetraarylporphyrins [22] and other studies reported yields of 35% [23] and 22% [24] following this method. The structure of the obtained porphyrin was confirmed by nuclear magnetic resonance (NMR), ultraviolet-visible (UV-Vis) spectroscopy, mass spectrometry (MS) and elemental analysis (EA). First, the formation of the porphyrin macrocycle was supported by UV-Vis spectroscopy; the electronic absorption spectrum contains a single intense maximum attributed to the Soret band at 405 nm and four Q-bands: Qy(1,0), Qy(0,0), Qx(1,0) and Qx(0,0) showing much smaller absorption intensities, between 500 and 650 nm, characteristic of the tetra-*meso*-substituted porphyrin chromophore in the visible region [4]. The mass spectrum showed the molecular ion peak at m/z 764 [M]⁺. The ¹H NMR spectrum of the porphyrin **1** shows a highly shielded peak around -2.81 ppm corresponding to the protons of the N-H groups in the porphyrin core and peaks at 4.11 ppm and 5.81 ppm corresponding to the methoxyl and hydroxyl groups, respectively. The spectrum furthermore shows well resolved signals of the aromatic protons at 7.22-7.62 ppm corresponding to the protons of the 2-hydroxy-3-methoxyphenyl group, 7.70 and 8.10 ppm corresponding to the protons of *p*-chlorophenyl groups and finally two peaks at 8.81 and 8.90 ppm corresponding to the β-protons of the pyrrole rings. The integration ratio of all signals corresponds to the expected for the different protons of the synthesized molecule.

The results of phototoxicity and intrinsic cytotoxicity of the unsymmetrical porphyrin **1** on HeLa cells are presented in percentages of viability (Table 1). Figure 1 shows the dose-

Table 1. Percentages of viability values[a] of phototoxicity and intrinsic cytotoxicity of the unsymmetrical porphyrin A_3B NS1 on Hela cells.

μM	phototoxicity (viability % ± SD)	intrinsic cytotoxicity (viability % ± SD)
13	7.1 ± 1.1	9.6 ± 2.3
6.5	14.4 ± 5.1	67.7 ± 5.2
1.3	55.2 ± 5.9	84.1 ± 5.2
0.13	94.4 ± 12.1	90.1 ± 13.7
0.013	95.5 ± 8.7	93.5 ± 3.9

[a] Percentages of viability values are reported as means of three independent experiments ± SD (standard deviation).
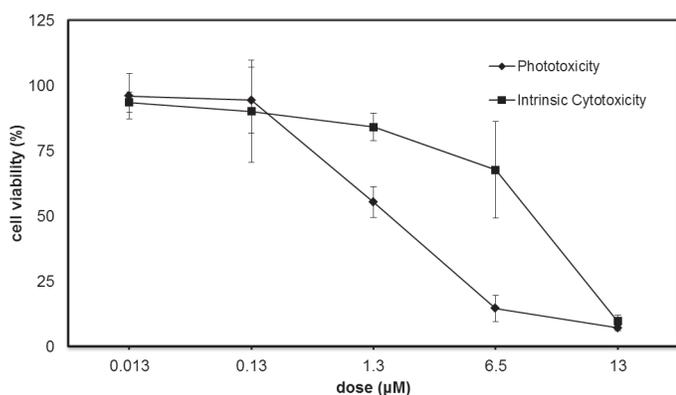
Fig. 1 Phototoxicity dose-response curve obtained in Hela cells following exposure to porphyrin **1** for 24 h and irradiation with visible light for 2 h. The intrinsic cytotoxicity obtained as described above, but omitting the light irradiation. The colorimetric WST-1 test was used for cell death estimation.

response curves obtained in HeLa cells following exposure to the unsymmetrical porphyrin **1** for 24 h and further irradiation with visible light for 2 h (phototoxicity) and also by omitting the irradiation step (intrinsic cytotoxicity). Phototoxicity and intrinsic cytotoxicity curves show similar values of cytotoxicity at porphyrin **1** concentration of 0.013 and 0.13 μM, while at concentration of 6.5 μM, the phototoxicity has a viability percentage of 14.4 and intrinsic cytotoxicity has a viability percentage of 67.7, showing a marked difference in cytotoxic activity. The highest concentration of porphyrin **1** dissolvent (0.5% dichloromethane in minimum essential medium (MEM)) was non-toxic. Phototoxicity and intrinsic cytotoxicity values were used to calculate the IC_{50} and a considerable difference was found: 1.5 μM for phototoxicity and 7 μM for intrinsic cytotoxicity. This difference is in agreement with the main requirements of the cytotoxic activity of a PS, which needs to be active in the presence of irradiation and inactive in the absence of light, thereby reducing side effects.

The results of phototoxicity presented here are similar to other studies where synthesized porphyrins were tested in different cell lines. In a study reported in 2006 by Banfi *et al*. [17], seven *meso*-tetrarylporphyrins were synthesized and exhibited IC_{50} values between 0.053 and 1.8 μM; these authors compared

its phototoxic effects against temoporfin ($IC_{50} = 0.0057$ μM) in HCT 116 cells (colorectal carcinoma). The porphyrin **1** presented here shows similar phototoxic effect ($IC_{50} = 1.5$ μM) compared with this study. On other hand, in a study presented in 2012 by Banerjee *et al*. [25], Photofrin® shows an IC_{50} value of 4.3μM in HeLa cells; accordingly with these data, porphyrin **1** is more phototoxic than Photofrin®. The intrinsic cytotoxicity profile observed in this study shows a similar behavior to that presented by Cheng-Liang *et al*. [8] in 2008.

Apoptosis requires transcriptional specific gene activation, including activation of endonucleases, DNA degradation and activation of caspases [26]. The apoptotic index was determined by T.U.N.E.L. assay. One of the major characteristics of apoptosis is the degradation of DNA after the activation of Ca^{2+}/Mg^{2+}-dependent endonucleases. This DNA cleavage leads to strand breaks within the DNA. The T.U.N.E.L. method identifies cells with DNA strand breaks detected by the activity of the terminal deoxynucelotidyl transferase, an enzyme that catalyzes the addition of dUTPs. Those nucleotides are previously labeled, allowing for the detection of apoptotic cells by a developing color assay with streptavidin-biotin-peroxidase substrate. HeLa cells treated with the unsymmetrical porphyrin **1** to a final concentration of 80 μM showed 15% apoptotic cells, whereas the control showed only 2.5%, which indicates the significant presence of apoptosis in treated cells compared with the control (Figure 2). Figure 3A shows the control cells where viable cells can be microscopically observed and apoptotic cells that were treated with unsymmetrical porphyrin **1** for 24 h and further irradiated with visible light for 2 h are shown in Figure 3B.

Some studies have documented the presence of apoptosis generated by the treatment of similar PSs. A work introduced in 2004 by Banfi *et al*. [18] shows that *meso*-tetrarylporphyrins induced cell death mainly by apoptosis in HCT 116 cells, confirming the results obtained in this study, which also are in agreement with observations reported by other authors [27-29]. Cells in apoptosis are characterized by cell shrinkage; meanwhile organelles and plasma membrane retain their integrity over a long period and the cells are ultimately fragmented
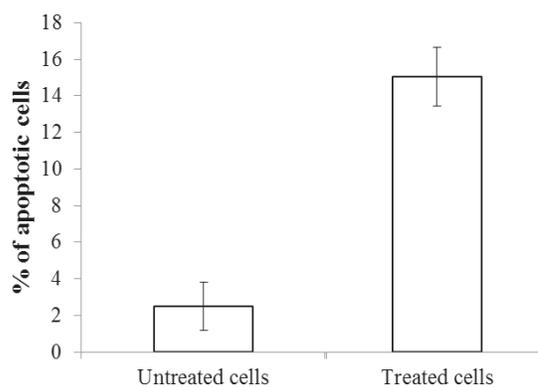
Fig. 2 Determination of apoptotic index of unsymmetrical porphyrin **1** in HeLa cells following exposure to porphyrin for 24 h and irradiation with visible light for 2 h, the control cells were not irradiated.
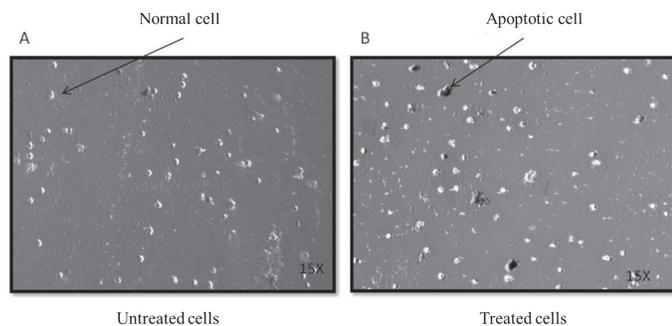
Fig. 3 Microscope photographs after determining the apoptotic index by TUNEL. Panel A shows untreated cells and panel B shows the cells treated with the unsymmetrical porphyrin **1**.

into apoptotic bodies, which are ingested by phagocytes, thereby preventing inflammation.

Conclusions

We have accomplished the synthesis and characterization of 5,10,15-Tris(*p*-chlorophenyl)-20-(2-hydroxy-3-methoxyphenyl) 21H,23H-porphyrin, evaluated its phototoxic activity and the induction of apoptosis. We found that this compound was more active than Photofrin® and similar to other *meso*-tetraryl-porphyrins. These results allowed us to consider porphyrin **1** as a potential photosensitizer in photodynamic therapy due to the structural synergistic effect of the hydrophobic and hydrophilic groups distributed unsymmetrically on the porphyrin macrocycle.

Experimental

The aromatic aldehydes were purchased from Sigma–Aldrich® (México). Pyrrole was distilled just before use. Dichloromethane, used as a reaction solvent, was distilled from LiAlH$_4$ directly into the reaction flask. Analytical thin-layer chromatography (TLC) was performed on Merck 60 F254 silica gel. Silica gel 60 (230-400 mesh, Merck) was used for column chromatography. The UV-vis absorption spectra were measured on a Perkin Elmer Lambda 12 spectrophotometer. NMR spectra were measured in CDCl$_3$ on a Varian-NMR System 500MHz spectrometer. Mass spectrometric measurements were performed on a JEOL JMS700 instrument. Elemental analysis was performed on a CHNS/O Perkin Elmer 2400 analyzer.

Synthesis and characterization of 5,10,15-Tris(*p*-chlorophenyl)-20-(2-hydroxy-3-methoxyphenyl)-21H,23H-porphyrin 1

BF$_3$•O(Et)$_2$ (0.012 mmol) and trifluoroacetic acid (TFA) (0.35 ml, 0.45 mmol) were added to a solution of 0.5266 g (3.75 mmol) of 4-chlorobenzaldehyde, 0.1902 g (1.25 mmol) of 2-hydroxy-3-methoxybenzaldehyde and 0.35 ml (5 mmol) of

freshly distilled pyrrole in 300 mL of CH$_2$Cl$_2$; the mixture was kept at room temperature for 2 h. Next, 0.570 g (0.251 mmol) of DDQ was added and the mixture was kept at room temperature for 2 h. The solvent was evaporated and the raw material was purified by column chromatography (SiO$_2$; CH$_2$Cl$_2$). Porphyrin **1** (yield 17.8 %) was obtained as a purple solid. UV-Vis (CH$_2$Cl$_2$): 405, 514, 548, 589 and 645 nm; ^{1}H NMR (500 MHz, CDCl$_3$): δ -2.81(2H, s, pyrrole-NH), 4.11 (3H, s, -OCH3), 5.81 (1H, s, -OH), 7.22 (1H, d, J=8.2 Hz, Ph), 7.29 (1H, d, J=8.2 Hz, Ph), 7.62 (1H, t, J=7.2 Hz, Ph), 7.70 (6H, d, J=7.8 Hz, Ph), 8.10 (6H, d, J=6.3 Hz, Ph), 8.81 (6H, s, pyrrole-H), 8.90 (2H, d, J=4.4 Hz, pyrrole-H)-, ^{13}C NMR (126 MHz, CDCl$_3$): δ 146.42, 145.95, 140.53, 140.48, 135.48, 134.29, 134.25, 128.04, 127.94, 126.96, 118.93, 118.67, 118.32, 115.04, 110.95, 56.28; MS: m/z 764 [M$^+$]; Anal. C 70.56 %; H 3.88 %, N 7.12 %; calculated for C$_{45}$H$_{29}$Cl$_3$N$_4$O$_2$, C 70.73 %; H 3.83 %; N 7.33 %.

Cytotoxicity studies

The cytotoxic effects of the 5,10,15-Tris(*p*-chlorophenyl)-20(2-hydroxy,3-methoxyphenyl)-21H,23H-porphyrin on human adenocarcinoma HeLa cells (ATCC: CL-2) maintained in MEM supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO$_2$ atmosphere, were assessed using the WST-1 assay. Briefly, 5 X 10^3 cells were seeded on to 96-well plates and incubated for 24 h to grow. After this incubation period, the cells were treated with different concentrations of the compound (porphyrin was first dissolved in 50 µl of dichloromethane and then diluted in MEM medium to raise a stock concentration of 130 µM; the final concentration of dichloromethane in each reaction well was 0.5% v/v or less) and incubated for 24 h. Later, the medium was replaced by PBS and the cells were irradiated under visible light (LED Lamp ATLED Apollo BR40 35W true white, 0.3 m away from the culture plate) for 2 h. At the end of this time, the cells were incubated for 24 h; WST-1 was then added to each well (10 µL) for 2 h at 37°C. Optical densities were measured at 450 nm using a Universal Microplate Reader ELx800 (Biotek Instrument, Vermont, USA). The intrinsic cytotoxic effects of the porphyrin were assessed as described above, but omitting cell irradiation [18, 30]. Triton X-100, 1% was used as a control of 0% viability; non-treated cells were used as a control of 100% viability.

Apoptotic index

T.U.N.E.L. assays were carried out using a DeadEnd™ Colorimetric T.U.N.E.L. System (Promega, WI, USA). The treated and untreated cells were attached to poly (L-lysine)-coated glass-bottomed dishes and fixed for 30 min in 4% paraformaldehyde in PBS (pH 7.4). After the cells were washed three times with PBS, the endogenous peroxidase was inactivated by incubation with 3% H$_2$O$_2$ in methanol for 30 min at room temperature. The cells were again washed with PBS and subsequently permeabilized for 2 min on ice with 0.1% Triton X-100. After two PBS washing steps, the cells were labeled with the biotinylated nucleotides for 60 min at 37°C, washed and

stained with peroxidase-streptavidin-biotin for 30 min at 37°C. DNA fragmentation was detected by developing dark brown color with diaminobenzidine substrate and observed under light microscope (Leica, IL, USA) [31].

Acknowledgments

References

1. Zhu, T. C.; Finlay J. C. *Med. Phys.* **2008**, *35*, 3127-3136.
2. Triesscheijn, M.; Baas, P.; Schellens J.; Stewart F. *Oncologist*. **2006**, *11*, 1034-1045.
3. Castano, A. P.; Mroz, P.; Hamblin, M. R. *Nat. Rev. Cancer*. **2006**, *6*, 535-545.
4. Milgrom, L.R., in: The colours of life. An introduction to the chemistry of porphyrins and related compounds, vol. 9*,* Ed., Oxford University Press: Oxford, **1997**, 225.
5. Senge, M. O. *Acc. Chem. Res.*, **2005**, *38*, 733-743.
6. Machado, A.; Gomes, W.R.; Araújo, D.M.; Miglio, H.S.; Ueno, L.T.; De Paula, R.; Cavaleiro J.A.; Newton, B.N. *Molecules*, **2011**, *16*, 5807-5821.
7. Calzavara-Pinton, P.G.; Venturini, M.; Sala, R. *J. Eur. Acad. Dermatol. Venereol*. **2007***, 21*, 293-302.
8. Cheng-Liang, P.; Ping-Shan, L.; Ming-Jium, S. *Biomed. Eng-App. Bas. C*. **2008**, *20*, 9-17.
9. O'Connor, A. E.; Gallagher, W. M.; Byrne, A. T. *Photochem. Photobiol*. **2009**, *85*, 1053-174.
10. Agostinis, P.; Berg, K.; Cengel, K.; Foster, T.; Girotti, A.; Gollnick, S.O.; Hahn, S.M; Hamblin, M.R; Juzeniene, A.; Kessel, D.; Korbelik, M.; Moan, J.; Pawel, M.; Nowis, D.; Piette, J.; Wilson, B.C; Golab, J. *C.A. Cancer J. Clin.* **2011**, *61*, 250–281.
11. Dougherty, T. J.; Gomer, C. J; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Natl. Cancer. Inst.* **1998**, *90*, 889-905.
12. Webber, J.; Herman, M.; Kessel, D.; Fromm, D. *Ann Surg*. **1999**, *230*, 12-23.
13. Dougherty, T. J.; Kaufman, J.E.; Goldfarb, A.; Weishaupt, K.R; Boyle, D.; Mittleman, A. *Cancer Res*. **1978**, *38*, 2628-2635.
14. McCaughan, J.; Guy J.; Hawley, P.; Hicks, W.; Inglis, W.; Laufman, L.; May, E.; Nims, T.A.; Sherman, A. *Lasers Surg. Med*. **1983**, *3*, 199-209.
15. McCaughan, J.; Hicks, W.; Laufman, L. *Cancer* **1984**, *54*, 2905-2910.
16. Wiedmann, M. W.; Caca, K. *Curr. Pharm. Biotechnol. 2004*, *5*, 397-408.
17. Banfi, S.; Caruso, E.; Buccafurni, L.; Murano, M.; Monti, E.; Gariboldi, M.; Papa E.; Gramatica, P. *J. Med. Chem*. **2006**, *49*, 3293-3304.
18. Banfi, S.; Caruso, E.; Caprioli, S.; Mazzagatti, L.; Canti, G.; Ravizza, R.; Gariboldi, M.; Monti, E. *Bioorg. Med. Chem*. **2004**, *12*, 4853-4860.
19. Łopuszyńska, B.; Piechocka, K.; Mikus, A.; Ostrysz, S.; Ostrowski, S. *Macroheterocycles*. **2013**, *6*, 245-250.
20. Nifiatis, F.; Athas, J. C.; Gunaratne, K. D. D.; Gurung, Y.; Monette, K.M.; Shivokevich, P.J. *The Open Spectroscopy Journal* **2011**, *5*, 1-12
21. Lindsey, J. S.; Schreiman, I. C; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem*. **1987**, *52*, 827-836.
22. Wang, C.; Yang, G.; Li, J.; Mele, G.; S1ota, R.; Broda, M.; Duan, M.; Vasapollo, G.; Zhang, X.; Zhang, F. *Dyes Pigm*. **2009**, *80*, 321–328.
23. Milanesio, M. E.; Alvarez, M. G.; Yslas, E. I.; Borsarelli, C. D.; Silber, J. J.; Rivarola, V.; Durantini, E. N. *Photochem. Photobiol*. **2001**, *74*, 14-21.
24. Azenha, E. G.; Serra, A. C.; Pineiro, M.; Pereira, M. M.; Melo, J. S.; Arnaut, L.; Formosinho, S. J.; Rocha, A. M. d´A. *Chem. Phys*. **2002**, 2*80*, 177-190.
25. Barnajje, S.; Prasad, P.; Hussain, A.; Khan, I.; Kondaiah, P.; Chakravarty, A. R. *Chem. Commun*. **2012**, *48*, 7702–7704.
26. Mroz, P.; Yaroslavsky, A.; Kharkwal, G. B.; Hamblin, M. R. *Cancers*. **2011**, *3*, 2516-2539.
27. Wawrzynska, M.; Kałas, W.; Biały, D.; Zioło, E.; Arkowski, J.; Mazurek, W.; Strządala, L. *Arch. Immunol. Ther. Exp*. **2010**, *58*, 67-75.
28. Králová, J.; Bříza, T.; Moserová, I.; Dolenský, B.; Vašek, P.; Poučkova, P.; Kejik, Z.; Kaplanek, R.; Martasek, P.; Dvořak, M.; Kral, V. *J. Med. Chem*. **2008**, *51*, 5964-5973.
29. Szurko, A.; Krämer-Marek, G.; Widel, M.; Ratuszna, A.; Habdas, J.; Kuś, P. *Acta Biochim. Pol*. **2003**, *50*, 1165-1174.
30. Durantini, E. N. *Molecules* **2001**, *6*, 533-539.
31. Khan, M. A. S.; Chock, P. B.; Stadtman, E. R. *Proc. Natl. Acad. Sci. U.S.A*. **2005**, 102.