



Research Paper

**PHOTODYNAMIC EFFECT OF NEW PORPHYRIN DERIVATIVE FOR
CANCER TREATMENT**

Meharban Faiza¹, Xue-Xue Zhu¹, Man-Yi Li¹, Le Mi¹, Yi-Jia Yan² and Zhi-Long Chen¹

¹Department of Pharmaceutical Science & Technology, College of Chemistry and
Biology,

Donghua University, Shanghai 201620, China,

²Shanghai Xianhui Pharmaceutical Co., Ltd., Shanghai 200433,
China.

Abstract

The substitution of porphyrin photosensitizers (PS) with amino acid molecules is a promising method to increase the efficiency and selectivity of photodynamic therapy (PDT). The covalent bond between porphyrin and amino acid molecule can produce phototoxic species *in vivo* that increases the rate of oxidative damage towards the cancerous cells. In this paper photophysical, photochemical properties, intracellular localization, *in vitro* and *in vivo* photocytotoxicity of TPPB-1 had been evaluated. The compound showed characteristic long wavelength absorption peak at 649 nm. After laser irradiation at 650 nm TPPB-1 inhibited the cell proliferation in Eca-109 cell *in vitro*. TPPB-1 significantly inhibited the growth of Eca-109 cells of Balb/c nude mice *in vivo*. The results showed that TPPB-1 could be used as a potential agent for photodynamic therapy of its reduced dark toxicity and selective accumulation in tumor cells.

Key words: Photodynamic therapy, photosensitizer, tumor.

INTRODUCTION

Cancer is among the most lethal disease and leading cause of illness and mortality on global scale. In 2012, nearly 14 million cancer cases were diagnosed, and documented cancer-related deaths were about 8.2 million, which is expected to increase by 70% over the coming two decade [1]. At present, medical treatments for cancer contain surgical treatment, radiation treatment, chemotherapy, immunotherapy and other targeted therapies [2]. PDT and boron neutron capture therapy (BNCT) are two therapies for cancer treatment which include the initiation of a photosensitizer (PS)

with red light (in PDT) or with low energy neutrons (in BNCT) [3 - 5]. The cytotoxic agents produced in PDT are, mainly $^1\text{O}_2$, whereas in BNCT $^4\text{He}^{2+}$ and $^7\text{Li}^{3+}$ are formed by ^{10}B -neutron capture nuclear reaction and travel little distance through tissue. These cytotoxic species have restricted ranges in tissue (about $0.1\ \mu\text{m}$ for $^1\text{O}_2$, $9\ \mu\text{m}$ for $^4\text{He}^{2+}$, and $5\ \mu\text{m}$ for $^7\text{Li}^{3+}$), hence, the toxic effect is limited to their site of production. As a result, the biological ability of porphyrin based photosensitizers equally depends on their selectivity and their intracellular distribution [6, 7]. In order to enhance the tumor specificity and water solubility of photosensitizers (PS) several methods have been developed [8]. The ideal photosensitizer should have, maximum purity, high singlet oxygen quantum yield, strong absorption in red region with high extinction coefficient, effective accumulation in the infective area, chemically stability, must have good solubility in body fluids and can be excrete from the body after the treatment [9]. PDT has been used with restrained success in the treatment of a variety of solid tumors and many non-malignant illnesses. PDT is a two-step process (1) administration of the drug called photosensitizer (PS) (2) initiation of the photosensitizer with light of specific wavelength [10, 11]. After irradiation of the photosensitizer it goes to a triplet excited state. There are three possible reactions that can occur at triplet excited state called type I, type II and type III reaction [12].

The biological activity of the activated PS is limited to the specific areas of tissue which are illuminated by the light. PDT has been permitted by the United States Food and Drug Administration for the treatment of endesophageal and endobronchial [13, 14] and also for the treatment of skin disease (actinic keratosis), premalignant and early malignant diseases, breast, stomach, oral cavity and bladder cancer [15, 16].

Porphyrin-type photosensitizers create a key class of pharmacological agents that is under clinical trials for the early treatment and diagnosis of cancer by PDT and a vast range of other diseases [14]. Porphyrins are tetrapyrrole molecules, consisting of four pyrrole units connected by methine linkage (=CH-). Porphyrins consists of delocalized 18-p electron system that is responsible for strong emission and large absorption in the visible region [17].

Several examples of coupling of porphyrin with amino acid have been reported in the literature but most of these porphyrins are designed as the model of haem protein or studies in electron transfer. In this paper a novel amino acid derivative TPPB-1 had been examined for photodynamic therapy. Its photo-physical and photochemical properties *in vivo* and *in vitro* were examined along with compatibility for PDT.

MATERIALS AND METHODS

Reagents and Instruments

All the chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. and Bide Pharmatech Ltd., and used without further purification. UV-visible absorption spectra were recorded on an ultraviolet visible spectrophotometer (Model V-530, Japan). Fluorescence spectrum was measured by Fluorescence Spectrophotometer (FluoroMax-4, France). Slits were kept narrow to 1 nm in excitation and 2 nm in emission. All the measurements were carried out at room temperature in quartz cuvettes with path length of 1 cm. DMSO was used as a solvent for all the measurements. All the measurements were done in a sterilized environment without the direct exposure of sunlight.

Singlet oxygen detection

The singlet oxygen quantum yield of TPPB-1 was determined by 1, 3-diphenylisobenzofuran (DPBF) in the DMF solution. Characteristically, 3 mL DMF solution containing 60 μ M DPBF and 2 μ M TPPB-1 were placed in a sealed quartz cuvette and respectively irradiated with 650 nm laser (5 mW/cm²). The natural logarithm values of absorption of DPBF at 410 nm were plotted against the irradiation time and fit by a first-order linear least squares model to get the singlet oxygen generation rate of the photosensitized process.

Cell culture

Eca-109 was obtained from the Type Culture Collection of the Chinese Academy of Sciences. All cell culture related reagents were purchased from Shanghai Ming Rong Bio-Science Technology Co., Ltd. Cells were cultured in normal RPMI1640 culture medium with 10% fetal bovine serum (FBS), 50 units per mL penicillin and 50 mg mL⁻¹ streptomycin in 5% CO₂ at 37°C.

MTT assay

Eca-109 cells were cultured in RPMI-1640 medium with 10% (v/v) FBS, collected with 0.25% (w/v) trypsin, and seeded in 96-well plates at 5×10^4 cells per well. The cells were allowed to attach to the bottom of the wells for 24 hours prior to start the experiment. RPMI1640 medium containing TPPB-1 in different concentrations (range from 0 to 30 μ M) was administered to cells and allowed to uptake for 24 hours. Non irradiated cells were used to investigate the dark cytotoxicity. RPMI-1640 medium containing TPPB-1 was removed and cells were washed with fresh PBS before irradiation with different concentrations (range from 0 to 30 μ M) and light doses (range from 2 to 12 J/cm²) with 650 nm laser. The cell viability was evaluated by 3-(4,5-

dimethyl-2-thiazolyl)-2,5-diphenyl- 2H-terazolium bromide (MTT) colorimetric assay 24 h after treatment.

In vivo experiment

Four-week-old female Balb/c nude mice were obtained from Shanghai SLAC Laboratory Animal Company and housed in dedicated pathogen-free barrier facilities. The 5×10^6 Eca-109 cells were injected subcutaneously in 200 μ L PBS into the right forelimb. All animal protocols were approved by the Animal Care and Use Committee of Donghua University. When the size of the tumor reached approximately 150 mm³ (about 14 days after vaccination), the tumor-bearing mice were divided into 4 groups containing 4 mice per group: control group, light (without drug), TPPB-1 (without light) and TPPB-1-PDT (with drug and Light). After PDT, tumor regeneration or healing of the mice was observed daily.

RESULTS AND DISCUSSION

UV- Visible absorption and florescence spectra

Porphyrins consists of four pyrrole rings which are connected via methine bridges (=CH-). The porphyrins contain delocalized 18- π electron system which is responsible for strong fluorescence and UV-visible absorption at room temperature [18]. The UV-visible and florescence spectrum of TPPB-1 were examined in DMSO at room temperature. As shown in **Figure 1 a**, TPPB-1 showed characteristic soret and Q band at 420 nm (soret), 517 nm, 552 nm, 592 nm and 649 nm (Q) in DMSO. Molar absorption coefficients (ϵ) were calculated in **Table 1**. When TPPB-1 was excited at 418 nm wavelength it exhibited strong emission peaks at 651 nm and 718 nm approximately as shown in **Figure 1 b**.

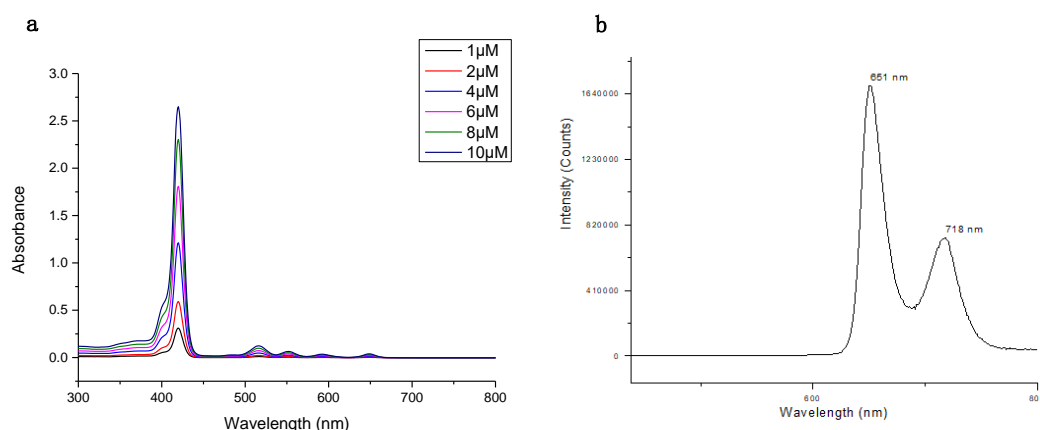


Figure 1. (a) UV-visible absorption spectra of TPPB-1 in DMSO with different concentration (range from 1 to 10 μM). (b) Fluorescence spectrum of TPPB-1 in DMSO at 2 μM .

Table 1. Molar absorption coefficients of TPPB-1

| Wavelength (nm) | $\epsilon \times 10^4 (\text{L} \cdot \text{mol}^{-1} \text{cm}^{-1})$ |
|-----------------|--|
| 420 | 29.4 |
| 517 | 1.18 |
| 552 | 0.599 |
| 592 | 0.283 |
| 649 | 0.349 |

Singlet oxygen

Singlet oxygen quantum yield is one of the determining factor to check efficiency of the photosensitizer for PDT. In this paper we used photochemical method for the determination of singlet oxygen quantum yield. Photochemical method uses DPBF as chemical quencher for the determination of $^1\text{O}_2$ [19]. For the determination of reactive oxygen 60 μM of DPBF and 2 μM solution of TPPB-1 were prepared in DMF in dark. The

disappearance spectra of DPBF were recorded at 410 nm with UV-visible spectrophotometer. DPBF absorption intensity was continuously decreased by increasing irradiation time. The data was plotted as $\ln [A_0]/[A_t]$ versus irradiation time t , TPPB-1 gave straight line, after applying the linear function slope was obtained for TPPB-1 shown in **Figure 2**. The singlet oxygen production rate K (min^{-1}) of TPPB-1 and HMME were 0.0625 min^{-1} and 0.0205 min^{-1} respectively. The experiment proved that TPPB-1 had better singlet oxygen quantum yield.

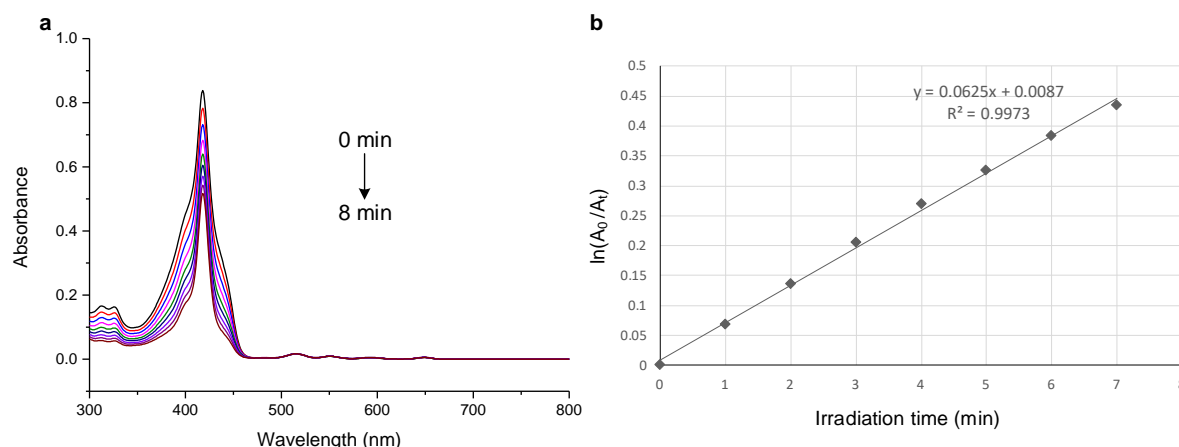


Figure 2. (a) Photodegradation curves of DPBF in the presence of TPPB-1 under 650 nm irradiation. (b) First-order plot for the photodecomposition of DPBF photosensitized by TPPB-1.

Cytotoxicity and intracellular localization

Effectiveness of the photosensitizer depends on the fact that it should have high phototoxicity and low dark toxicity. Phototoxicity and the dark toxicity of TPPB-1 were examined by MTT assay [20]. TPPB-1 showed no dark toxicity when exposed up to $10 \mu\text{M}$ against Eca-109 cells. When the concentration was increased to $15 \mu\text{M}$, the sustainability of Eca-109 cells dropped from 85% to 67%. After irradiation with 650 nm

laser (0 - 12 J/cm²) at concentration 5 µM, a substantial cytotoxicity was noticed as shown in **Figure 3**. These results proposed that TPPB-1 was an effective photosensitizer *in vitro*.

Intracellular localization of the photosensitizer is of great importance, because it determines the site of initial photodamage and initial response of the cell. The intracellular localization of TPPB-1 was determined by confocal microscopy using 10 µM TPPB-1 on Eca-109 cells for 4 hours and staining with Hoechst 33342, Lyso-Blue and Mitro-Tracker for nucleus, lysosome and mitochondria, respectively. The results indicated that TPPB-1 was mainly assembled in lysosome and mitochondria, which might cause necrosis and apoptosis of tumor cells.

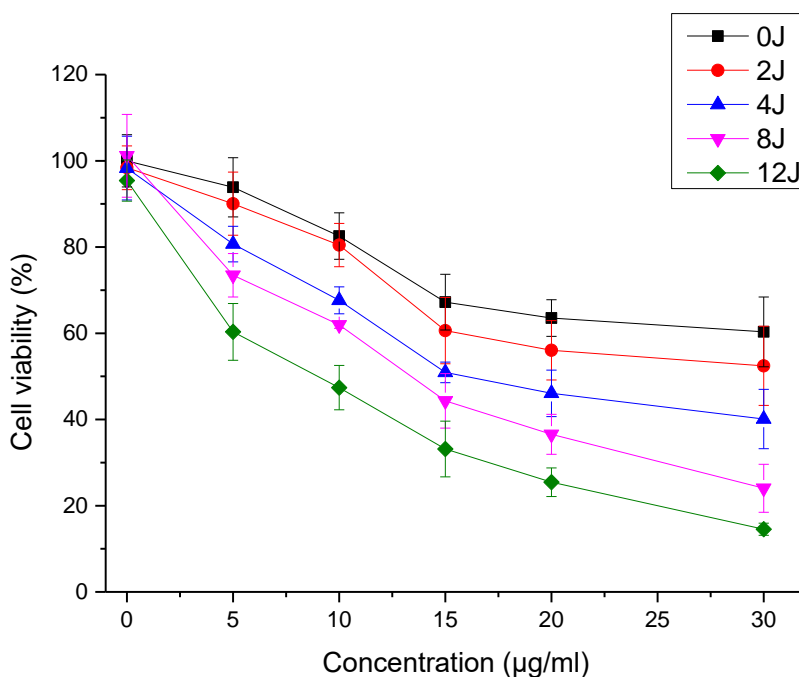


Figure 3. Cytotoxicity of TPPB-1 at different concentrations and light doses.

In vivo photodynamic therapy

In vivo efficacy of PDT using TPPB-1 was examined in Eca-109 tumor bearing Balb/c nude mice. When tumor sizes had reached 4-7 mm in diameter, Balb/c nude mice were injected intravenously via tail vein, TPPB-1 at a dose of 5 mg/kg. Tumor infected area was irradiated with 650 nm, 100 J/cm², 180 mW/cm². Tumor inhibition rates of TPPB-1 at 14 days post PDT was shown in **Figure 5**. There was no substantial difference between the control group (without treatment), the light group (without drug) and the TPPB-1 group (without light). The tumors in the control group, the light group and the TPPB-1 group were larger than the tumors in the TPPB-1-PDT group. TPPB-1-PDT exhibited great antitumor performance. The tumor volume examined to be increased approximately 8 fold for 14 days in the control group, the light group and the TPPB-1 group without any treatment. TPPB-1 mediated PDT decreased volume of tumor at the 3rd day post treatment, and the tumor growth was noted to be slowed down as compared to the other groups. These results indicated that the growth of Eca-109 tumor cells in Balb/c nude mice was notably inhibited by TPPB-1 mediated PDT.

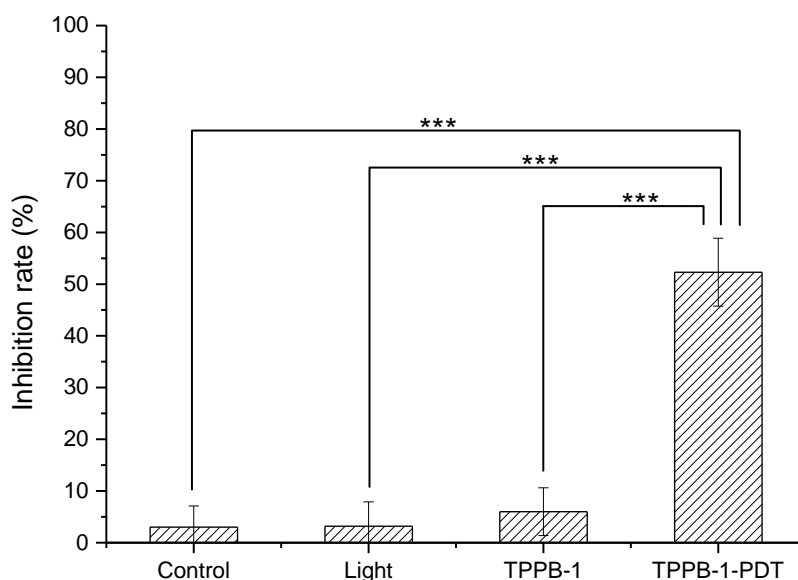


Figure 4. Inhibition rates of TPPB-1 against Eca-109 cells *in vivo*. Results are expressed as mean values \pm SD of three independent experiments. The significant differences were signed as $p < 0.001$ (***)

CONCLUSIONS

In this paper the photochemical and photophysical properties of amino acid porphyrin derivative TPPB-1 was examined. *In vivo* and *in vitro* experiments were performed to evaluate the ability of newly synthesized photosensitizer to destroy tumor. The studies showed that the target compound TPPB-1 has high phototoxicity and low dark toxicity. Hence TPPB-1 can be used as a potential photosensitizer (PS) for photodynamic therapy (PDT) of cancer.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No. 21977016), Foundation of Shanghai Science and Technology Committee (No. 17430741800, 17430711900, 18430713000, 18430731600 ; 19410711000).

REFERENCES

- [1] World Health Organization. Cancer. [Cited 30 March 2017]. Available from: <http://www.who.int/mediacentre/factsheets/fs297/en/>.
- [2] Lucky SS, Soo KC, Zhang Y. (2015) Nanoparticles in photodynamic therapy. *Chem Rev* 2015; 115:1990–2042.
- [3] Pandey, R. K., Zheng, G. Porphyrins as photosensitizers in photodynamic therapy. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K 2000; Vol. 6, pp 157–230.
- [4] Brown, S. B.; Brown, E. A.; Walker, I. The present and future role of photodynamic therapy in cancer treatment. *Lancet Oncol.* 2004, 5, 497–58.
- [5] Barth, R. F.; Coderre, J. A.; Vicente, M. G. H.; Blue, T. E. Boron neutron capture therapy of cancer: current status and future prospects. *Clin. Cancer Res.* 2005, 11, 3987–4002.
- [6] Kessel, D. Correlation between subcellular. *J. Porphyrins Phthalocyanines* 2004, 8, 1009–1014.
- [7] Rosenkranz, A. A.; Jans, D. A.; Sobolev, A. S. Targeted intracellular delivery of photosensitizers to enhance photodynamic efficiency. *Immunol. Cell Biol.* 2002, 78, 452–464.

-
- [8]. Osterloh, J.; Vicente, M. G. H. Mechanisms of Porphyrinoid Localization in Tumors. *J. Porphyrins Phthalocyanines* 2002, 6, 305–324.
- [9] Pushpan, S.K.; Venkatraman, S.; Anand, V.G.; Sankar, J.; Parmeswaran, D.; Ganesan, S.; Chandrashekar. Porphyrins in Photodynamic Therapy - A Search for Ideal Photosensitizers *Med. Chem. Anti-Cancer Agents* 2002, 2, 187–207.
- [10] B. W. Henderson, T. J. Dougherty. How does photodynamic therapy works? *Photochem. Photobiol.* 1992, 55, 145.
- [11] M. R. Hamblin and P. Mroz. *Photodynamic Therapy in Advances in Photodynamic Therapy*, Artech House, Boston, 2008.
- [12] I. J. MacDonald and T. J. Dougherty. Basic principle in photodynamic therapy. *J. Porphyrins Phthalocyanines*, 2001, 5, 105–129
- [13] J. G. Levy, *Photodynamic Therapy*. *Trends Biotechnol.* 1995, 13, 14.
- [14] M. B. Vrouenraets, G. Visser, G. Snow, G. Van Dongen, Basic principles applications in onocology and improved selectivity of photodynamic therapy. *Anticancer Res.* 2003, 23(1B):505-22.
- [15] T. J. Dougherty, *J.Clin.* An update on photodynamic applications *Laser. Med. Surg.* 2002, 20, 3.
- [16] N. L. Oleinick, H. H. Evans. *The Photobiology of Photodynamic Therapy: Cellular Targets and Mechanisms*. *Radiat. Res.* 1998, 150, S146
- [17] Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korblik, M.; Moan, J.; Peng, Q. *Photodynamic Therapy*. *J. Natl. Cancer Inst.* 1998, 90, 889–905
- [18] H. Ali, J. E. Van Lier. *Handbook of Porphyrin Science*, Vol. 4 (Eds.: K. M. Kadish, K. M. Smith, R. Guilard), World Scientific, New Jersey, 2010, pp. 1–119
- [19] Spiller, W.; Kliesch, H.; Wohrle, D.; Hackbarth, S.; Roder, B.; Schnurpfeil, G. Singlet oxygen quantum yields of different photosensitizers in polar solvents and micellar solutions. *J. Porphyrins Phthalocyanines* 1998, 2 (2), 145-158.
- [20] Lovell, J. F.; Liu, T. W. B.; Chen, J.; Zheng, G. Activatable Photosensitizers for Imaging and Therapy. *Chem. Rev.* (Washington, DC, U. S.) 2010, 110 (5), 2839-2857.