The biological activities of 5,15-diaryl-10,20-dihalogeno porphyrins for photodynamic therapy

Man Yi Li1 · Le Mi1 · Gennady Meerovich2 · Thin Wut Soe1,3 · Ting Chen1 · Ni Ni Than3 · Yi Jia Yan1 · Zhi Long Chen1,4

Received: 17 March 2022 / Accepted: 19 April 2022 / Published online: 6 May 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Purpose Esophageal cancer is the most common gastrointestinal tumor and is difficult to be eradicated with conventional treatment. Porphyrin-based photosensitizers (PSs) mediated photodynamic therapy (PDT) could kill tumor cells with less damage to normal cells. As the most widely used porphyrin-based photosensitizer in clinics, Photofrin II has excellent anti-tumor effect. However, it has some disadvantages such as weak absorption at near infrared region, the complexity of components and prolonged skin photosensitivity. Here series novel 5,15-diaryl-10,20-dihalogeno porphyrin derivatives were afforded and evaluated to develop more effective and safer photosensitizers for tumor therapy.

Methods The photophysical properties and singlet oxygen generation rates of 5,15-diaryl-10,20-dihalogeno porphyrins (I1-6, II1-4) were tested. The cytotoxicity of I1-6 and II1-4 were measured by MTT assay. The pathway of cell death was studied by flow cytometry. In vivo photodynamic efficacy of I3 and II2-4 in Eca-109 tumor-bearing BABL/c nude mice were measured and histopathological analysis were examined.

Results 5,15-Diaryl-10,20-dihalogeno porphyrins I1-6 and II1-4 were synthesized. The longest absorption wavelength of these halogenated porphyrins (λmax = 660 nm) displayed a red shift around 30 nm compared to the unhalogenated porphyrins PS1 (λmax = 630 nm). The singlet oxygen generation rates of I1-6 and II1-4 were significantly higher than PS1 and HMME. All PSs mediated PDT showed obvious cytotoxic effect against Eca-109 cells compared to HMME in vitro and in vivo. Among these PSs, II4 exhibited appropriate absorption in the phototherapeutic window, higher 1O2 generation rate (k = 0.0061 s⁻¹), the strongest phototoxicity (IC50 = 0.4 μM), lower dark toxicity, high generation of intracellular ROS in Eca-109 cells and excellent photodynamic anti-tumor efficacy in vivo. Besides, cell necrosis was induced by compound II4 mediated PDT.

Conclusion All new compounds have obvious photodynamic anti-esophageal cancer effects. Among them, the photosensitizer II4 showed excellent efficacy in vitro and in vivo, which has the potential to become a photodynamic anti-tumor drug.

Keywords Photodynamic therapy · Photosensitizer · Halogenated porphyrin · Anti-tumor

Abbreviations

PDT Photodynamic therapy
PSs Photosensitizers
ROS Reactive oxygen species
1O2 Singlet oxygen
ISC Intersystem crossing
DFT Density functional theory
TLC Thin layer chromatography
NMR Nuclear magnetic resonance
MS Mass spectra
FMOs Frontier molecular orbitals
HMME Hematoporphyrin monomethyl ether
DMSO Dimethyl sulfoxide
DMF Dimethylformamide
DPBF 1,3-Diphenylisobenzofuran
DDQ 2,3-Dicyano-5,6-dichlorobenzoquinone
H&E reagent        Hematoxylin–eosin reagent
FBS                Fetal bovine serum
PBS                Phosphate buffered saline
MTT                3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-terazolium bromide

Introduction

Esophageal cancer (Eca) is the common type of cancer and the leading cause of cancer deaths worldwide according to GLOBOCAN 2018. More than 570,000 people are newly diagnosed with esophageal carcinoma, and over 500,000 deaths are recorded annually (Bray et al. 2018; Uhlenhopp et al. 2020). PDT is a clinically approved novel method for the treatment of this cancer with many advantages including prominent selectivity, flexibility, minimum injury, and negligible toxicity to normal tissues (Wu et al. 2020). Exogenous light could activate the PSs from ground state to excited state whose energy could be transferred to surrounding state oxygen to generate reactive oxygen species (ROS), such as singlet oxygen (1O2), which in turn could cause oxidative damage and kill cancer cells by reacting with biomolecules (Wu et al. 2020; Li et al. 2021a, b; Du et al. 2021; Li et al. 2021a, b; Ormond et al. 2013).

Porphyrin, also called the “color of life”, has drawn considerable attention in photodynamic therapy, cancer diagnosis, optoelectronic materials and other aspects because of its unique physical and chemical properties (Yu et al. 2020). Currently, as the representatives of porphyrin-based photosensitizers, Photofrin II and Hematoporphyrin monomethyl ether (HMME) are commonly used in photodynamic therapy (Massiot et al. 2018; Li et al. 2020; Zhu et al. 2018, Liao et al. 2017). Photofrin II is the first commercially available and most widely used photosensitizer due to its excellent anti-tumor effect. However, its clinical application is limited by the complexity of components and the prolonged skin photosensitivity (Li et al. 2021a, b). HMME, developed by our group, possesses a pair of isomers which has been clinically used for the treatment of nevus flammeus and tumors. The photophysical and photochemical properties of Photofrin II and HMME, such as the absorption ability at red light region, the 1O2 generation ability and the photophysical and photochemical properties of Photofrin II and HMME, were reported about meso-diarylporphyrins, namely 5,15-diarylporphyrins. The preliminary study was proceeded by Banfi et al. (2006) and Wiehe et al. (2001) showed that 5,15-diarylporphyrins exhibited significantly higher photocytotoxicity than Photofrin II. The dicationic 5,15-bis-[4-(3-trimethylammoniopropoxy)phenyl] porphyrin (XF-73) was developed for the treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections with satisfactory efficacy, safety and tolerability in clinical trials (Maisch et al. 2005). Constant and abundant amino acids supply is required for tumor survival to support numerous biochemical reactions, and for tumor proliferation to synthesize structural and functional proteins (Martinez-Outschoorn et al. 2017; Hosios et al. 2016). The approval of talaporfin (Laserphyrin®) which is the novel photosensitizer obtained by coupling chlorin e6 with aspartic acid proves that the introduction of amino acids into porphyrin-based derivatives is an effective strategy to improve the hydrophilicity and the biocompatibility (Kwitniewski et al. 2009; Serra et al. 2010; Tamiaki et al. 2014; Wang et al. 2008; Allison et al. 2004), A series of chlorin p6-based water-soluble amino acid derivatives were synthesized by Meng et al (2016), and it was found that the aqueous solubility of aspartylchlorin p6 dimethylster was significantly better than its lead compound chlorin p6 dimethylster.

In the present study, 5,15-diaryl-10,20-dihalogeno porphyrins I1_4 and II1_4 were synthesized by introducing halogen atoms and amino acids groups into 5,15-diarylporphyrins, and their photodynamic activities in vitro and in vivo against esophageal cancer were investigated (Fig. 1).

Materials and methods

Materials

5,15-Diaryl-10,20-dihalogeno porphyrins (I1_4, II1_4) were synthesized in our laboratory (Fig. 2). All solvents and reagents were purchased from commercial suppliers and dichloromethane was used after further distillation. HMME was donated by Shanghai Xianhui Pharmaceutical Co. Ltd. Thin-layer chromatography (TLC) analysis was carried out on silica gel plates GF254 and column chromatography was performed on silica gel (300–400 mesh). 1H NMR and 13C NMR spectra were recorded on a Bruker 400 or 600 MHz spectrometer. MALDI-TOF mass spectra were recorded using a JEOL JMS-S3000 Spiral-TOFMS (JEOL, Tokyo, Japan). UV–Vis absorption spectra were recorded on a Cary 5000 spectrophotometer (Agilent Technologies, CA, USA). Fluorescence spectra were measured on a PerkinElmer LS-45 spectrophotometer (PerkinElmer, Waltham, MA, USA).

Among the porphyrin derivatives, numerous researches on meso-tetraarylporphyrins (Chatterjee et al., 2017) have been carried out. However, only very few studies were reported about meso-diarylporphyrins, namely 5,15-diarylporphyrins. The preliminary study was proceeded by Banfi et al. (2006) and Wiehe et al. (2001) showed that 5,15-diarylporphyrins exhibited significantly higher photocytotoxicity than Photofrin II. The dicationic
by Bonferroni correction for multiple testing. \( P < 0.05 \) was considered statistically significant.

**Density functional theory (DFT) studies**

The geometry optimization of the compounds (PS\(_1\), I\(_2\), I\(_5\), I\(_6\)) in DMSO was carried out by DFT calculations using the B3LYP functional with a 6-31G basis set for carbon, hydrogen, nitrogen, oxygen, chlorine, bromine atoms and LANL2DZ for iodine atoms. And then the FMOs of HOMO and LUMO energy (eV) level for the optimized structure of the products were calculated to evaluate the influence of the halogen atoms (Zhao et al. 2021).

**Photophysical properties**

UV–Vis absorption spectra of all PSs in DMSO were recorded on an ultraviolet visible spectrophotometer (Jasco Model V-530, Japan) at wavelengths from 300 to 800 nm. Fluorescence spectra were carried out using a fluorescence spectrometer (FluoroMax-4, France) in the range 500–800 nm using 425 nm excitation wavelength. All the measurements were carried out at room temperature.

**Singlet oxygen generation**

1,3-Diphenylisobenzofuran (DPBF) supplied by J&K Scientific was used for the detection of the singlet oxygen.
In vivo photosensitizing efficacy

Cell lines and culture conditions

Human esophageal cancer cell line Eca-109 was obtained from the Type Culture Collection of the Chinese Academy of Sciences. All cell culture reagents were purchased from MesGen Biotech (Shanghai, China). Cells were cultured in normal RPMI-1640 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.

Cytotoxicity

The dark- and photocytotoxicity of PSs were analyzed in Eca-109 cells following the procedures as described in the literature (Tang et al. 2005). 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 × 10⁴ cells per well. The cells were allowed to attach to the bottom of the wells for 24 h prior to starting the experiment. RPMI-1640 medium containing I₁, I₂, I₃ and I₄ was measured by fluorescent probe 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) (Wang et al. 2014; Rubio et al. 2014; Kim et al. 2011). Eca-109 cells were successively incubated with 1 μM photosensitizer for 4 h, and then irradiated by 650 nm laser (2 J/cm², 40 mW/cm²) for 50 s, followed by incubating with DCFH-DA for 30 min. The fluorescence imaging was monitored by inverted fluorescence microscope (Leica, DMI8, Germany).

Intracellular ROS generation

The intracellular ROS generation of compounds I₁, I₂, I₃ and I₄ was measured by fluorescent probe 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) (Wang et al. 2014; Rubio et al. 2014; Kim et al. 2011). Eca-109 cells were successively incubated with 1 μM photosensitizer for 4 h, and then irradiated by 650 nm laser (2 J/cm², 40 mW/cm²) for 50 s, followed by incubating with DCFH-DA for 30 min. The fluorescence imaging was monitored by inverted fluorescence microscope (Leica, DMI8, Germany).

Flow cytometry analysis

Flow cytometry analysis was conducted using Annexin V-EGFP/PI Apoptosis Detection Kit (KeyGEN BioTECH, China) following the manufacturer’s protocol. Briefly, Eca-109 cells were seeded in a six-well plate and incubated with the compounds at the concentration of 4 μM for 4 h and then irradiated with a light dose of 2 J/cm² (λ = 650 nm), followed by incubation for 6 h at 37 °C. After the treatment, the media and cells were collected for each sample. The cells were resuspended with binding buffer in a 12 × 75 test tube, and then 5 μL of Annexin V and 5 μL of PI staining solution were added to each tube followed by incubation for 20 min at room temperature in the dark. The fluorescence of the cells was immediately determined by BD FACS Calibur Flow cytometer (Franklin Lakes, NJ).

Different PSs mediated PDT in vivo

Animals model

The BALB/c nude mice (female, 18–20 g) were obtained from Shanghai SLAC Laboratory Animal Company and housed in an air-conditioned room at 23 ± 2 °C with dedicated pathogen-free barrier facilities. For the establishment of tumor model, the 5 × 10⁶ Eca-109 cells in 1 mL PBS were subcutaneously injected into the right region of BALB/c mice. When tumors reached a volume range from 100–150 mm³, the following experiments were carried out. All animal procedures were performed according to the Guidelines of the Laboratory Protocol of Animal Handling and with approval from the Animal Care and Use Committee of Donghua University.

In vivo photosensitizing efficacy

The tumor xenograft mice were randomly divided into the following groups of five each: (1) control, (2) 120 J/cm² light alone, (3) 2 mg/kg HMME + 120 J/cm² light, (4) 2 mg/kg I₁ + 120 J/cm² light, (5) 2 mg/kg I₂ + 120 J/cm² light, (6) 2 mg/kg I₃ + 120 J/cm² light, (7) 2 mg/kg I₄ + 120 J/cm² light.
light. The control mice were injected with the same dose of PBS. Five minutes after the caudal vein injection, the tumor sites of mice were irradiated with laser light (400 mW/cm²) for 5 min, and the total light dose was 120 J/cm². After the treatment, the length (L) and width (W) of tumors were measured with a caliper every other day. The tumor volume was calculated using the formula: \( V = L \times W^2 \times 0.5 \). After 14 days post-treatment, the mice were sacrificed and the tumors were removed and weighed.

**Histological analysis**

After 1 day-post treatment, tumor tissues from different groups were fixed with 4% paraformaldehyde fix solution for 24 h, dehydrated through graded alcohols, and embedded in paraffin. The paraffin-embedded section (6 μm thick) were stained with hematoxylin and eosin (H&E). Histopathological changes were observed under a light microscope (Nikon E600, Japan).

**Results**

**Chemistry**

5,15-Diaryl-10,20-dihalogeno porphyrins \( \text{I}_{1-6} \) were obtained by halogenation and hydrolysis of porphyrins synthesized by standard Lindsey protocol in 30–50% total yields, as shown in Scheme S1 (Vinogradova et al. 2009). The synthesis of 5,15-diaryl-10,20-dibromoporphyrins \( \text{II}_{1-4} \) was shown in Scheme S2. An amide condensation reaction occurred among compounds \( \text{I}_1, \text{I}_2 \) and amino-acid esters. The compounds \( \text{I}_{4a-d} \) were hydrolyzed to give the target compounds \( \text{II}_{1,4} \) in 56–70% total yields. The structures of these porphyrins \( \text{I}_{1,6}, \text{II}_{1,4} \) were characterized by \(^1\text{H} \) NMR, \(^{13}\text{C} \) NMR and HR-MS (Figure S1–25).

**Density functional theory (DFT) studies**

In general, a smaller gap of HOMO and LUMO energy level (\( \Delta E \)) corresponds to a stronger driving force toward the electron-transfer state. Frontier molecular orbitals (FMOs) of the HOMO and LUMO energy (eV) levels for the optimized structures of \( \text{PS}_1, \text{I}_2, \text{I}_5 \) and \( \text{I}_6 \) (Fig. 3) were calculated. According to our DFT calculations, \( \Delta E \) of compounds \( \text{I}_2 \) (0.09637 eV), \( \text{I}_5 \) (0.09675 eV) and \( \text{I}_6 \) (0.09400 eV) were smaller than compound \( \text{PS}_1 \) (0.10184 eV) due to the existence of halogen atoms (Fig. 3). In addition, it was observed that their corresponding \( \Delta E \) values decreased with the increase of relative atomic mass of halogen atoms of meso-substituents.

**Photophysical properties**

The UV–Vis absorption spectrum of the 5,15-diaryl-10,20-dihalogeno porphyrins \( \text{I}_{1-6} \) and \( \text{II}_{1-4} \) in DMSO were presented in Fig. 4A. All compounds exhibited an intense B band at ~ 425 nm, and four Q bands at around 523, 558, 602 and 660 nm. Among these compounds, \( \text{I}_6 \) showed the longest absorption wavelength at 664 nm. The unhalogenated porphyrins \( \text{PS}_1 \) had fluorescence while 5,15-diaryl-10,20-dihalogeno porphyrins \( \text{I}_{1-6} \) and \( \text{II}_{1,4} \) have no fluorescence (Fig. 4B).

![Fig. 3 FMOs and \( \Delta E \) of \( \text{PS}_1, \text{I}_2, \text{I}_5, \text{I}_6 \)](image-url)
Singlet oxygen generation

To evaluate the ability to produce singlet oxygen, 1,3-diphenylisobenzofuran (DPBF) was used as the quencher. The generation rate of singlet oxygen was measured by monitoring the decrease in absorbance at 415 nm with regular intervals of 10 s upon irradiation with 650 nm laser light. As shown in Fig. 5A, decomposition of DPBF did not occur in the absence of singlet oxygen. The representative halogenated porphyrins (I2, I6, II4) were irradiated with 650 nm laser and their absorbance did not decrease with the increase of irradiation time, which indicated that halogenated porphyrins had good photostability. Time-dependent changes of the absorption spectra of various PSs containing DPBF under light irradiation were shown in Fig. 5B, C and Figure S26. The singlet oxygen generation rates of all halogenated compounds were summarized in Table 1. The results showed that the ability of singlet oxygen generation of halogenated porphyrins PS1 and HMME.

In vitro photosensitizing efficacy

MTT assays were used to test the in vitro cytotoxicity of the target compounds against Eca-109 cells. As shown in Fig. 6A and Table 2, the cell viabilities after the treatment with compounds I3,4, and II1,4 at 10 μM were higher than 80% under dark condition while other compounds were lower than 80% under the same conditions, which suggested that compounds I3,4, and II1,4 had negligible dark cytotoxicity. There was little difference between the IC50 values of halogenated porphyrins (I2, I5 and I6) with Cl, Br and I under dark or light irradiation, as shown in Figure S27. The IC50 values of all target compounds were evidently smaller than HMME under light irradiation of 12 J/cm2 (Table 2). It was noteworthy that significant decrease in cell viability of Eca-109 cells after the treatment with compounds I1,3, I5,6, and II1,4 at concentration of 4 μM and a light dose of 12 J/cm2 was observed (Figure S27 and Fig. 6B), which indicated that these compounds had obvious photodynamic activities against Eca-109 cells.
Intracellular ROS generation

To confirm ROS generation ability of compounds in Eca-109 cells, fluorescence probe DCFH-DA was chosen as the indicator of $^{1}\text{O}_2$ after PDT treatment. As illustrated in Fig. 7, the cells in the control group, light alone group and PSs alone group exhibited dim green fluorescence, while the cells in PDT groups showed intense green fluorescence under same irradiation condition. It indicated that compounds I$_3$, I$_2$, I$_4$, and I$_4$ could generate ROS under irradiation in Eca-109 cells. The fluorescence intensity of I$_4$-PDT group was visually higher than I$_3$-PDT group, I$_2$-PDT group and I$_3$-PDT group.

Table 1 Singlet oxygen generation rate of compounds I$_1$-I$_6$, II$_1$-II$_4$

| Compound | $k \times 10^{-2}$ (s$^{-1}$) | Compound | $k \times 10^{-2}$ (s$^{-1}$) |
|----------|-----------------|----------|-----------------|
| I$_1$    | 0.27            | II$_1$   | 0.29            |
| I$_2$    | 0.60            | II$_2$   | 0.43            |
| I$_3$    | 0.16            | II$_3$   | 0.30            |
| I$_4$    | 0.64            | II$_4$   | 0.61            |
| I$_5$    | 0.68            | PS$_1$   | 0.014           |
| I$_6$    | 0.64            | HMME     | 0.026           |

Fig. 5 The singlet oxygen generation of compounds. A Photodegradation of DPBF and compounds I$_2$, I$_6$, II$_4$ under 650 nm laser irradiation (5 mW/cm$^2$) every 10 s. B Photodecomposition of DPBF in the presence of II$_4$ under 650 nm laser irradiation (5 mW/cm$^2$) every 10 s. C First-order plot for the photodecomposition of DPBF after PDT with the compounds.

Fig. 6 The efficacy of compounds against Eca-109 cells in vitro. A Dark cytotoxicity of compound I$_1$-I$_6$, II$_1$-II$_4$ at concentrations ranging from 0 to 30 μM in Eca-109 cells. B The cell viabilities treated by II$_4$ at concentrations ranging from 2 to 10 μM under different light doses. Data represents mean ± SD.
Flow cytometry analysis

Tumor cells are killed by PDT through apoptotic and/or non-apoptotic (necrosis, autophagy) pathways, through damaging tumor vasculature and immune response. The effects relied on the type, dosage and localization of PSs, incubation time, oxygen level, genotype of cells subjected to PDT etc. Apoptosis is likely caused by the reactive oxygen radicals produced by the photodynamic effect of the PSs localized in mitochondria, while necrosis is the major form of tumor cells death induced by photosensitizers that localize in plasma membrane and lysosomes (Chilakamarthi and Giribabu 2017). To investigate the cell death induced by different PSs, Eca-109 cells after PDT were incubated for 5 h and stained with the Annexin V-FITC/PI apoptosis assay kit for flow cytometry analysis. As shown in Fig. 8, no necrotic cells were detected in the control group. After 5 h post-PDT, the proportion of living cells were decreased in all PDT-treatment groups, especially dropped to 48.40 and 45.58% in $I_3$ and $I_4$ groups respectively. $I_3$-PDT mainly induced cell necrosis while $I_3$-PDT could induce cell necrosis and apoptotic.

**Different PSs mediated PDT in vivo**

Since compounds $I_3$, $I_2$, $I_3$ and $I_4$ had more obvious photodynamic cytotoxicity than others under the same conditions, their photodynamic efficacy in Eca-109 tumor-bearing BALB/c nude mice were evaluated. The compounds at a dose of 2 mg/kg were injected intravenously into mice, followed by irradiation of 120 J/cm² light at tumor site for single time. As shown in Fig. 9A–C, almost negligible inhibition of tumor growth was observed from the mice treated with light only. Notably, $I_2$ and $I_4$ showed more prominent anti-tumor PDT effect compared to the control group, light group and HMME group, especially $I_4$. After 1-day post treatment, the tumor tissue sections were separated then stained with hematoxylin and eosin (H&E) reagent. As shown in Fig. 9D, significant tumor tissue damage in the PDT group could be observed under microscopy compared to the control group. The results of the tumor growth curve and histological examination showed that $I_4$ had the best photodynamic antitumor activity in vivo.

**Discussion and conclusion**

In this study, series novel photosensitizers were prepared and their photodynamic activities were investigated. The longest absorption wavelength of halogenated porphyrins was superior to unhalogenated porphyrins as shown in Fig. 4, which was correlated with their small $\Delta E$ calculated by the DFT. It is interesting that all halogenated porphyrins showed no fluorescence emission, which is caused by the corresponding smaller $\Delta E$ values to transfer most of the first excited state populations of PSs into triplet states.
excited state. In addition, as shown in Table 1, the singlet oxygen generation rates of all halogeno compounds were remarkably higher than PS1 and HMME, because the increase of the triplet excited state populations of halogenated porphyrins was beneficial to the generation of singlet oxygen. The singlet oxygen generation rates of porphyrin-amino acid conjugates have been improved to a certain extent, especially bromoporphyrins linked with 5-aminolevulinic acid (II2, II4). However, the singlet oxygen generation rates of bromoporphyrin (I3), chloroporphyrin (I5) and iodoporphyrin (I6) are slightly different. It was also showed that all compounds (I1, I2, I3, I4, II1, II2, II3, II4) had more obvious photodynamic anti-Eca-109 cell activities in vitro than compounds HMME at the same treatment conditions, and the compounds II3, II4 had negligible dark cytotoxicity. Moreover, with 12 J/cm² irradiation of 650 nm laser, compounds II4 at 4 μM had the excellent anti-tumor effect and low dark toxicity in vitro. Besides, compounds I3, II2, II3 and II4 could generate intracellular ROS under irradiation, especially II4-PDT showed high generation level of intracellular ROS. Necrosis is the major form of tumor cells death induced by PSs that localize in plasma membrane and lysosomes. Cell necrosis rate of II4-PDT was significantly higher than that of I3-PDT, which indicated their subcellular localization may be different. II4-PDT mainly induced cell necrosis, while I3-PDT could induce cell necrosis and apoptotic. In addition, compounds II3 and II4 with amino acid groups have higher cell necrosis rates compared to compound I3 when the substituents located at the same positions, while compound II4 with substituents on the meta position of the benzene ring had higher one compared to compound II2 with substituents on the para position when the substituents were the same (Fig. 8). These results implied that the kind and position of the substituents on benzene ring greatly affected the cell death pathway. Notably, compound II4 showed more prominent anti-tumor photodynamic efficacy in Eca-109

Fig. 8 The extent and mode of cell death induced by different PSs-PDT. A Flow cytometric assay of I3, II2, II3 and II4 at 4 μM exposed to 2 J/cm² of light. LL: annexin V (−) PI (−), lived cell; LR: annexin V (+) PI (−), early apoptotic cells; UR: annexin V (+) PI (+), late apoptotic cells; UL annexin V (−), necrotic cells. B Histogram of apoptotic cells, necrosis cells and live cells after the treatment. Data represents mean ± SD.
tumor-bearing BABL/c nude mice (Fig. 9), which was consistent with the phototoxicity in vitro and intracellular ROS generation level. In summary, II₄, with 5-aminolevulinic acid group at periphery of tetrapyrrole ring, showed excellent photodynamic efficacy on Eca-109 cells in vitro and in vivo. So it has the potential to act as a photodynamic anti-tumor drug.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00432-022-04037-7.

**Acknowledgements** This work was supported by the National Natural Science Foundation of China (No. 21977016), Foundation of Shanghai Science and Technology Committee (No. 19410711000, 20430730900, 20490740400, 21430730100).

**Author contributions** The manuscript was written through contributions of all authors. All authors have read and agreed to the final version of the manuscript.

**Declarations**

**Conflict of interest** The authors have declared no conflict of interest.

**References**

Allison RR, Downie GH, Sibata CH et al (2004) Photosensitizers in clinical PDT. Photodiag Photodyn 1(1):27–42. https://doi.org/10.1016/S1572-1000(04)00007-9

Banfi S, Caruso E, Gramatica P et al (2006) Comparison between 5,10,15,20-tetraaryl- and 5,15-diarylporphyrins as

**Fig. 9** Evaluation of tumor growth inhibition in the Eca-109-tumor-bearing model mediated by PDT. **A** Tumor images after 14 days. **B** Tumor growth curves after different treatments. *P < 0.05, **P < 0.01, ***P < 0.001 vs HMME-PDT group. **C** Tumor weight. **D** Histological sections of tumor tissues stained with hematoxylin and eosin. Scale bar = 50 μm
Zhu W, Gao YH, Chen ZL et al (2018) Comparison between porphin, chlorin and bacteriochlorin derivatives for photodynamic therapy: synthesis, photophysical properties, and biological activity. Eur J Med Chem 160:146–156. https://doi.org/10.1016/j.ejmech.2018.10.005

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.