International Journal of Pharmaceutics 505 (2016) 369–375

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Intracellular uptake and fluorescence imaging potential in tumor cell of zinc phthalocyanine

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ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 5 April 2016
Accepted 12 April 2016
Available online 13 April 2016

Keywords:
Nuclear imaging
Breast cancer
^{131}I
Radiolabeling
Phthalocyanine
Dual imaging

ABSTRACT

A near IR absorbing phthalocyanine bearing four binaphtyl group has been synthesized in order to investigate its cytotoxicity and intracellular uptake of sensitizer on MCF-7 (human breast cancer), MDAH (ovarian cancer), HeLa (human epitheloid cervix carcinoma), EMT-6 (mouse breast cancer) and WI-38 (human fibroblast lung) cell lines. ZnPc showed four time higher intracellular uptake in carcinoma cells (MCF-7) than normal (WI-38) cell lines. With the aim of studying in detail the biodistribution feature and tumor nuclear imaging capacity, ZnPc was also labeled with I-131. The efficiency of radiolabeled compound was $95 \pm 4.6\%$. In addition, ZnPc reveals to be very efficient singlet oxygen generators ($\Phi\Delta = 0.612$ in DMSO) and promising PS for PDT application. *In vitro* fluorescence imaging study with MCF-7 cells showed that ZnPc localized in cytoplasm of the cells. This results showed that synthesized ZnPc is promising candidate for dual fluorescence/nuclear imaging breast cancer and shows potential PS for PDT application.

1. Introduction

Photodynamic therapy (PDT) has attracted great attention as a medical treatment, in which light energy absorbed by chromophores is transferred to oxygen molecules in the cell to produce toxic singlet oxygen, which causes cell death (Dolmans et al., 2003). PDT has a number of considerable advantages in terms of tumor selectivity, low toxicity and good repeatability over the conventional anti-cancer treatments such as radiation, surgery and chemotherapy that exhibit deleterious side effects (Wilson and Patterson, 2008). In the process of PDT, a non-toxic photosensitizer (PS), which locates in the tumour tissue, absorbs a specific wavelength of light, and then leads the formation of cytotoxic reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) through energy transfer processes to the surrounding oxygen, which induces damage and death of cancer cells (Juarranz et al., 2008). From PDT treatment point of view the photosensitizer plays a crucial role in improving the efficacy of PDT. Ideal photosensor should have high absorption coefficient in the near–infrared (NIR) region in order to penetrate deeper into tissues, thus causes less damage than shorter wavelength light. Moreover, photosensitizers should be selectively accumulated in the target tissue (Nyman and Hynninen, 2004). Phthalocyanines (Pcs), thus, fulfil many essential requirements of efficient sensitizers for PDT (Bonnett, 1995). Up to now, several Pc derivatives have been extensively studied as notable second-generation photosensitizers in photodynamic therapy applications due to their suitable physical and chemical properties. They exhibit intense absorption in the red visible region with high extinction coefficients ($\varepsilon > 10^5\,M^{-M}\,cm^{-c}$) which is required to penetrate deeply into tissues (Allen et al., 2001).

Zinc phthalocyanines present intense fluorescence in the near infrared region which render them an excellent photosensitizer for NIR fluorescence imaging method that has been used the diagnosis of cancer (Moan et al., 1998; Witjes et al., 1996; Nesterova et al., 2009). It is known that radiolabeled phthalocyanines can be used

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http://dx.doi.org/10.1016/j.ijpharm.2016.04.023
0378-5173/

as nuclear imaging agent (Ranyuk et al., 2013a,b; Ma et al., 2003; Scasnar and Vanlier, 1993). Lipophilic photosensitizers are preferentially transported by lipoproteins, which are uptaken directly by tumor cells (Jori, 1989).

In this context, ZnPc bearing four binaphtyl group has been synthesized in order to investigate its cytotoxicity and intracellular uptake of sensitizer on MCF-7 (human breast cancer), MDAH (ovarian cancer), HeLa (human epitheloid cervix carcinoma), EMT-6 (mouse breast cancer) and WI-38 (human fibroblast lung) cell lines. ZnPc was also labeled with I-131. The singlet oxygen generation potential was tested. The ZnPc was labeled with ^{131}I using iodogen method in order to investigate intracellular uptake in different cells for as a tumor fluorescence/nuclear imaging agent potential.

2. Materials and methods

2.1. Materials

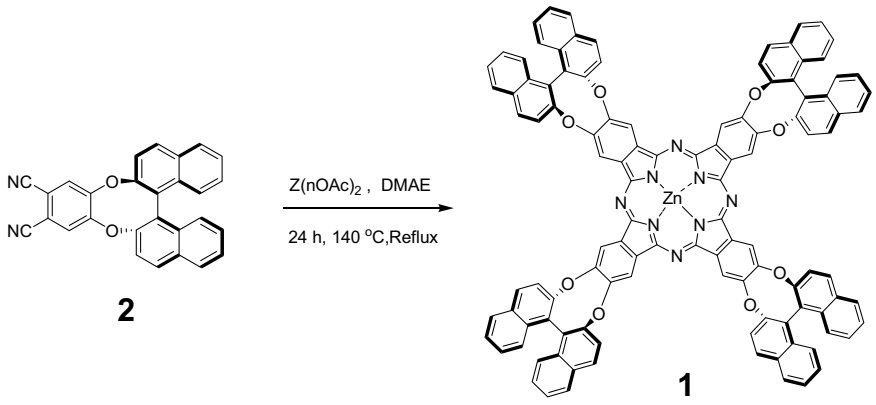
Thin-layer chromatography-cellulose gel (ITLC-F plastic sheets 20 × 20) was supplied from Merck. Iodogen was purchased from Sigma-Aldrich. Radiolabeling experiments were analyzed using a Bioscan AR2000 TLC Scanner. All chemicals used for the *in vitro* studies were supplied from Biological Industries; all other chemicals were provided from Merck. Cell culture studies were performed in a Thermo MSC Advantage 1.2 laminar air flow cabinet. An Olympus Japan inverted light microscope was used for counting cells. A Thermo Multimode microplate reader was used to determine the IC_{50} values of cell cultures. All chemicals and 1,3-diphenylisobenzofuran (DPBF) were purchased from Aldrich Chemical Co. and used without further purification. The monitoring of the reactions has been carried out by thin layer chromatography (TLC), was carried out on aluminum sheets coated with silica gel type 60 F254 (E. Merck). Purification and separation of the synthesized products were performed by column chromatography, using silica gel Merck-60 (230–400 mesh, 60 Å). Infrared spectra (IR) were performed with Perkin-Elmer, FT-IR/MIR-FIR (ATR, Attenuated total reflectance) spectrophotometer. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) MS spectra were determined on a BRUKER Microflex LT. Nuclear magnetic resonance spectra (NMR) (^{1}H NMR) spectra were recorded with a Bruker AC-400 instrument. UV/Vis spectra were recorded with an Analytic JENA S 600 UV–vis spectrophotometer. 4,5-Dichlorophthalonitrile (Wöhrle et al., 1993) and (*R*)/(*S*)-benzo[b]dinaphtho[2,1-e:1′,2′-g][1,4]dioxocine-5,6-dicarbonitrile (Wang et al., 2012) were prepared according to literature procedures. (*R*)- and (*S*)-2,2′-dihydroxy-1,1′-binaphthyl was commercially available. The ZnPc was prepared following a modification of the synthesis of such compounds by Wang et al., (2012).

2.2. Synthesis of (R)/(S)-tetrakis(dinaphtho)[1,2-e:1′,2′-g]-1,4-(dioxocine)[2,3-b;2′,3′-k;2″,3″-t;2‴,3‴-c′] phthalocyaninato zinc complex

A mixture of (*R*)/(*S*)-benzo[b]dinaphtho[2,1-e:1′,2′-g][1,4]dioxocine-5,6-dicarbonitrile (100 mg, 0.243 mmol) and $(ZnOAc)_2$ (12 mg, 0.061 mmol) in DMAE (3 mL) was stirred at 140 °C under argon atmosphere for 24 h. After cooling to room temperature, the solvent was removed and the residue was washed with a MeOH/H_2O (5:1) mixture. The crude product was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 10:0.1) to give **ZnPc** (70 mg, 0.04 mmol) as a green solid. Yield: 17%.^{1}HNMR (d_8-DMSO, 400 MHz): δ (ppm) = 9.49 (s, 8H), 8.23 (d, J = 8, 8H), 8.14 (d, J = 8, 8H), 8.1–8.0 (m, 8H), 7.62–7.51 (m, 24H). IR (ATR): ν (cm^{-1}) = 2920, 2850, 1588, 1442, 1395, 1262, 1215, 1088, 950, 830, 740. UV/Vis (DMSO): λ_{max} (log ε) = 680 (5.3), 613 (4.6), 359 (4.9). MS (MALDI-TOF, ATRA): m/z calcd for $C_{112}H_{56}N_8O_8Zn$: 1704.35; found: 1704.3.

2.3. Singlet oxygen measurements

The singlet oxygen quantum yield (ΦΔ) is a measurement of the conversion of molecular oxygen into reactive oxygen when a photosensitizer irradiated by the light. In this study, the singlet oxygen quantum yield (ΦΔ) was determined by using 1,3-diphenylbenzofuran (DPBF) as a quencher, according to the literature procedure (Ince et al., 2016). Non-substituted **ZnPc** in DMSO was used as the reference (ΦΔ (DMSO) = 0.67). A halogen lamp (300 W, Optel) was used as a light source. To filter of light under 515 nm and far infrared radiations, a glass (Jenger Glaswerk Schott & Gen., Mainz OG 515) and water filters were used, respectively. DPBF was dissolved in DMSO (2 mL, with an absorbance of ca. 1) in a 1 × 1 cm quartz optical cell and bubbled with oxygen for 60 s. The ZnPc solution in DMSO (with an absorbance of ca. 0,1) was then added to solution of DPBF, to give an absorbance of ca. 0.1. The working solution was placed at 60 cm from the light source and irradiated for defined time intervals by using a halogen lamp (300 W). The experiment was repeated three times. The decrease of DPBF concentration with irradiation time was monitored by UV/vis absorption spectroscopy at 418 nm due to formation of singlet oxygen (Scheme 1).



Scheme 1. Synthetic route to ZnPc.