Journal of Luminescence 205 (2019) 385–392

Contents lists available at ScienceDirect

Journal of Luminescence

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

Evaluation of the photosensitizing properties of zinc and indium tetra cinnamic acid phthalocyanines linked to magnetic nanoparticles on human breast adenocarcinoma cells

Gauta Gold Matlou, David O. Oluwole, Tebello Nyokong*

Centre for Nanotechnology Innovation, Department of Chemistry, Rhodes University, Grahamstown 6140, South Africa

ARTICLE INFO

Keywords:
Photodynamic therapy
Cinnamic acid phthalocyanine
Magnetic nanoparticles
Singlet oxygen quantum yield

ABSTRACT

This work reports on the synthesis, photophysico-chemical properties and photodynamic therapy activity of novel zinc (**1**) and indium (**2**) tetra substituted cinnamic acid phthalocyanine (Pc) complexes linked to amino functionalized magnetic nanoparticles (AMNPs) through an amide bond. Asymmetric ZnPc complex (**3**) showed better triplet and singlet oxygen quantum yields as compared to its symmetrical analogues (**1** and **2**). The AMNPs (**1**-AMNPs and **2**-AMNPs) linked conjugates depicted increased triplet quantum yields in comparison to their unlinked Pcs, while **3**-AMNPs showed a decrease compared to **3**. The complexes showed increased *in-vitro* photocytotoxic effect against MCF-7 cells with an increase in drug concentration. At 80 μg/mL, **2** and **3**, **2**-AMNPs and **3**-AMNPs with higher singlet oxygen quantum yields caused more cytotoxic effect on the cancer cells in the presence of light as compared to **1** and **1**-AMNPs respectively.

1. Introduction

Photodynamic therapy (PDT) is an attractive treatment method for cancer. This treatment modality is dependent on the synergy between photosensitizer (PS) and light in the presence of oxygen to selectively destroy the tumorigenic cells [1]. Phthalocyanines (Pcs) are macrocycles commonly used as photosensitizer in PDT [1,2] due to their excellent ability to generate cytotoxic singlet oxygen specie, known for its efficacious tumor elimination [3]. The diverse physico-chemical functions of the Pcs can be achieved by varying the ring substituents and insertion of a heavy atom in the Pc macrocycle cavity to promote rapid intersystem crossing to the excited triplet state [4,5].

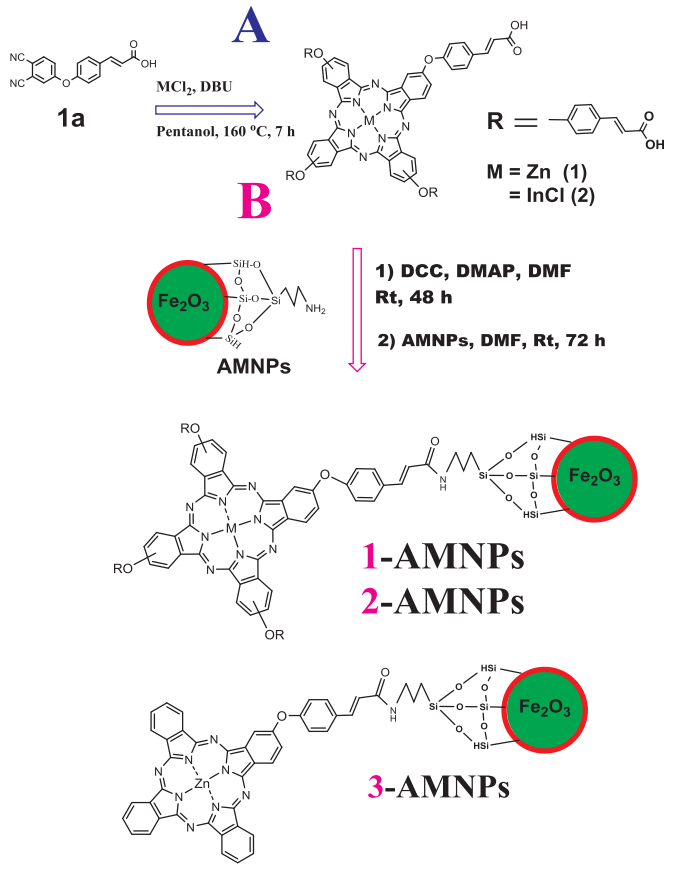
Cinnamic acids (3-phenyl-2-propenoic acid) are natural carboxylic acid group containing compounds found in plants [6]. They are precursors of many metabolites which include coumarins, flavonoids, phenyl-propanoids, stilbenes and spermidines [7]. Cinnamic acid derivatives are proven therapeutic agents with remarkable inhibitory activity against fungi, tumor and parasites [8,9] making them important drug candidates. In this work, cinnamic acids are employed as ring substituents for zinc (**1**) and indium (**2**) phthalocyanines (Pc) since they both possess antitumor activity. The –COOH moiety of the cinnamic acid of the substituted ZnPc and InPc will be covalently linked to amino functionalized magnetic nanoparticles (AMNPs), Scheme 1. Zn and In will be used as central metals for the Pc due to their size which encourages spin orbit coupling (also known as heavy atom effect), which foster rapid intersystem crossing to the triplet state [10].

On the other hand, iron oxide magnetic nanoparticles have shown potential as efficient platform for drug loading in nanocarrier formulations due to their unique physicochemical properties [11]. In cancer therapy, nanoparticles loaded with drug complexes can deliver the drugs at a tumor site by taking advantage of the enhanced permeability and retention (EPR) effects [12]. More so, the magnetic nanoparticles used in this work are known to foster enhancement of the physico-chemical properties of Pc complexes when in dyad forms [13,14]. PS attached to MNPs also possess a further advantage of a targeted PDT mode combined with magnetic resonance imaging [15]. This motivated the choice to link Pc complexes to AMNPs for PDT applications in this work.

In our previous work, we reported on the photophysical behavior of low symmetry ZnPc having a single cinnamic acid as its ring substituent (**3**) and its amide bond conjugates with AMNPs (**3**–AMNPs), Scheme 1 [16]. Amide bonds have an advantage of being stable even above physiological conditions [17]. PDT is administered within physiological conditions, hence is not expected to denature the amide bond or healthy bodily functions [18]. This work reports on the synthesis and comparison of the photophysico-chemical properties of symmetrical cinnamic acid substituted Zn (ZnTCAPc, **1**) and In (InTCAPc, **2**) Pcs when alone and in conjugates with AMNPs (**1**-AMNPs and **2**-AMNPs), Scheme 1.

* Corresponding author.
E-mail address: t.nyokong@ru.ac.za (T. Nyokong).

https://doi.org/10.1016/j.jlumin.2018.09.054
Received 10 August 2018; Received in revised form 20 September 2018; Accepted 22 September 2018
Available online 24 September 2018



Scheme 1. A) Synthesis of zinc (1) and indium (2) tetra cinnamic acid phthalocyanines and B) chemical linkage of 1 and 2 to AMNPs to afford 1-AMNPs and 2-AMNPs. 3-AMNPs [16] have been previously reported.

The *in vitro* dark cytotoxicity and PDT effect of complexes **1**–**3** against human breast adenocarcinoma cell will be compared with their conjugates: **1**-AMNPs, **2**-AMNPs and **3**-AMNPs. The effect of symmetry will be evaluated by comparing the tetra substituted complex **1** with the monosubstituted complex **3**.

2. Experimental

2.1. Materials

Zinc (II) chloride, indium (III) chloride, absolute ethanol, methanol, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), N, N′- dicyclohexylcarbodiimide (DCC), 4-(di-methyl amino) pyridine (DMAP), 1.3-diphenylisobenzofuran (DPBF), 1-pentanol, and 2′,5′ dihydroxy acetophenone (used as a MALDI-TOF matrix) were purchased from Sigma–Aldrich. Tetrahydrofuran (THF) was obtained from MINEMA. 1, 8-Diazabicyclo [5.4.0] undec-7-ene (DBU) was purchased from Fluka. Human breast adenocarcinoma cell cultures (MCF–7 cells) were procured from Cellonex®. Dulbecco Modified Eagle's Medium (DMEM) and Dulbecco phosphate-buffer saline (DPBS) were purchased from Lonza®. Heat-inactivated fetal bovine serum (FBS) and 100 unit/mL penicillin-100 µg/mL streptomycin-amphotericin B were obtained from Biowest®. The syntheses of cinnamic acid phthalonitrile (3-(4-(3,4-dicyanophenoxy) phenyl) propenoic acid) (**1a**) [16], **3** (Zn mono cinnamic acid phthalocyanine) [16], **3**-AMNPs [16], and iron oxide magnetic nanoparticles functionalized with amino groups (AMNPs) [19], have been reported before.

2.2. Equipment

The ground state electronic absorption was measured using a Shimadzu® UV-2550 spectrophotometer. Fluorescence excitation and emission spectra were collected on a Varian Eclipse® spectrofluorometer using a 360–1100 nm filter. The Excitation spectra were measured using the Q-band of the emission maxima. Bruker® Alpha FT-IR spectrophotometer with universal attenuated total reflectance (ATR) was used to measure the FT-IR spectra. Bruker® Autoflex III smartbeam TOF/TOF Mass spectrophotometer was used to measure the mass spectra of the complexes using 2′,5′ dihydroxy acetophenone as a matrix. Elemental analysis (CHN microanalysis) was recorded using a Vario-Elementar® Microcube ELIII. Bruker® AMX 600 MHz NMR spectrometer was used to measure the proton (^{1}H) spectra.

X-ray powder diffraction (XRD) patterns were recorded on a Bruker® D8 Discover equipped with a Lynx Eye detector, using Cu K α-radiation (l = 1.5405 Å, nickel filter). The XRD data were fitted using Eva (evaluation curve fitting) software. Baseline corrections were performed on each diffraction pattern. Dynamic light scattering (DLS) experiments were conducted on a Malvern® Zetasizer Nanoseries, Nano-ZS90.

Fluorescence lifetimes were measured on a time correlated single photon counting (TCSPC) setup (FluoTime 300, Picoquant® GmbH). The excitation source was a diode laser (LDH-P-670 driven by PDL 800-B, 670 nm, 20 MHz repetition rate, 44 ps pulse width, Picoquant® GmbH). Triplet quantum yields were determined using a laser flash photolysis system. The excitation pulses were produced using a tunable laser system consisting of an Nd: YAG laser (355 nm, 135 mJ/4–6 ns) pumping an optical parametric oscillator (OPO, 30 mJ/ 3–5 ns) with a wavelength range of 420–2300 nm (NT-342B, Ekspla). The fitting of the triplet lifetimes was determined by exponential fitting of the kinetic curve using OriginPro® 8 software. For laser flash photolysis studies, the samples and unsubstituted ZnPc standard solutions had an equal absorbance at the Q band. The solutions were then introduced into a spectrophotometer cell of 1 cm path length, de-aerated using argon, sealed and illuminated using an appropriate excitation wavelength (crossover wavelength at Q-band of the sample and the standard, which was ~ 670).

Photocatalysis for singlet oxygen quantum yield studies was carried out using irradiation from a halogen lamp (300 W), 600 nm glass (Schott) and water filters were used to filter off ultra-violet and far infrared radiation, respectively. An interference filter (Intor, 670 nm with bandwidth of 40 nm) was placed in the light path just before the sample. Light intensities were measured with a POWER MAX 5100 (Molelectron detector incorporated) power meter and were found to be 9.54×10^{18} photons/cm^2 s for singlet oxygen studies. For singlet oxygen, samples and standards were separately mixed with DPBF in DMSO at a volume ratio of 1:1 and the rate of DPBF degradation was monitored at 416 nm and were used to calculate the singlet oxygen quantum yields.

The illumination source for the PDT studies was obtained from Modulight® Medical Laser System (MLS) 7710–680 channel Turnkey laser system coupled with a 2 x 3 W channel at 680 nm, cylindrical output channels, aiming beam, integrated calibration module, foot/hand switch pedal, subminiature version A connectors, and safety interlocks. The illumination kit for *in vitro* PDT studies has capacity to hold 127.76 x 85.48 mm 96 well tissue culture plate [20]. Culturing of the MCF-7 cells was achieved in a 75 cm^2 vented flask (Porvair) in a humidified atmosphere incubator with ~ 5% CO_2 and a physiological temperature of 37 °C (HealForce®). The *in vitro* dark and PDT cytotoxicity studies were performed in triplicates. Zeiss® Axiovert. A1 Fluorescence LED (FL-LED) inverted microscope was used to view the cells under phase contrast. WST1 cell proliferation neutral red reagent (Roche®) with BioTek® Synergy 2 multi-mode microplate reader was used to measure the viable cells.