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Photodynamic therapy activity of zinc phthalocyanine linked to folic acid and magnetic nanoparticles

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ABSTRACT

In this work, the photodynamic therapy (PDT) activities (using human carcinoma adherent MCF-7 cells) of zinc phthalocyanine derivatives: complexes **1** (Zn mono cinnamic acid phthalocyanine) and **2** (zinc mono carboxyphenoxy phthalocyanine) when covalently linked to folic acid (FA) and amine functionalized magnetic nanoparticles (AMNPs) are reported. The covalent linkage of asymmetric zinc cinnamic acid Pc (**1**) to FA (**1**-FA) through an amide bond is reported for the first time. Complex **1** is insoluble in water, but upon linkage to FA, (to form **1**-FA) the molecule become water soluble, hence the UV–Vis spectrum and singlet oxygen quantum yield for **1**-FA were also done in water since water solubility is essential for biological applications. The reported **2**-FA is also water soluble. Linking complexes **1** and **2** to FA and AMNPs decreased the dark toxicity of **1** and **2** on MCF-7 cells. Pc-FA (**1**-FA and **2**-FA) conjugates had better singlet oxygen quantum yields (Φ_Δ) in DMSO as compared to Pc-AMNPs (**1**-AMNPs and **2**-AMNPs). The water- soluble **1**-FA and **2**-FA also achieved a better photodynamic therapy (PDT) activity as compared to **1**-AMNPs and **2**-AMNPs. Folic acid targeting on the tumor cells may have also facilitated better bioavailability of **1**-FA and **2**-FA and improved PDT activity on MCF-7 cells over AMNPs carriers.

1. Introduction

Metallophthalocyanines (MPcs) are well-known photosensitizers (PS) for photodynamic therapy (PDT) due to their ability to generate singlet oxygen in high yields [1, 2]. In PDT, exposure of the PS to light of suitable wavelength, results in PS being excited to the triplet state. This is followed by energy transfer from the excited triplet state of the PS to ground state molecular oxygen resulting in the generation of singlet oxygen which is responsible for the cytocidal activity against tumor cells [3, 4]. The main drawback of PDT comes with the non-specificity and non-selectivity of the PS agents to cancerous cells over normal cells [5]. Retention and specificity can be achieved by linkage of PS to cancer specific molecules such as folic acid and monoclonal antibodies [6–8] or to nanomaterials such as quantum dots, gold nanoparticles, liposomes and magnetic nanoparticles (MNPs) [9–12]. As a result, MPcs have been linked to nanocarriers for improved targeting of tumors through the enhanced permeability and retention (EPR) effect [13]. This work focuses on the PDT activity (on human breast adenocarcinoma cells, MCF-7 cells) of conjugates of asymmetric zinc phthalocyanine complexes (ZnPc) when covalently linked to amino functionalized magnetic nanoparticles (AMNPs) and folic acid (FA). Attachment of FA and MNPs to ZnPc complexes is achieved by amide bond linkage using the COOH groups of the ZnPc and amino groups on AMNPs and FA.

ZnPc complexes are known to generate high triplet and singlet oxygen quantum yields [14], hence ZnPc derivatives (Scheme 1 and Fig. 1) are used in this work. Depending on the central metal and ring substituents, asymmetry in porphyrin type complexes is known to result in improved triplet state properties of PSs [15]. Therefore, the use of asymmetric Pc complexes offers better PS properties while giving defined chemical bonding to FA or AMNPs due to their specific attachment. Iron oxide nanoparticles (Fe_2O_3) are MNPs which have attracted attention in nanotherapeutics due to their biocompatibility, biodegradability and high surface biofunctionality. In cancer therapy, their magnetic properties can be used to guide and localize the therapeutic agents at the tumor site with an aid of an external magnetic field which improves the efficacy of the therapeutic agents [16]. PS attached to MNPs also possess a further advantage of a targeted PDT mode combined with magnetic resonance imaging [17]. This motivated the choice to link Pc complexes to AMNPs for PDT applications in this work.

A number of malignant cells have folate receptors expressed on their surfaces, which helps them to multiply rapidly during cell proliferation

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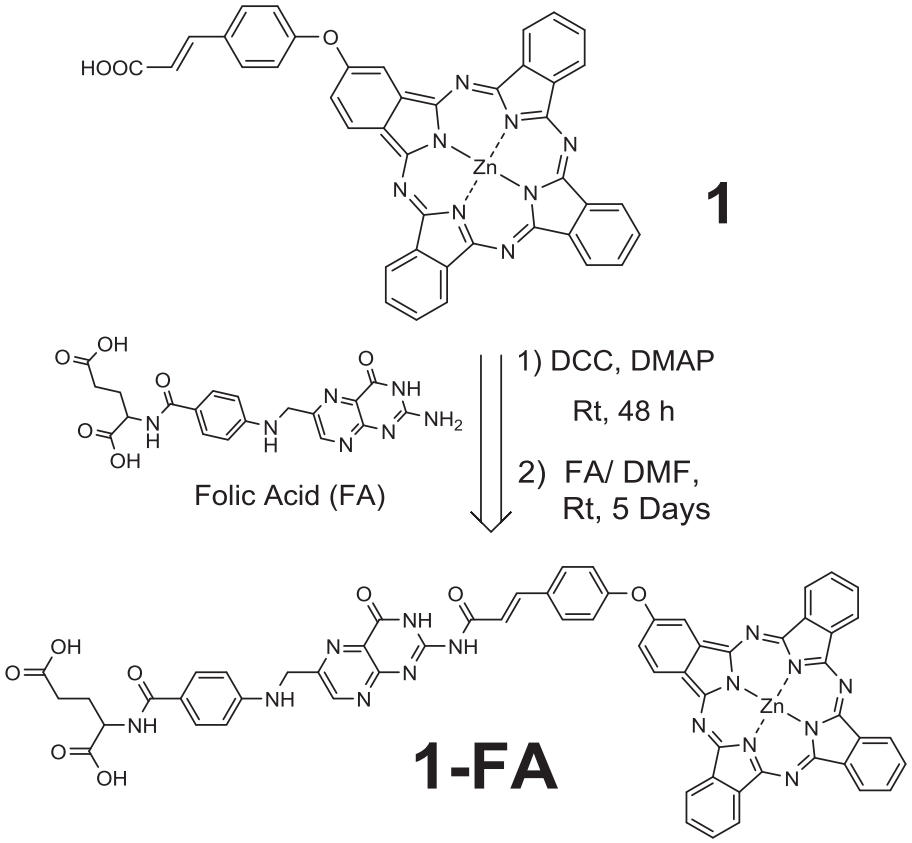

Scheme 1. Chemical bond linkage between the carboxylic group of **1** and the amino group folic acid (FA) to afford linked **1**-FA. Rt = room temperature.

[18, 19]. Linking Pc complexes to FA, presents an attractive target for tumor-selective drug delivery [20] that could improve the PDT activity of the Pc on the cancer cells.

The syntheses of complexes **1** and **2** and their conjugates with AMNPs (**1**-AMNPs and **2**-AMNPs) have been reported [21, 22] as well as the linkage of complex **2** to FA (**2**-FA) [21], the structures are shown in Fig. 1. The linkage of complex **1** to FA is reported in this work for the first time (Scheme 1). The PDT activity of complexes **1** and **2** and their conjugates is reported for the first time. Composites (not covalently linked) of phthalocyanines with magnetic nanoparticles have been employed for PDT [23, 24], this is the first time that covalently linked Pc-MNPs are employed for PDT. Covalent linkage ensures that the two components do not separate during PDT. This work compares complexes **1** and **2**, and their conjugates on their cytotoxic efficiency on MCF-7 cells. The difference in complexes **1** and **2** is the extra double bond on the cinnamic acid group of the former, Fig. 1. This study hopes to bring an insight on PDT efficacy of Pcs linked to FA and AMNPs.

2. Experimental

2.1. Materials

Dicyclohexylcarbodiimide (DCC), folic acid (FA), 4-(dimethyl amino) pyridine (DMAP), anthracene-9,10-bis-methylmalonate (ADMA), and 1,3-diphenylisobenzofuran (DPBF) were purchased from Sigma Aldrich®. Dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) were purchased from SAARChem®. Dichloromethane (DCM) was purchased from B & M Scientific®. Acetonitrile and methanol were purchased from Merck®. AlPcSmix (containing a mixture of sulfonated Pc derivatives and used as a standard for singlet oxygen quantum yields in water) was synthesized according to reported method [25]. Human breast adenocarcinoma cell cultures (MCF–7 cells) were purchased from Cellonex®. Dulbecco Modified Eagle's Medium (DMEM) with phenol red and Dulbecco's modified eagle's medium (DMEM) without phenol red, 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 complexes **1** (Zn mono cinnamic acid phthalocyanine) and **2** (zinc mono carboxyphenoxy phthalocyanine), and of **1**-AMNPs, **2**-AMNPs and **2**-FA have been reported [21, 22].

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. Time correlated single photon counting (TCSPC) setup (FluoTime 300, Picoquant GmbH) was used for the fluorescence lifetime studies. 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).

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 collect mass spectra data using α-cyano-4-hydrocinnamic acid as the matrix in the positive ion mode. Bruker® AVANCE 600 MHz Hz NMR spectrometer were used to measure the ^{1}H NMR spectra.

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). Triplet lifetimes were determined by exponential fitting of the kinetic curve using OriginPro® 8 software. For laser flash photolysis studies, the samples and the ZnPc standard solutions had an absorbance of ~1.5 at the Q