Development of Novel Molecular- and Nano-sensitizers for Sonodynamic Therapy

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Background of Research

Ultrasound has been extensively utilized in clinical diagnostics owing to its manageability, non-invasive, and high tissue-penetrating capability. Sonodynamic therapy (SDT) is a novel and minimally invasive therapeutic modality for the treatment of cancer [1, 2]. It has been garnering significant attention in recent years due to its potential therapeutic outcomes. SDT involves the use of ultrasound waves to stimulate the sonosensitizers to induce reactive oxygen species (ROS), cavitation, gas bubbles, and hyperthermia. When the sonosensitizer is exposed to ultrasound waves, it undergoes a transition to an excited state. Upon returning to the ground state, it transfers energy to molecular oxygen, leading to the formation of ROS, including singlet oxygen and free radicals. These ROS can cause oxidative damage to cellular components, leading to cell death. Moreover, SDT can induce cavitation, which is a complex process that causes the oscillation of gas in the liquid, resulting in the generation, expansion, and collapse of microbubbles. This process can produce heat, potentially triggering a pyrolysis reaction that results in the formation of ROS. Additionally, it can create pressure, which may inflict physical damage on the cell membrane. Contrary to photodynamic therapy (PDT), which employs light to activate photosensitizers, SDT uses ultrasound waves that exhibit superior tissue penetration ability. Ultrasound can penetrate soft tissue to a depth of more than 10 cm, which is significantly deeper than what can be achieved with light, making SDT a more effective therapy for treating tumors located deep within the body and overcoming the challenge for PDT. Moreover, SDT can overcome the limitations of hypoxia in solid tumors, as it does not mainly rely on the presence of oxygen to exert its cytotoxic effects. Although SDT is promising alternative for cancer treatment, there are still some challenges yet to be overcome. The lack of fully satisfactory sonosensitizers, despite some possessing desirable features, is a key issue. Commonly used sonosensitizers [3], such as porphyrins and their derivatives, and certain chemotherapeutic drugs like doxorubicin and curcumin, often have skin sensitivity or high dose-dependent toxicity, limiting their clinical use. Their hydrophobic nature can also lead to aggregation in physiological environments, reducing bioavailability and ROS generation, thus diminishing therapeutic effects. Apart from the molecular analogues, various types of nanomaterials have been explored for use in SDT, such as titanium dioxide nanoparticles, gold nanoparticles, quantum dots, magnetic nanoparticles, and metal-organic frameworks (MOFs) [4]. However, they are there are several challenges, including lack of biocompatibility, high cytotoxicity, poor pharmacokinetics, instability, and the difficulty in scale-up and reproducibility. Therefore, the development of advanced sonosensitizers to address these limitations is urgently needed.

Over the last decade, we have been putting our research effort into the development of advanced photosensitizers for targeted PDT. To achieve active targeting, various tumor-directing ligands have been conjugated to zinc(II) phthalocyanine (ZnPc) and boron dipyrromethene (BODIPY) based photosensitizers, such as the cyclic RGD peptide [5], the dodecapeptide GE11 [6], and folate [7], which can target the $\alpha_v\beta_3$ integrin, epidermal growth factor receptor (EGFR), and folate receptor, respectively. The targeting can also be achieved through bioorthogonal chemistry using a BODIPY-based photosensitizer substituted with both tetrazine and alkyne moieties as the bioorthogonal handles [8]. To control the photodynamic action, a series of acid, thiol, and enzyme-responsive photosensitizers have also been prepared, including several dual stimuli-activated analogues [9, 10]. These active-targeting and activatable approaches have also been integrated to further enhance the tumor specificity. For example, compound **1** is a GSH- responsive self-quenched phthalocyanine trimer containing a biotin targeting group [11], while compound **2** is another GSH-responsive and biotinylated photosensitizer, which can localize in the endoplasmic reticulum (ER) of target cancer cells, inducing ER stress and cell death upon irradiation [12]. In addition, we have also synthesized a ZnPc-based photosensitizer conjugated with a cyclic bactenecin peptide (compound **3**) via a one-pot procedure, which can induce

synergistic cytotoxic effects due to the ZnPc and the antimicrobial peptide against a spectrum of Gram-positive and Gram-negative bacterial strains [13] (see the molecular structures of **1**-**3** in Figure S1 in the Supporting Information).

Recently, we have extended the study to enzyme-responsive photosensitizers. By connecting two or three GSH-responsive 2,4-dinitrobenzenesulfonate (DNBS)-substituted ZnPc units via one or two cathepsin B-cleavable GFLG peptide linker(s), two dual GSH and cathepsin B activated photosensitizers were prepared [14]. Owing to the photoinduced electron transfer (PET) effect of the DNBS substituent and the self-quenching of the ZnPc units, these agents were fully quenched in the native state. Upon internalization into cancer cells, these quenching pathways were relaxed by the two stimuli in the cells, resulting in restoration of the fluorescence emission and singlet oxygen formation. To extend this study further, we introduced a cyclic peptide of the sequence CMYIEALDKYAC, which showed high affinity toward the EGFR, to the trimeric analogue [15]. It represents the first molecular photosensitizer that can achieve both tumor targeting and dual-stimuli activation for precise PDT. The mechanistic action of these multifunctional agents is shown in Figure S2.

More lately, we have been interested in NO-releasing photosensitizers for NO-mediated PDT. Compound **4**, which contains a ZnPc core substituted with two NO donors, is one of these candidates (Figure S1) [16]. This photosensitizer can release NO effectively in phosphate-buffered saline (PBS) upon addition of GSH. It can also internalize into HT29 cells and generate NO in a time-dependent manner. The released NO can lower the oxygen consumption rate, thereby decreasing the adenosine triphosphate (ATP) level and relieving the hypoxic condition of the cells. More interestingly, we found that this compound can also induce immunogenic cell death (ICD). To demonstrate this, the PDT-treated HT29 cells were co-cultured with immature myeloid dendritic cells (iMoDCs) generated from monocyte THP-1 cells at a ratio of 20:1 (HT29:iMoDCs) for 24 h. The RNA of the cells was extracted and examined by quantitative reverse transcription PCR (RT-qPCR). The mRNA expression of the surface markers of mature DCs (CD80, CD83, CD40, CD86, MHC I, and MHC II) and inflammatory cytokines [interleukin 1β (IL-1β), interleukin 12 (IL-12p40), and tumor necrosis factor α (TNF-α)] were significantly up-regulated upon co-incubation of the PDT-treated HT29 cells and iMoDCs, indicating the stimulation and activation of iMoDCs into mature DCs (Figure S3). It was found that the dying tumor cells induced by PDT could release immunogenic signals and elicit ICD.

Apart from these molecular-based photosensitizers, we have also developed a series of stimuli-responsive nano counterparts. For example, a mesoporous silica nanoparticle (MSN)-based nanoplatform was used to encapsulate molecules of a ZnPc conjugated with doxorubicin (DOX) via a hydrazone linker (Figure S4a) [17]. It was cleaved inside the acidic compartments of cancer cells to release ZnPc and DOX moieties for combined PDT and chemotherapy. In addition, polymeric micelles were also used as carriers for controlled release of these two therapeutic agents. In one of the nanosystems, ZnPc and DOX were connected to the polymeric backbone via a GSH-cleavable disulfide bond and an acid-sensitive hydrazone linker, respectively (Figure S4b) [18]. Hence, the resulting nanoparticles exhibited a dual-responsive property, functioning as an efficient nanodrug for synergistic anticancer therapy. As another example, a hydrazone-linked ZnPc-DOX conjugate and the hypoxia-activated prodrug tirapazamine were co-encapsulated into polymeric micelles for multimodal cancer therapy against tumor hypoxia [19]. Recently, we have extended the study to carrier-free nanodrugs based on the self-assembly of amino acids and peptides. By using Fe^{3+} ions to promote the self-assembly of amino acids and encapsulation of a ZnPc-based photosensitizer and the hypoxia-inducible factor 1 (HIF-1) inhibitor acriflavine (ACF) during the self-assembly, a series of catalase-like photosensitizing nanozymes with a self-oxygen-supplying ability were prepared (Figure S4c) [20]. Upon internalization into cancer cells, these nanosystems were disassembled, releasing ZnPc, Fe^{3+}, and ACF. The photoactivities of ZnPc were restored upon disaggregation. The released Fe^{3+} could promote a catalase-like reaction of the endogenous H_2O_2 to generate oxygen that could enhance the photodynamic activities of ZnPc in hypoxic

cancer cells. Moreover, the released ACF could inhibit the HIF-1 β/α dimerization and damage the hypoxic cancer cells through a different mechanism, resulting in synergistic therapy.

Research Impact:

The development of sonosensitizers is a crucial step in translating the concept of SDT from the laboratory to the clinic. Effective sonosensitizers can enhance the therapeutic efficacy of SDT, making it a more viable treatment option for various types of cancer. This could lead to new clinical trials and potentially, new treatment protocols, expanding the arsenal of tools available to oncologists. Moreover, traditional cancer therapies, such as chemotherapy, surgery, and radiation therapy, are often associated with severe side effects and can harm healthy tissues along with cancer cells. They may also carry risks such as infection and complications from surgery. In contrast, SDT, aided by targeted sonosensitizers, offers a more selective approach, potentially reducing collateral damage to healthy cells. This selectivity could lead to fewer side effects, improved patient quality of life, and potentially better treatment outcomes. This could have a profound impact on cancer care and patient outcomes, underscoring the importance and urgency of this research.

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Supporting Information

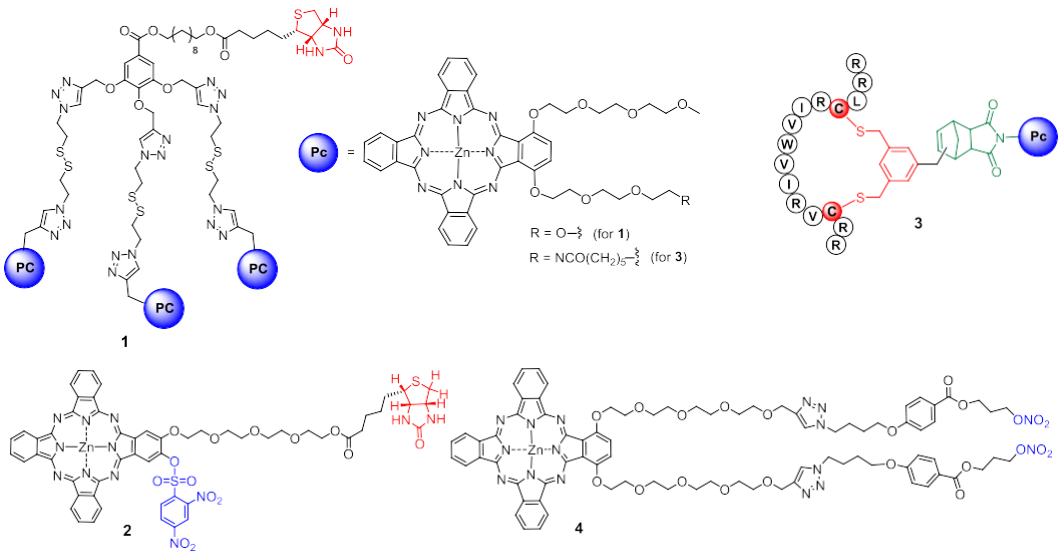


Figure S1. Molecular structures of ZnPc-based photosensitizers **1-4**.

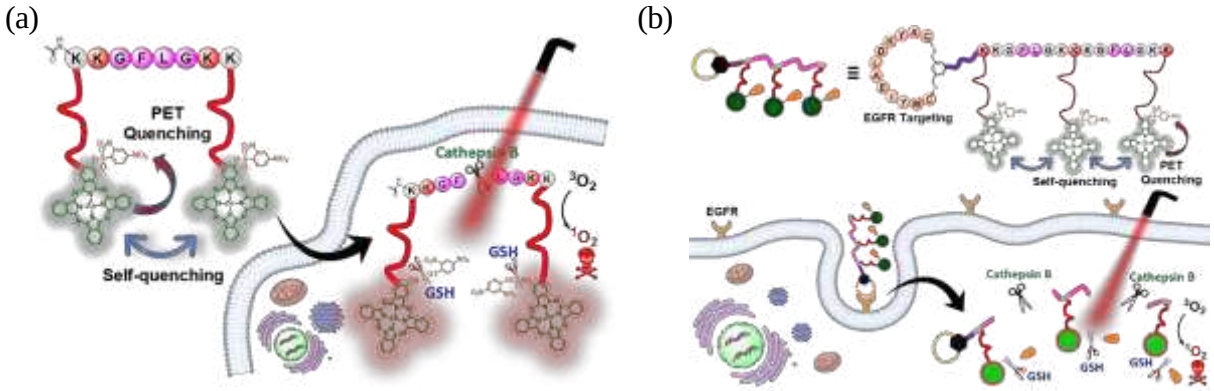


Figure S2. Mechanistic action of (a) the dual GSH and cathepsin B activated ZnPc dimer and (b) the EGFR-targeting dual-stimuli-activated ZnPc trimer.

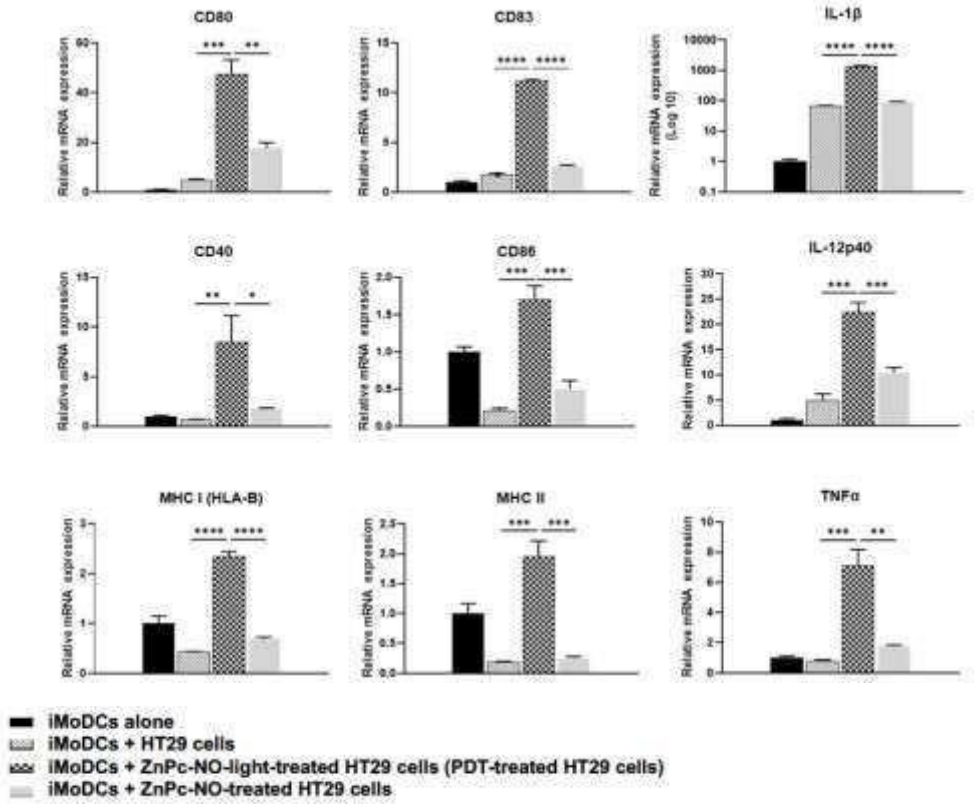


Figure S3. DC maturation and production of pro-inflammatory cytokines and IFNs induced by the PDT-treated HT29 cells. Data have been normalized to the untreated 'Blank' values. (*P<0.05, **P<0.002, ***P<0.0002, ****P<0.0001).

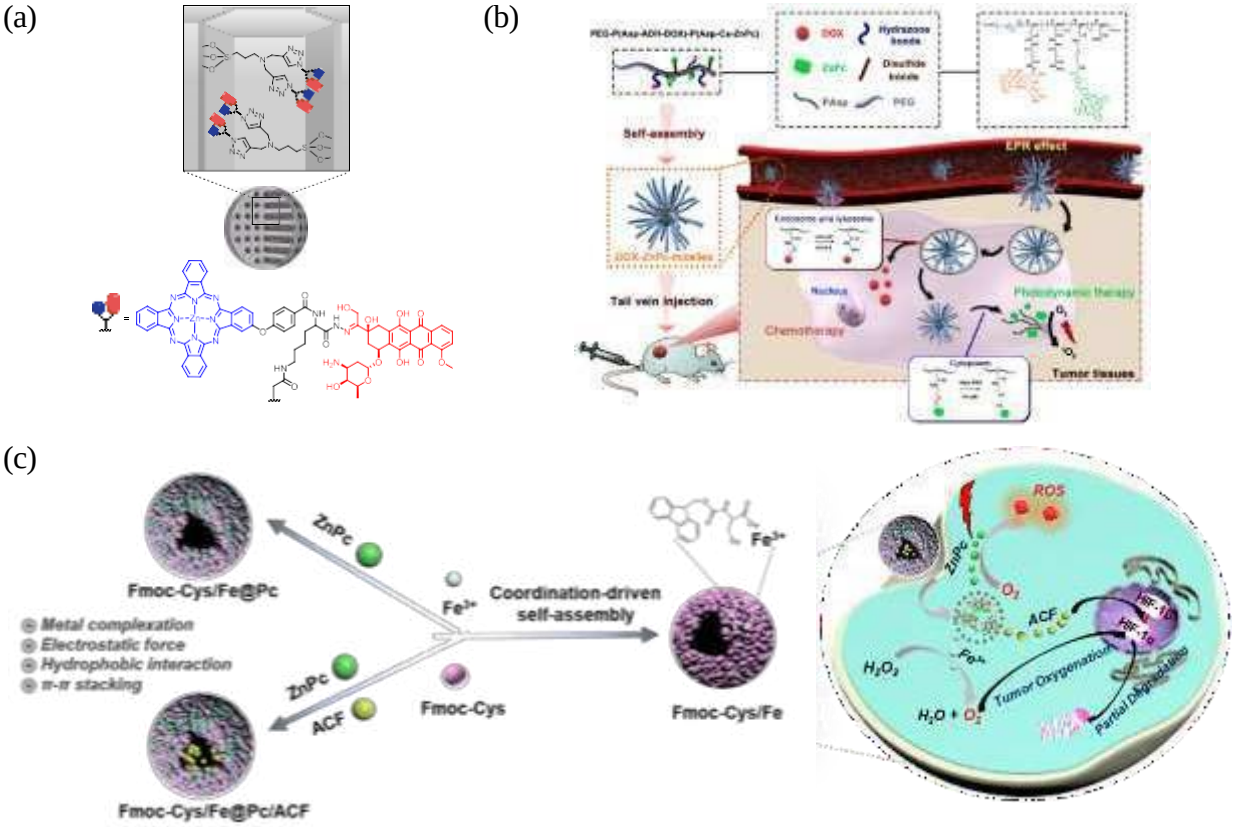
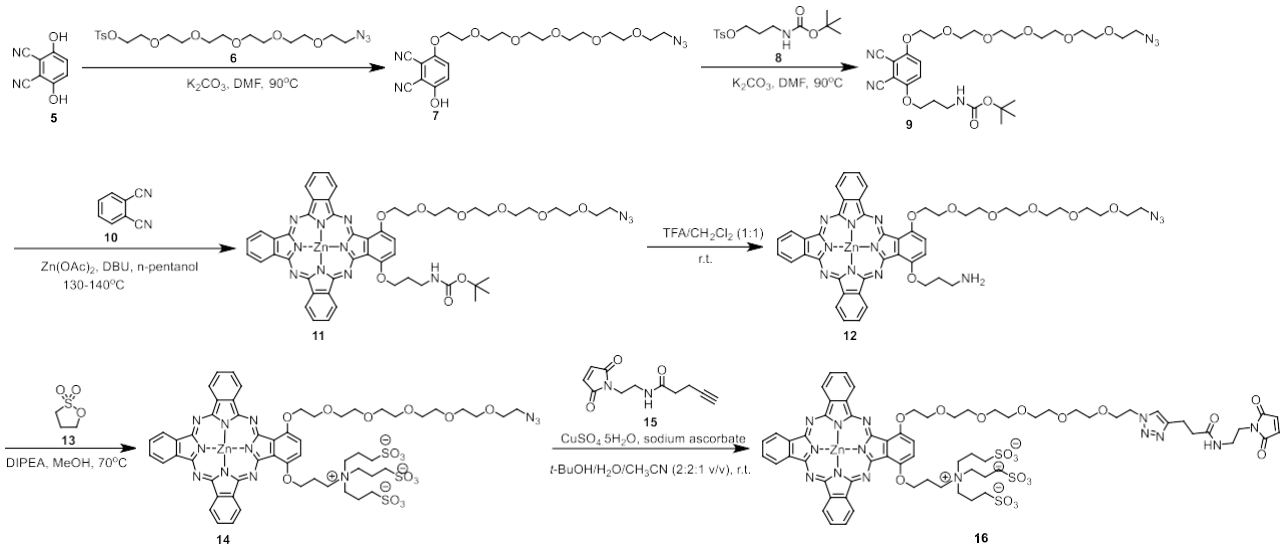


Figure S4. (a) Structure of ZnPc-DOX-encapsulated MSNs. (b) Schematic diagram showing the structure and therapeutic action of ZnPc-DOX-encapsulated polymeric micelles. (c) Design and mechanistic action of the self-assembled catalase-like photosensitizing nanozymes.



Scheme 1. Proposed synthetic route for zinc(II) phthalocyanine substituted with maleimide moiety.