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Smart activatable and traceable dual-prodrug for image-guided combination photodynamic and chemo-therapy

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ABSTRACT

Activatable photosensitizers (PSs) and chemo-prodrugs are highly desirable for anti-cancer therapy to reduce systemic toxicity. However, it is difficult to integrate both together into a molecular probe for combination therapy due to the complexity of introducing PS, singlet oxygen quencher, chemo-drug, chemo-drug inhibitor and active linker at the same time. To realize activatable PS and chemo-prodrug combination therapy, we develop a smart therapeutic platform in which the chemo-prodrug serves as the singlet oxygen quencher for the PS. Specifically, the photosensitizing activity and fluorescence of the PS (**TPEPY-SH**) are blocked by the chemo-prodrug (Mitomycin C, **MMC**) in the probe. Meanwhile, the cytotoxicity of **MMC** is also inhibited by the electron-withdrawing acyl at the nitrogen position next to the linker. Upon glutathione activation, **TPEPY-S-MMC** can simultaneously release active PS and **MMC** for combination therapy. The restored fluorescence of **TPEPY-SH** is also used to report the activation for both PS and **MMC** as well as to guide the photodynamic therapy.

1. Introduction

Chemo-prodrugs are drug derivatives with low systemic toxicity, which are expected to be activated only at the disease site [1]. However, clinical efficiency of simple chemo-drugs is severely inhibited by drug resistance [2]. To address this issue, combination therapy, such as the combination of photodynamic therapy (PDT) and chemotherapy has been introduced, which is proved effective in overcoming drug resistance [3]. PDT is a therapeutic modality based on photosensitizers (PSs) which can generate cytotoxic singlet oxygen under light illumination [4]. One simple strategy is to load both PSs and chemo-drug into nanoparticles (NPs) for combination therapy [5,6]. These NPs have also been designed to offer stimuli-responsive and controlled release of both PSs and chemo-drugs [7]. Although chemo-prodrugs have been incorporated into NPs [8], so far, the PSs in almost all the combination PDT and chemo-therapy platforms do not offer activation process, which have the risk to damage healthy tissues upon exposure to light [9]. As activatable PSs can offer additional advantages, such as the minimized side effect [10], it is of great interest to integrate activatable PS and chemo-prodrug to offer dual activated combination therapy. This is especially challenging for molecular probes because of the complexity of introducing PS, singlet oxygen quencher, chemo-drug, chemo-drug inhibitor and tumor stimuli-responsive linker into one probe.

Mitomycin C (**MMC**) is a broad-spectrum DNA cross-linking anticancer drug [11] containing a quinone structure, which can serve as a photo-induced electron transfer (PET) fluorescence quencher [12]. Since the rate of PET process is faster than spin-forbidden intersystem crossing (ISC), the PET fluorescence quencher can serve as a singlet oxygen quencher at the same time [13]. Meanwhile, as the cytotoxicity of **MMC** is directly related to the ring-opening possibility of the three-membered aziridine, introducing electron with-drawing group at the nitrogen position can effectively decrease the basicity of the aziridine nitrogen atom, resulting in diminished ring-opening reaction with low cell cytotoxicity [14]. As a result, introducing a modified **MMC** to a PS via a tumor-responsive linker is likely to yield a dual-prodrug with

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activatable PS and chemo-prodrug in a simple probe design.

Conventional PSs are subjected to varying degrees of reduced ROS generation and emission quenching in aggregated state on account of aggregation-caused quenching (ACQ) effect [15]. In contrast, PSs with aggregation-induced emission (AIE) exhibit intensive fluorescence [16,17] and efficient ROS generation [18–21] in aggregated state. So far, AIE PSs have been explored for image-guided PDT and antibacterial studies [22,23]. More recently, an activatable AIE PS has also been successfully synthesized, which is selective to specific cancer cells and exhibits low photo-toxicity for cancer cell ablation [24].

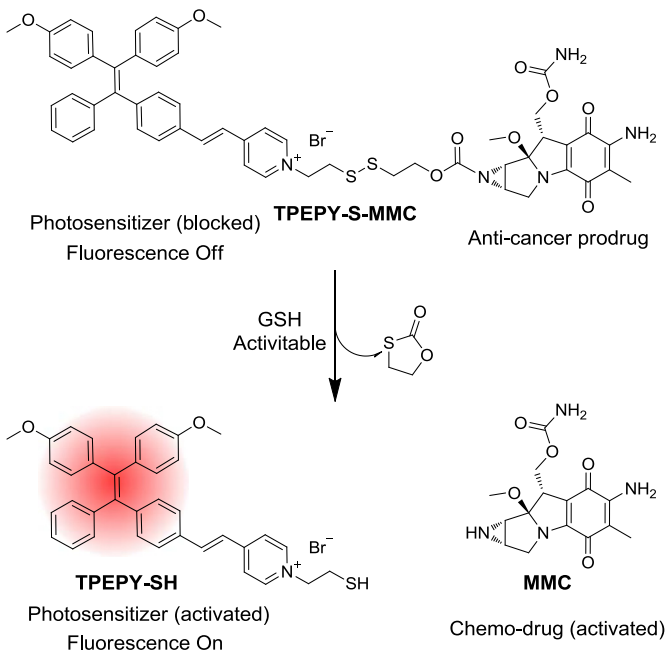
In this contribution, we report a dual-prodrug by integration of **MMC** and vinyl pyridinium-substituted tetraphenylethylene (**TPEPY**, an AIE based PS) through a disulfide bond to accomplish activatable PS and chemo-prodrug combination therapy with traceable activation. **TPEPY** exhibits bright fluorescence and high ROS generation efficiency in aggregated state. The obtained **TPEPY-S-MMC** does not show fluorescence or ROS generation due to the quenching effect from the quinone component in **MMC** moiety. Meanwhile, the electron-withdrawing acyl at the nitrogen position of **MMC** is able to reduce the system toxicity of **TPEPY-S-MMC** [14]. After reaction with glutathione (GSH) [25], **TPEPY-S-MMC** is activated into PDT active **TPEPY-SH** and chemo-drug **MMC**. Moreover, the restored fluorescence of **TPEPY-SH** can serve as an indicator for tracing the activation of the dual-prodrug (Scheme 1). To the best of our knowledge, this is the first design for dual-prodrug with activatable PS and chemo-prodrug for combination therapy.

2. Methods

2.1. Synthesis

2.1.1. Synthesis of *TPEPY-S-MMC*

Compounds **2** (45 mg, 0.09 mmol) and **3** (25 mg, 43 μmol) were dissolved in DMF (3 mL) and reacted at 70 °C for 14 h. After evaporation, the residue was separated with chromatography directly (Eluent: hexane/ethyl acetate (EA) = 1/5, v/v, dichloromethane (DCM)/methanol = 10/1, v/v) to yield **TPEPY-S-MMC** as brown powder (34 mg, yield 75%).



Scheme 1. Proposed route for the traceable activation of dual-prodrug.

2.1.2. Synthesis of *TPEPY-C-MMC*

Compound **4** (34 mg, 50 μmol) and 4-nitrophenyl chloroformate (12 mg, 60 μmol) were dissolved in anhydrous DCM (10 mL) at 0 °C. 40 μL pyridine in 2 mL DCM was injected dropwise. The solution was stirred at 20 °C for 3 h. Then the reaction was washed by water (20 mL × 3). The organic phase was dried with Na_2SO_4 and the solvent was removed to yield the crude compound **5**. The obtained compound **5**, MMC (20 mg, 60 μmol) and DMF (5 mL) were mixed at 0 °C. Pyridine (40 μL, 0.5 mmol) in DMF (0.5 mL) was injected dropwise. The mixture was stirred at 20 °C for 3 h. Then DCM (20 mL) was added, and the solution was washed by water (20 mL × 3). The organic phase was dried by Na_2SO_4 and the solvent was removed. The obtained residue was separated with chromatography (DCM/methanol = 9/1, v/v) to obtain **TPEPY-C-MMC** as brown powder (23 mg, yield 45%).

2.1.3. Synthesis of NPs

Mixture of **TPEPY-S-MMC** (1 mg) or **TPEPY-C-MMC** (1 mg) with DSPE-PEG_{2000} (2 mg) in 1 mL THF was poured into water (9 mL). The solution was rapidly blended by a sonicator at 10 W output. Then, the solution was stirred for THF evaporation. The suspension was further purified by filtering through a 200 nm filter to get **TPEPY-S-MMC NPs** and **TPEPY-C-MMC NPs** in water, vapored to 1 mL as stock solution.

3. Results and discussion

3.1. Synthesis and photoproperties of probe

The synthetic route to **TPEPY-S-MMC** is shown in Scheme 2. Heck coupling between **1** and 4-vinylpyridine yielded the intermediate **2**. Reaction between 2-((2-bromoethyl)disulfanyl) ethyl (4-nitrophenyl) carbonate and **MMC** yielded the intermediate **3**. **TPEPY-S-MMC** was subsequently obtained by heating the mixture of **2** and **3** in dimethyl formamide. Meanwhile, a control probe with an uncleavable linker ("C-C"), **TPEPY-C-MMC**, was also synthesized. Starting from **2**, upon reaction with 6-bromohexanol, **4** was obtained, which was then activated by 4-nitrophenyl chloroformate and further reacted with **MMC** to yield **TPEPY-C-MMC**. All the intermediates and targeted compounds were well characterized (Figs. S14-29 in supporting information). The NPs of **TPEPY-S-MMC** and **TPEPY-C-MMC** were also prepared through nanoprecipitation method, which have average sizes of ~21 nm and ~23 nm, respectively, evaluated by laser light scattering (Fig. S1C). The morphologies were examined by high-resolution transmission electron microscopy, both show spherical shapes with a mean size of ~20 nm (Fig. S1A-B).

We first investigated the dual-prodrug activation upon treatment of **TPEPY-S-MMC** with GSH. **TPEPY-S-MMC** (10 μM) was almost non-emissive in PBS/DMSO (v/v = 199/1, pH = 7.4) solution. After addition of 0.5 mM GSH, the fluorescence intensity was significantly enhanced by more than 10-fold within 30 min upon excitation at 430 nm (Fig. S2A), which was ascribed to the formation of emissive **TPEPY-SH** aggregates. Meanwhile, the fluorescent signals at 660 nm gradually intensify along with the increase of GSH concentration (0–220 μM, Fig. 1A) and they show a linear relationship in the concentration range of 0–100 μM (Fig. S2B). In contrast, the control probe of **TPEPY-C-MMC** with uncleavable alkane bond exhibits no response to GSH treatment (Fig. 1B), indicating that the fluorescence enhancement of **TPEPY-S-MMC** comes from the cleavage of disulfide bond by GSH. In addition, **TPEPY-S-MMC** shows highly selective response to biological thiols such as cysteine (Cys) and GSH compared to some other relevant analytes