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A photosensitizer-drug conjugate for synergistic photodynamic therapy and photo-triggered camptothecin release

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Combination therapy has emerged as a promising strategy to enhance treatment efficacy at reduced doses and overcome the limitations of monotherapy by harnessing complementary therapeutic modalities, while external stimulus-controlled therapeutic activation can localize therapeutic activity to tumors and reduce systemic toxicity. Here, we present a chemo-photodynamic combination nanotherapy strategy based on a red light-responsive photosensitizer-drug conjugate. This prodrug integrates the photosensitizer 5-mono(4-carboxyphenyl)-10,15,20-triphenylporphyrin (TPP) with an anticancer drug camptothecin (CPT) through a singlet oxygen-cleavable thioketal linker. The prodrug exhibited minimal dark toxicity *in vitro* and was readily encapsulated into PLGA-PEG nanoparticles, affording nanoparticles that exhibited uniform morphology, high stability, and efficient cellular uptake. Upon red light irradiation, TPP generates singlet oxygen ($^1\text{O}_2$) to induce photodynamic cytotoxicity and simultaneously cleaves the thioketal linker, triggering the controlled release of CPT. The intrinsic PDT cytotoxicity of the photo-generated $^1\text{O}_2$, together with the CPT released *via* $^1\text{O}_2$ -mediated thioketal cleavage, synergistically enhanced cytotoxicity in photo-irradiated HeLa cells compared with either PDT or chemotherapy alone. Collectively, this study demonstrates a chemo-photodynamic combination nanotherapy strategy that enables red-light-guided, spatiotemporal control of cancer therapy.

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Introduction

Cancer remains one of the leading causes of mortality worldwide, yet conventional cancer therapies are often limited by suboptimal therapeutic efficacy, off-target toxicity in healthy tissues, and acquired drug resistance that diminish long-term efficacy.^{1,2} Spatiotemporally controlled treatment can localize therapeutic activity to the tumors to reduce the nonspecific exposure of healthy tissues,^{3–5} while combination therapy harnesses complementary therapeutic modalities to enhance efficacy at reduced doses, thereby reducing systemic toxicity relative to monotherapy and mitigating the emergence of drug resistance.^{6–8}

Photodynamic therapy (PDT) is a light-activated treatment in which a photosensitizer, under irradiation at an appropriate wavelength of light, is photoexcited and transfers energy (or electrons) to generate cytotoxic reactive oxygen species (most commonly singlet oxygen ($^1\text{O}_2$); type II PDT) that induce localized oxidative damage in the light-irradiated regions.^{9–12} Since PDT operates primarily *via* oxidative damage to biomolecules

and cellular components (*e.g.*, membranes),¹³ it is mechanistically orthogonal to many conventional therapies, and therefore, PDT enables complementary and potentially synergistic treatment efficacy and is less susceptible to common drug-resistance mechanisms. Accordingly, combining PDT with conventional cancer therapy modalities is a promising strategy to achieve therapeutic synergy and overcome the limitations associated with monotherapy.^{14–19}

A prodrug is a pharmacologically inactive compound that, upon administration, is enzymatically or chemically converted to release active therapeutic agents at targeted sites.^{20–22} Building upon this concept, stimuli-responsive prodrug platforms have been developed to achieve spatiotemporally controlled drug activation by incorporating stimulus-cleavable linkers that respond to tumor-associated microenvironmental cues (*e.g.*, acidic pH,^{23–25} elevated ROS,^{26,27} overexpressed enzymes,^{28,29} high intracellular glutathione (GSH),^{30,31} and hypoxia^{32,33}) or externally applied triggers (*e.g.*, light irradiation,^{34–38} ultrasound,^{39,40} and ionizing radiation^{41–43}). By integrating responsive chemistry with prodrug design, these systems offer improved systemic stability and tumor-selective drug release. However, systems that rely on endogenous tumor-associated stimuli often suffer from insufficient specificity and tumor heterogeneity as these triggers may also be present at low levels

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in normal tissues, leading to undesired drug activation and limited control over activation selectivity. Photo-cleavage chemistry has been widely adopted to regulate prodrug activation,⁴⁴ and increasing efforts have been devoted to developing photo-cleavage chemistry with red-shifted excitation wavelengths for enhanced tissue penetration.^{45–47} An emerging strategy in photo-mediated cleavage chemistry leverages PDT-generated reactive oxygen species (ROS) to trigger preprogrammed oxidative reactions, resulting in cascade bond cleavage and drug release.^{48–52} Such a PDT-mediated strategy significantly diversifies and expands the structural and functional scope of photocleavage chemistry,⁵³ given the rich library of photosensitizers responsive to wavelengths spanning the visible to the near-infrared. Of particular interest are thioketal linkers that undergo oxidative cleavage and have been used to construct ROS-activable prodrug systems.^{54–61} In this context, covalent conjugation of a chemotherapeutic agent with a photosensitizer through a ROS-responsive linker enables photo-activation of prodrugs using longer-wavelength light, while offering synergy between chemo- and photo-dynamic therapy modalities.

Nanocarriers offer a number of advantages over the direct usage of small-molecule drugs, such as protection of payloads from premature degradation, solubilizing hydrophobic drugs, reduced nonspecific drug exposure, and tunable control over cellular uptake.^{62–64} For example, PLGA-PEG is a widely used biocompatible diblock copolymer comprising a hydrophilic PEG segment and a biodegradable PLGA segment, and can readily self-assemble into core-shell nanoparticles to encapsulate hydrophobic drugs under appropriate formulation conditions.⁶⁵ Such PLGA-PEG nanoformulations have been extensively employed to improve pharmacokinetics and

biodistribution, thereby enhancing therapeutic performance in biological environments.^{66,67}

Herein, we report a chemo-photodynamic combination nanotherapy strategy based on a photosensitizer-drug conjugate comprising an anticancer drug CPT conjugated to a porphyrin derivative (the photosensitizer) through a ¹O₂-cleavable thioketal linker (Fig. 1). This conjugate exhibits minimal dark toxicity *in vitro* and can be stably encapsulated into PLGA-PEG nanoparticles, which exhibit uniform morphology, high stability, and efficient cellular uptake. Upon red light irradiation (> 600 nm), the photosensitizer TPP^{68–70} generates ¹O₂ and simultaneously induces PDT cytotoxicity while cleaving the thioketal linker to release CPT with restored chemotherapeutic toxicity. Combination index-fraction affected (CI-Fa) analysis demonstrates synergistic effects of this combined chemo-PDT nanotherapy system, enabled by light-triggered ¹O₂ generation that drives phototoxicity and prodrug activation. By combining synergistic chemo-PDT cytotoxicity with light-controlled (> 600 nm) spatio-temporal resolution, this nanotherapy platform holds promise to improve localized therapeutic efficacy while potentially mitigating systemic toxicity.

Materials and methods

Synthesis of the ¹O₂-responsive prodrug ProCPT

The synthetic procedure for ProCPT is illustrated in Scheme 1 (see SI for synthetic details). Briefly, a ¹O₂-responsive thioketal diol linker (compound 2) was synthesized from 2-mercaptoethanol through sequential thioketal formation and deprotection steps. CPT was reacted with triphosgene in the presence of DMAP and

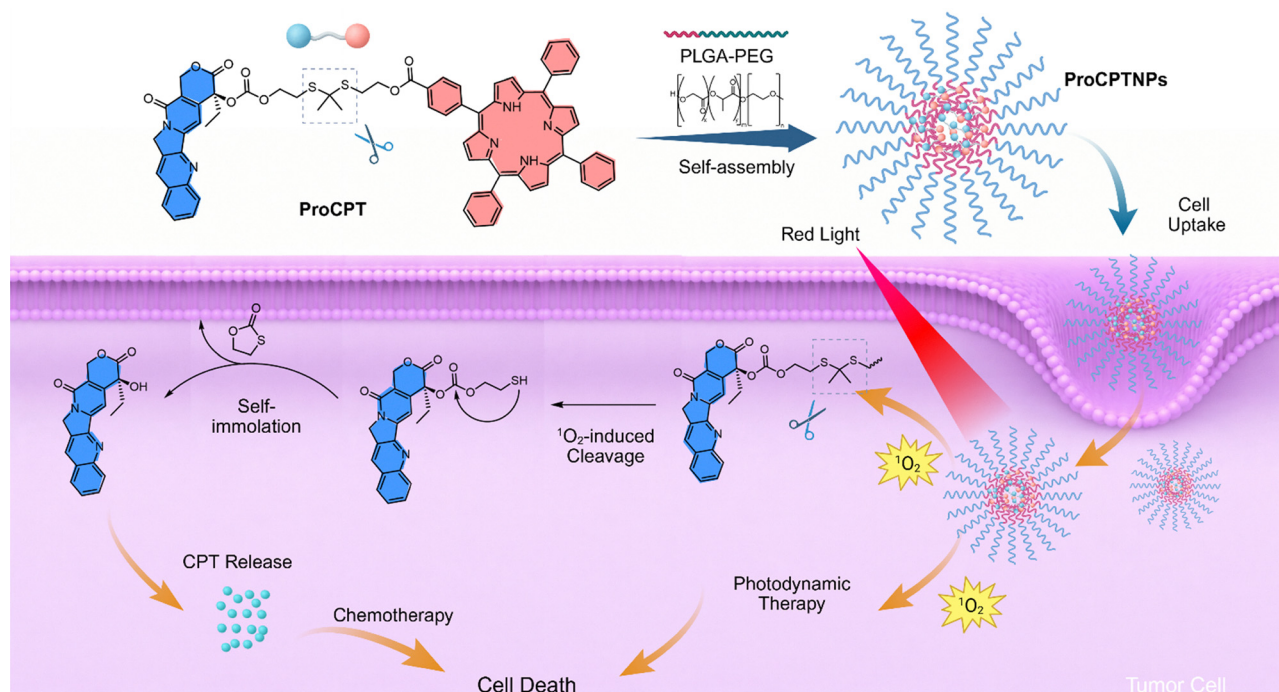
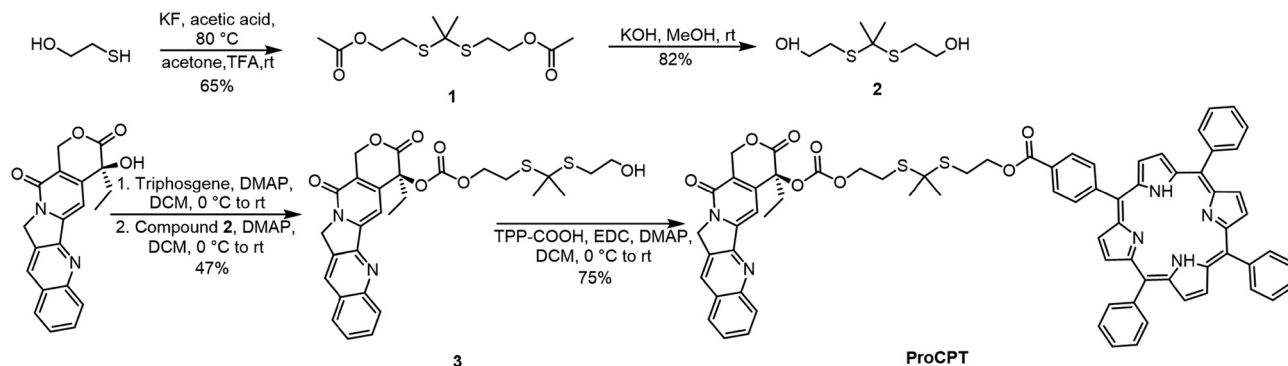


Fig. 1 Schematic of the preparation of the ProCPTNPs and their therapeutic effects.





Scheme 1 Synthesis of the $^1\text{O}_2$ -responsive prodrug **ProCPT**.

coupled with the thioketal linker to afford the CPT-linker intermediate (compound 3). Compound 3 was conjugated to 5-mono(4-carboxyphenyl)-10,15,20-triphenylporphyrin (TPP-COOH) *via* EDC/DMAP-mediated coupling to yield the target prodrug **ProCPT**. Synthetic intermediates and products were structurally confirmed by ^1H and ^{13}C NMR and high-resolution mass spectrometry.

Preparation of ProCPTNPs and TPPNPs

ProCPT (1 mg) or TPP (0.5 mg) and PLGA (10 kDa)-mPEG (5 kDa) (10 mg) were dissolved in THF (1 mL). The resulting solution was rapidly added into deionized water (10 mL) within 30 s under stirring (1000 rpm). The organic solvent (THF) was removed under reduced pressure to ensure complete solvent removal. The obtained nanoparticles were concentrated to 1 mg mL^{-1} and stored in a dark environment at $4\text{ }^\circ\text{C}$.

Size, zeta potential and colloidal stability measurements

Dynamic light scattering (DLS) measurements were carried out using a Zetasizer Nano ZS (Malvern PANalytical, UK) equipped with a 173° backscattering detector. All measurements were performed at $25\text{ }^\circ\text{C}$, and hydrodynamic diameters were reported as intensity-weighted size distributions. Samples were diluted to an appropriate scattering intensity in PBS prior to measurement. Each sample was measured in triplicate ($n = 3$), and the data are presented as mean \pm standard deviation. Zeta potential values were determined using the same instrument with folded capillary cells.

Colloidal stability was assessed by incubating **ProCPTNPs** and **TPPNPs** in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (FBS) at $37\text{ }^\circ\text{C}$ under gentle shaking. At predetermined time points, changes in the hydrodynamic diameter were monitored by DLS.

UV-vis and fluorescence spectroscopy

UV-vis absorption spectra were recorded using a Cary 60 UV-vis spectrophotometer (Agilent Technologies, USA). **ProCPT** and **ProCPTNPs** were dispersed in PBS at a concentration of $5\text{ }\mu\text{M}$ (equivalent to $66.5\text{ }\mu\text{g mL}^{-1}$ for **ProCPTNPs**) and measured at room temperature.

Fluorescence spectra were recorded using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA).

Samples were excited at 600 nm, and emission spectra were collected over the range of 620–800 nm.

In vitro singlet oxygen generation

Singlet oxygen sensor green (SOSG)^{71,72} was used as a fluorescent probe for singlet oxygen ($^1\text{O}_2$) detection. Briefly, $13.3\text{ }\mu\text{g mL}^{-1}$ **ProCPTNPs** (*i.e.*, $1\text{ }\mu\text{M}$ **ProCPT**) in PBS was mixed with SOSG ($5\text{ }\mu\text{M}$) and irradiated with red light ($>600\text{ nm}$, 40 mW cm^{-2}) for predetermined time intervals. The fluorescence intensity of SOSG at 530 nm ($\lambda_{\text{ex}} = 488\text{ nm}$) was recorded using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA). Each experiment was performed in triplicate ($n = 3$), and the data are presented as the mean \pm standard deviation.

CPT release study

A PBS solution of **ProCPT** ($5\text{ }\mu\text{M}$) was irradiated with red light ($>600\text{ nm}$, 40 mW cm^{-2}) for predetermined time intervals and subsequently incubated overnight at room temperature to allow complete reaction. The release of CPT was analysed by high-performance liquid chromatography (HPLC).

HPLC analysis was performed using a Shimadzu LC-20 system (Shimadzu, Japan) equipped with a Shimadzu Nexcol C18 column ($5\text{ }\mu\text{m}$, $4.6 \times 250\text{ mm}$). The mobile phase consisted of (A) acetonitrile containing 0.1% trifluoroacetic acid (TFA) and (B) water containing 0.1% TFA. A linear gradient was applied from 5% to 95% acetonitrile over 15 min at a flow rate of 1.0 mL min^{-1} . The column temperature was maintained at $25\text{ }^\circ\text{C}$, and detection was carried out at 254 nm using a UV detector.

Cell culture

HeLa cells were cultured in RPMI-1640 containing 10% FBS and 1% penicillin/streptomycin, and maintained at $37\text{ }^\circ\text{C}$ in a humidified atmosphere containing 5% CO_2 .

Cellular uptake

HeLa cells (1×10^4) were seeded in 96-well plates and cultured overnight to allow adherence. The medium was then replaced with fresh RPMI-1640 containing **ProCPTNPs** ($100\text{ }\mu\text{g mL}^{-1}$), and the cells were incubated at $37\text{ }^\circ\text{C}$ for 0, 4, 8 and 12 h. Subsequently, LysoTracker™ Deep Red (75 nM in RPMI-1640



medium) was added to the cells and incubated for 30 min at 37 °C to stain lysosomes. The nuclei were counterstained with Hoechst 33342 (5 $\mu\text{g mL}^{-1}$ in RPMI-1640 medium) for 10 min. After staining, cells were carefully washed three times with ice-cold PBS (1 \times) to remove excess dyes.

Fluorescence images were acquired using a Zeiss LSM 980 Airyscan 2 confocal super resolution microscope (Carl Zeiss, Germany) equipped with a GaAsP detector array. Excitation wavelengths were set at 405 nm for Hoechst 33342, 633 nm for LysoTracker™ Deep Red, and 561 nm for the **ProCPTNPs** fluorescence channel. Images were captured using a Plan-Apochromat 20 \times /0.8 objective lens. Detector settings, laser power, and acquisition parameters were kept constant across all samples to ensure comparability.

Measurement of intracellular ROS generation

HeLa cells (1×10^4) were seeded in 96-well plates and cultured overnight to allow adherence. Cells were then treated with either PBS (control) or **ProCPTNPs** (100 $\mu\text{g mL}^{-1}$ in RPMI-1640 medium) and incubated at 37 °C for 12 h. After incubation, the cells were washed three times with PBS (1 \times) and subsequently treated with 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA)⁷³ (20 μM in RPMI-1640 medium) for 20 min at 37 °C. The cells were then irradiated with light (> 600 nm, 40 mW cm^{-2}) for 5 min. After irradiation, the cells were washed three times with PBS (1 \times), and the fluorescence signals derived from oxidized 2',7'-dichlorofluorescein (DCF) were collected. Excitation and emission were set at 488 nm and 525 ± 20 nm for DCF detection.

Cytotoxicity test

HeLa cells were seeded in 96-well plates at a density of 5000 cells per well and incubated for 24 h to allow cell attachment. Subsequently, cells were treated with different concentrations of **ProCPTNPs** or **TPPNPs**. After 12 h of incubation, the **ProCPTNPs** and **TPPNPs** groups were exposed to light irradiation (> 600 nm, 40 mW cm^{-2}) for 15 min. For the chemotherapy group, **ProCPTNPs** were added to the cells immediately after light irradiation under the same conditions (> 600 nm, 40 mW cm^{-2} , 15 min). After a total culture time of 48 h, the supernatant was replaced with 100 μL of thiazolyl blue (MTT) solution (0.5 mg mL^{-1} in RPMI-1640 medium) and incubated at 37 °C for 4 hours. The supernatant was removed, 100 μL of DMSO was added to dissolve the formazan crystals, and the plate was shaken for 60 seconds. Absorbance at 490 nm was measured using a microplate reader, and the viability of the blank control group was set as 100% for normalization. Each experimental condition was performed in sextuplicate ($n = 6$).

Results and discussion

The photosensitizer TPP was covalently linked to CPT through a $^1\text{O}_2$ -cleavable thioketal linker, temporarily suppressing the intrinsic cytotoxicity of CPT in the prodrug state (**ProCPT**). The resulting hydrophobic prodrug was subsequently co-assembled with PLGA-PEG amphiphiles to construct a stimuli-responsive nanoplatform (**ProCPTNPs**) (Fig. 2a), ensuring structural stability in aqueous media while mitigating premature drug leakage

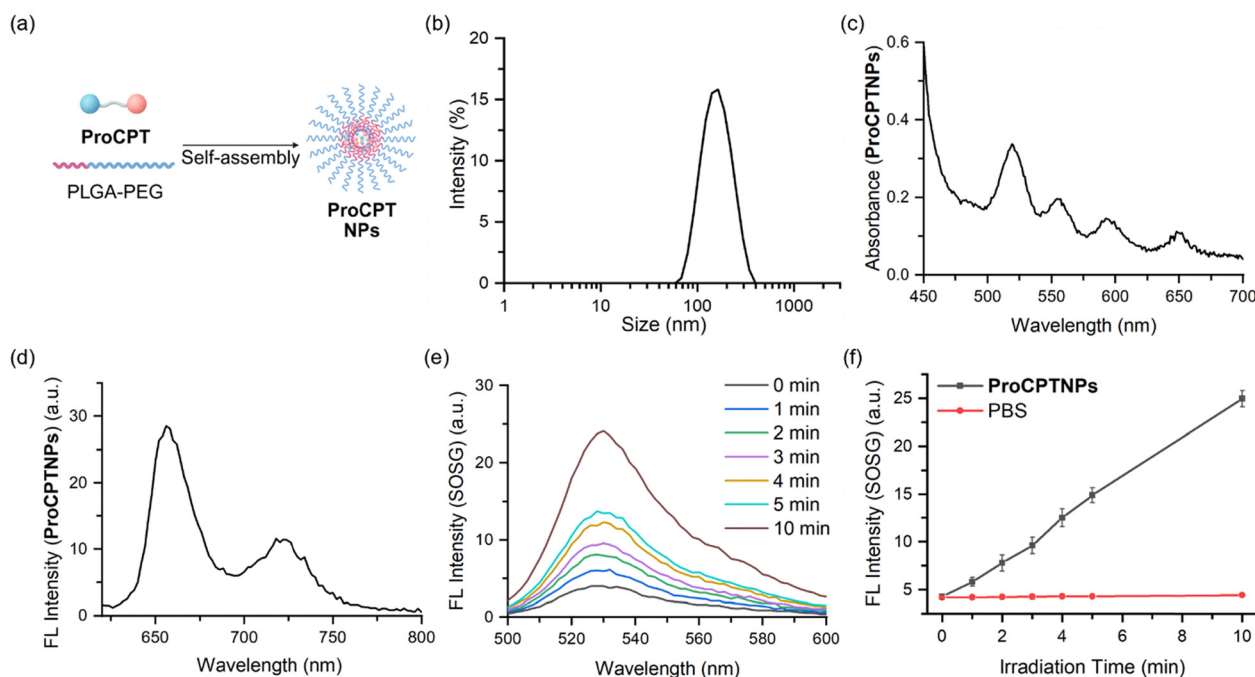


Fig. 2 Preparation and characterization of **ProCPTNPs**. (a) Schematic of the preparation of **ProCPTNPs**. (b) Size distribution of **ProCPTNPs** measured by DLS. (c) Absorption spectra and (d) fluorescence spectra of **ProCPTNPs** in a PBS buffer. (e) Generation of $^1\text{O}_2$ from **ProCPTNPs** as a function of the light irradiation time determined by SOSG assay. (f) Changes in the fluorescence intensity of SOSG (emission peak = 530 nm) with or without **ProCPTNPs** under light irradiation. Irradiation conditions: > 600 nm and 40 mW cm^{-2} .



under physiological conditions. Upon red light irradiation, TPP efficiently generates $^1\text{O}_2$, which induces cleavage of the thioketal bond and triggers the on-demand activation of CPT. TPP was selected not only for its high $^1\text{O}_2$ quantum yield, but also for its hydrophobic porphyrin framework, which promotes efficient packing of the hydrophobic prodrug and avoids undesired interaction with cells in the encapsulated form.

DLS measurements confirmed that **ProCPTNPs** exhibited a hydrodynamic diameter of approximately 156.4 nm (Fig. 2b) with a surface charge of ~ -17.8 mV. The nanoparticles displayed a narrow size distribution with a low polydispersity index (PDI) of 0.091. Scanning electron microscopy (SEM) images (Fig. S1) further revealed well-defined spherical morphology with a size range consistent with that indicated by DLS. Moreover, **ProCPTNPs** demonstrated excellent colloidal stability under physiological conditions, as negligible size changes were observed during long-term storage (\sim one week) in PBS (pH 7.4) (Fig. S2).

The optical and photodynamic properties of **ProCPTNPs** were systematically evaluated. Their UV-vis absorption spectra displayed characteristic peaks within the 550–650 nm region, originating from the porphyrin chromophore of TPP (Fig. 2c and Fig. S5). In addition, a distinct emission peak of TPP was observed at around 680 nm (Fig. 2d). To assess the $^1\text{O}_2$ generation capability of **ProCPTNPs**, the singlet oxygen probe SOSG was employed as a fluorescent probe. Upon irradiation with red

light, the SOSG emission at 530 nm gradually increased in the solution of **ProCPTNPs** (Fig. 2e). Quantitative analysis of SOSG emission at 530 nm revealed a 4.3-fold enhancement after 10 min of irradiation (Fig. 2f and S6), confirming the efficient $^1\text{O}_2$ generation capacity of **ProCPTNPs**.

The photo-generated reactive $^1\text{O}_2$ from **ProCPT** efficiently cleaves the thioketal linker within the prodrug, thereby triggering a cascade cyclization–elimination reaction to release free CPT (Fig. 3a). HPLC analysis revealed that, after 16 min of irradiation, the characteristic peak of **ProCPT** (retention time, $t_{\text{R}} = 8.75$ min) disappeared, accompanied by the emergence of a new elution peak at $t_{\text{R}} = 3.95$ min which matches the free CPT, thereby confirming the successful photo-controlled CPT release (Fig. 3b). Quantitative analysis demonstrated that ~ 1.2 μM CPT was released from 5 μM **ProCPT** after 16 min irradiation and overnight incubation, corresponding to a release percentage of $\sim 24\%$ (Fig. 3c).

We next evaluated the cellular uptake and photodynamic activity of **ProCPTNPs** in HeLa cells. After co-incubation with **ProCPTNPs**, cellular internalization was visualized using confocal laser scanning microscopy (CLSM). As the incubation time increased, the intracellular fluorescence from **ProCPT** progressively intensified (Fig. 4a and Fig. S7; **ProCPT** fluorescence depicted in green; $\lambda_{\text{ex}} = 561$ nm; $\lambda_{\text{em}} = 600$ –630 nm), indicating the time-dependent cellular uptake of **ProCPTNPs**, with the mean fluorescence intensity reaching 2.1-fold at 8 h (Fig. 4b).

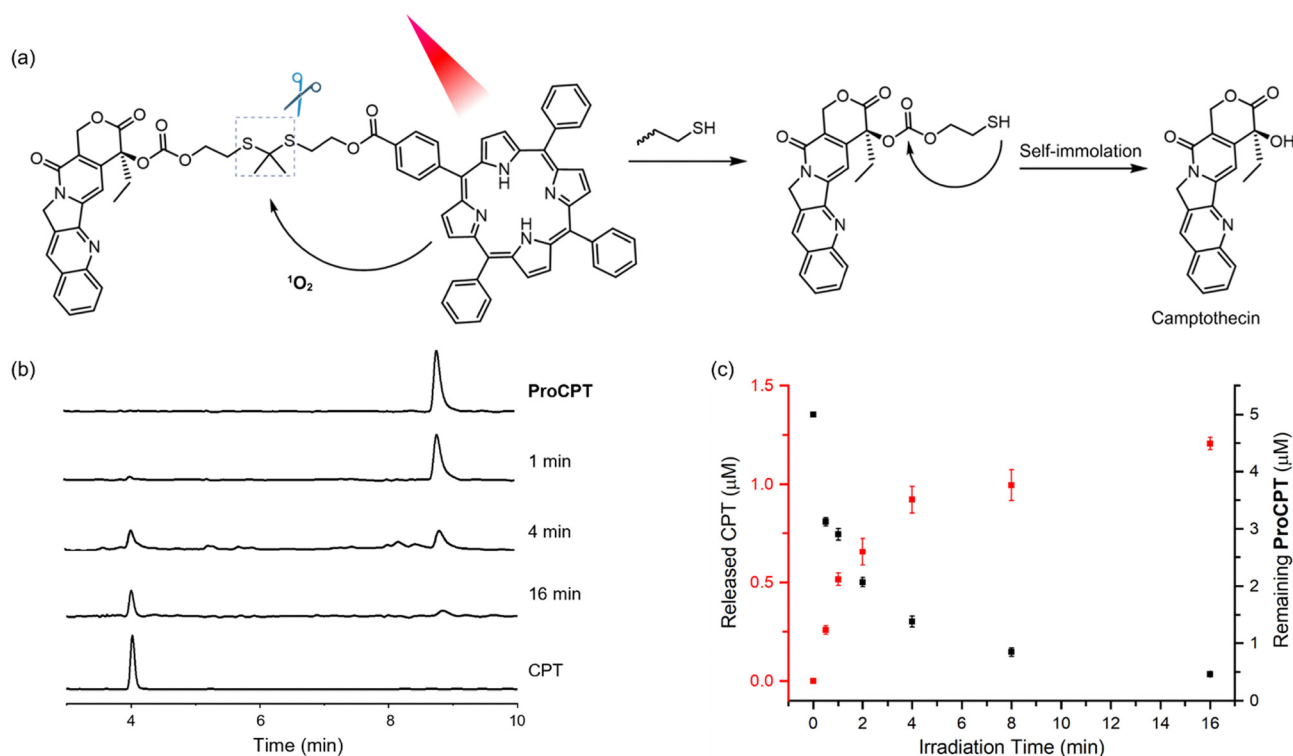


Fig. 3 (a) Schematic of the PDT-mediated CPT release from **ProCPT**. (b) HPLC profiles of **ProCPT** (5 μM) as a function of the light irradiation duration (0–16 min) compared against a standard solution of CPT (5 μM , bottom trace). (c) Photo-triggered release of CPT from **ProCPT** as a function of the light irradiation monitored by HPLC. Light irradiation conditions: > 600 nm and 40 mW cm^{-2} . All photo-irradiated samples were kept at room temperature overnight before HPLC analysis to ensure the completion of the cyclization–elimination cascade.



The PLGA-PEG nanoparticles (~ 150 nm) exhibit a narrow size distribution consistent with previously reported PLGA-based systems and are therefore expected to be internalized by cells through endocytosis.^{74,75} When lysosomes were stained with LysoTracker™ Deep Red, the fluorescence signal (depicted in red) showed clear colocalization with that of **ProCPT** after 8 h of incubation, suggesting lysosomal involvement and supporting endo-lysosomal trafficking following cellular uptake. The acidic lysosomal microenvironment is expected to promote the degradation of PLGA nanoparticles, thereby facilitating the release of encapsulated hydrophobic cargo molecules.⁶⁴ More importantly, lysosomes are known to be highly vulnerable organelles to photodynamic damage, which may further potentiate the therapeutic efficacy of **ProCPTNPs**.

Intracellular ROS production triggered by **ProCPTNPs** upon red light irradiation was further examined using H_2DCFDA as a fluorescent probe. H_2DCFDA is non-fluorescent until oxidized by ROS to yield the green-fluorescent DCF.⁷⁵ As shown in confocal fluorescence images (Fig. 4c), negligible DCF fluorescence was

observed in HeLa cells incubated with PBS or **ProCPTNPs** under dark conditions. In contrast, strong green fluorescence emerged in **ProCPTNPs**-treated cells upon red light irradiation. Quantitative analysis demonstrated that DCF fluorescence intensity in irradiated cells was approximately 25-fold higher than in non-irradiated controls (Fig. 4d). These findings unambiguously confirm that **ProCPTNPs** are capable of selectively generating substantial intracellular ROS in areas exposed to red light irradiation.

The chemo-PDT combination therapy of **ProCPTNPs** against HeLa cells was evaluated using the MTT assay. Such combination strategies are designed to enhance therapeutic efficacy through complementary mechanisms while reducing the likelihood of resistance associated with monotherapy. For comparison, nanoparticles containing only the TPP photosensitizer (**TPPNPs**) were also prepared. Without red light irradiation, both **ProCPTNPs** and **TPPNPs** exhibited negligible cytotoxicity, with cell viability remaining above 80%, confirming their good biocompatibility in the dark (Fig. 5a and b). Upon red light

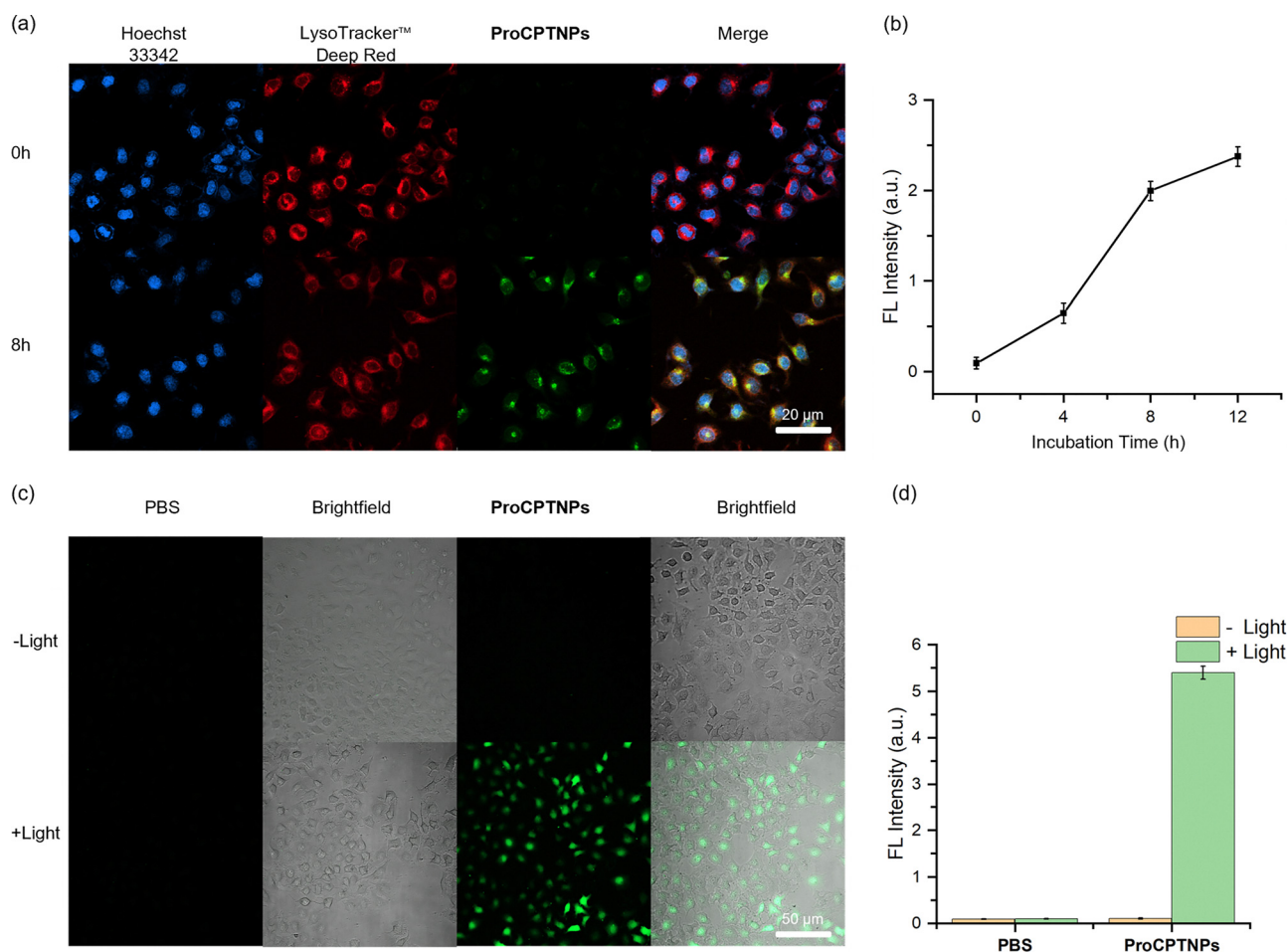


Fig. 4 (a) Confocal fluorescence images and (b) mean fluorescence intensity (MFI) profile of the HeLa cells treated with **ProCPTNPs** for 0, 4, 8 and 12 h. Arbitrary colors are shown in the figure for visual clarity only and do not represent the actual fluorescence emission colors: blue, Hoechst 33342 (nucleus); red, LysoTracker™ (lysosomes); and green, **ProCPTNPs**. Scale bar: 20 μm . (c) Confocal fluorescence/brightfield overlay images and (d) MFI profiles of the HeLa cells treated with **ProCPTNPs** for 12 h, followed by staining with H_2DCFDA and red light photoirradiation (>600 nm and 40 mW cm^{-2}) for 5 min. DCF fluorescence is indicated in green. Scale bar: 50 μm .



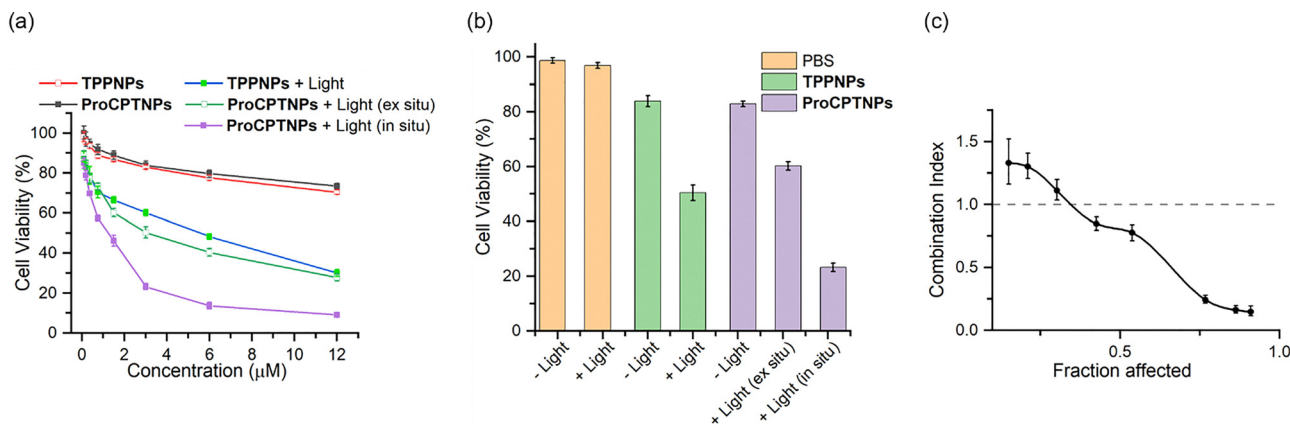


Fig. 5 (a) Cell viability following different treatments as measured by the MTT assay. Data are shown as mean \pm SD ($n = 6$). (b) Bar graphs showing the cell viability under different treatment conditions: PBS (negative control), **TPPNPs** (PDT only), and **ProCPTNPs** (*ex situ*: **ProCPTNPs** solutions irradiated in a vial before adding into the cell culture medium, isolating the cytotoxic effect of chemotherapy from PDT; *in situ*: **ProCPTNPs** added into the cell culture medium and then irradiated for combination therapy). Concentration of TPP/**ProCPT** = 3 μ M. Data are presented as mean \pm SD ($n = 6$). (c) Combination index (CI) analysis of the interactions between photodynamic therapy **TPPNPs** and chemotherapy CPT in the **ProCPTNPs** combination system. The error bars represent the 95% confidence intervals for the CI values. CI > 1 indicates antagonism, CI < 1 indicates synergism, and CI = 1 indicates an additive effect.

irradiation (> 600 nm, 40 mW cm⁻², 15 min), both groups showed enhanced cytotoxicity, while more significant photo-induced toxicity was observed for the **ProCPTNPs** group (IC₅₀ = 0.95 \pm 0.05 μ M) compared with the **TPPNPs** (IC₅₀ = 6.0 \pm 0.21 μ M). At a fixed concentration of 3 μ M, light irradiation induced only a moderate decrease in MTT viability (50%) for the **TPPNPs** group, whereas **ProCPTNPs** caused a markedly increased cytotoxicity (MTT viability = 23%). We attribute the enhanced cell-killing capability of **ProCPTNPs** to a synergistic effect between photodynamic oxidative damage and PDT-mediated prodrug activation (Fig. 5b). In addition, exposure of cells to the same light conditions in the absence of nanoparticles induced negligible cytotoxicity, confirming that the observed phototoxicity originated from the photo-responsive nanoparticles. Collectively, these results demonstrate that **ProCPTNPs** effectively integrate photodynamic therapy and chemotherapy, achieving superior cancer cell killing with minimal dark toxicity.

In another controlled experiment, chemo-PDT combination therapy (**ProCPTNPs**) was compared to a chemotherapy-only condition, in which **ProCPTNPs** were irradiated *ex situ* in a vial, and then this pre-activated solution was incubated with HeLa cells (Fig. 5a and b). This *ex situ* irradiation allows PDT-mediated prodrug activation in the nanoparticles, yet cells were not subjected to the PDT oxidative environment due to the short lifetimes of the ROS species. The suboptimal therapeutic effect (IC₅₀ = 3.2 \pm 0.31 μ M) of this chemotherapy-only control group underscores the direct contribution from the PDT cytotoxicity that is in synergy with the photo-released drugs.

The synergistic interaction between CPT and PDT was quantitatively evaluated using the Chou–Talalay method.⁷⁶ The combination index (CI) analysis was performed at a fixed molar ratio of CPT to TPP (~1:1), which was maintained across all tested concentrations based on the nanoparticle formulation. As shown in Fig. 5c, the CI–Fa curve remained consistently below 1.0 over a broad fraction affected (Fa) range,

confirming a synergistic rather than additive effect. At the 50% inhibitory fraction Fa = 0.5, the CI value was approximately 0.80, indicating that a reduced dose of each modality was sufficient to achieve half-maximal inhibition in combination. With increasing Fa, the CI values declined further, reaching below 0.5 at higher effect levels, which demonstrates a dose-dependent enhancement of synergy. This effect arises from the dual function of the **ProCPTNPs** system, where red-light-triggered ROS generation not only induces direct PDT cytotoxicity but also cleaves the thioketal linker to release CPT *in situ*. Mechanistically, PDT-induced cytotoxicity arises from the generation of reactive oxygen species that oxidatively damage cellular lipids, proteins, nucleic acids, and organelle membranes.^{77–79} In contrast, the anticancer agent CPT exerts its therapeutic activity through inhibition of DNA topoisomerase I, stabilizing the Topo I–DNA cleavage complex and inducing replication-associated DNA damage.⁸⁰ Owing to their mechanistically distinct yet complementary modes of action, the combination of PDT and CPT has the potential to overcome the intrinsic limitations of each monotherapy. Collectively, these findings demonstrate that **ProCPTNPs** enable spatiotemporally controlled chemo-photodynamic synergy, achieving enhanced therapeutic efficacy at reduced doses compared with either modality alone.

Conclusions

In conclusion, we have developed a red-light-responsive prodrug nanoplatform **ProCPTNPs** that rationally integrates PDT and CPT chemotherapy within a single molecular construct *via* a ¹O₂-cleavable, self-immolative thioketal linker. Because both the PDT cytotoxicity and prodrug activation are contingent upon PDT-mediated ROS production, therapeutic activity of this nanomedicine system is spatiotemporally controlled by deep-penetrating red light, thereby confining cytotoxic effects



on demand. Additionally, the PDT and chemotherapy cooperate to damage tumor cells *via* orthogonal yet complementary mechanisms, PDT-driven oxidative damage coupled with drug-mediated DNA disruption, resulting in enhanced cytotoxic pressure beyond that achievable by either monotherapy alone. Consistent with this dual-modality mechanism, combination index analysis demonstrated significant synergy between PDT-induced oxidative stress and CPT-mediated topoisomerase I inhibition, particularly at intermediate effect levels (CI (Fa = 0.5) \approx 0.80). Overall, this study reports a rationally designed photosensitizer-drug conjugate strategy harnessing the combined effects of chemo-photodynamic therapy, while enabling externally controlled therapeutic efficacy by red light to reduce systemic exposure.

Author contributions

B. Xu led the study and contributed to the manuscript writing. X. Fu, Z. Huang and C. Zhang contributed to the experimental work. X. Hu oversaw the project, secured funding and resources, and contributed to the manuscript writing.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: The supplementary information includes the experimental details, supporting figures, synthetic procedures, and UV-vis, fluorescence, HPLC, and NMR spectra. See DOI: <https://doi.org/10.1039/d6ma00384b>.

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