Nanoscale Covalent Organic Framework for Combinatorial Antitumor Photodynamic and Photothermal Therapy

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ABSTRACT: Despite the excellent photodynamic and photothermal properties of organic molecular photosensitizers (PSs) and photothermal agents (PTAs), such as porphyrin and naphthalocyanine, their poor water solubility severely impedes their biological applications. Covalent organic frameworks (COFs), as an emerging class of organic crystalline porous materials, possess free active end groups (bonding defects) and large inner pores, which make them an ideal type of nanocarriers for loading hydrophobic organic molecular PSs and PTAs by both bonding defect functionalization (BDF) and guest encapsulation approaches to obtain multifunctional nanomedicines for PDT/PTT combination therapy. In this work, we report a nanoscale COF (NCOF) prepared via a facile synthetic approach under ambient conditions. Furthermore, a dual-modal PDT/PTT therapeutic nanoagent, VONc@COF-Por (3), is successfully fabricated by stepwise BDF and guest encapsulation processes. The covalently grafted porphyrinic PS (Por) and the noncovalently loaded naphthalocyanine PTA (VONc) are independently responsible for the PDT and PTT functionalities of the nanoagent. Upon visible (red LED) and NIR (808 nm laser) irradiation, VONc@COF-Por (3) displayed high 1O2 generation and photothermal conversion ability (55.9%), consequently providing an excellent combined PDT/PTT therapeutic effect on inhibiting MCF-7 tumor cell proliferation and metastasis, which was well evidenced by in vitro and in vivo experiments. We believe that the results obtained herein can significantly promote the development of NCOF-based multifunctional nanomedicines for biomedical applications.

KEYWORDS: covalent organic frameworks, photodynamic therapy, photothermal therapy, nanotherapeutics, cancer

Breast cancer is the most common female cancer worldwide, and despite ongoing efforts to improve treatment, the number of breast cancer deaths each year is still high.1 Among various cancer therapeutic methods, phototherapy, as a promising clinical transformation, has attracted intense attention due to its ease of operation, high specificity, low invasiveness, and minimal side effects.2−4 In this context, photodynamic therapy (PDT) employs photosensitizers (PSs) to absorb light energy and excite oxygen into singlet oxygen (1O2), resulting in cell damage, necrosis, and apoptosis.5−6 Furthermore, photothermal therapy (PTT) utilizes photothermal agents (PTAs) to convert light energy into heat, resulting in elevated temperature at the tumor site to kill tumor cells.7−8

However, PDT or PTT monotherapy might cause unnecessary side effects under prolonged treatment time and increased laser power conditions because the higher power and longer illumination time could lead to normal tissue damage to a certain extent.9 As it is known, PTAs with near-infrared (NIR) absorption (>650 nm) have become a popular research...
topic because of their optimal depth of penetration of biological tissue.\textsuperscript{10,11} Common inorganic NIR-PTAs, such as carbon nanomaterials,\textsuperscript{12−14} black phosphorus,\textsuperscript{15−17} gold nanorods,\textsuperscript{18,19} MXene nanosheets,\textsuperscript{20,21} and metal oxides and sulfides,\textsuperscript{22−24} have been proven to metabolize slowly in organisms and are mostly retained in the reticuloendothelial system (e.g., liver, spleen, macrophages),\textsuperscript{25−27} which is a limitation for their potential clinical applications. Therefore, organic PTAs, such as BODIPY,\textsuperscript{28,29} phthalocyanine,\textsuperscript{30,31} cyanine,\textsuperscript{32,33} and diketopyrrolopyrrole,\textsuperscript{34,35} have become good candidates for PTT applications. The commonality of these molecules is that they have a large molecular size and extensive π-conjugated systems, which result in poor water solubility and photostability.

To overcome the above issues, PTA delivery systems based on nanocarriers, such as metal−organic frameworks (MOFs),\textsuperscript{36,37} mesoporous silica (m-SiO\textsubscript{2}),\textsuperscript{38,39} phospholipids,\textsuperscript{40} and albumin,\textsuperscript{41} have been developed in recent years. These studies reached the consensus that the encapsulation of an organic PTA in nanocarriers can improve its photostability and enhance its accumulation at the tumor site. However, as previously mentioned, organic PTAs, usually with large molecular sizes (typically greater than 20 Å),\textsuperscript{42} often have low loading in these nanocarriers, which severely limits their PTT effect. Therefore, the development of nanocarriers with high PTA loading for improved PTT efficacy is a huge challenge.

Recently, covalent organic frameworks (COFs),\textsuperscript{43,44} as highly crystalline and covalently linked organic porous networks, have attracted much interest. As a growing class of nanoagents, COFs have shown great potential in nanotherapeutics, although only a handful of examples of COF-based PDT\textsuperscript{45−48} and drug delivery\textsuperscript{49−51} have been reported so far. Unlike amorphous organic polymers, COFs feature a long-range ordered structure, in which the organic building blocks are positionally controlled in two or three dimensions.\textsuperscript{52,53} This structural feature leads to regular pores with large diameters and facilitates the loading of large-sized PTAs, such as porphyrins and phthalonitriles. In addition, COF carriers are metal-free and should be highly biocompatible; moreover, they could effectively avoid the potential toxicity caused by metal-containing nanomaterials.\textsuperscript{54,55} Therefore, the fabrication of COF-based host−guest systems can be an alternative approach to access PTT agents, although there has been no report on this topic yet.

Recent studies on nanoscale MOF (NMOF)-based PDT revealed that an NMOF with its surface decorated with organic PSs possessed higher PDT efficiency. For example, compared to interior-grafted TCPP⊂UiO-66 (TCPP = meso-tetrakis(4-carboxyphenyl)porphyrin), surface porphyrinic species-decorated UiO-66-TPP-SH (TPP-SH = 5-(4-(5-ethylthioureido)-thiobenzophenyl)-10,15,20-tris(4-chlorophenyl)porphyrin) displayed significantly higher singlet oxygen generation ability and greater PDT efficacy against cancer cells in vitro.\textsuperscript{56} As a promising alternative to MOFs, COFs are usually obtained via step-growth condensation polymerization between two or more monomers, and the free end groups from the monomer functionality are left after the COF synthesis. In principle, these residual bonding defects can be further functionalized by the bonding defect functionalization (BDF) approach;\textsuperscript{45} thus, organic PSs can be primarily grafted onto the COF nanocarrier surface via free end-group modification. This method is different from the existing postsynthetic modification (PSM);\textsuperscript{57,58} the BDF surface decoration approach does not change the inner pore size of the established COFs; therefore,
the accommodation of large-sized organic PSs should be feasible.

Given the above discussion, the combination of BDF and guest encapsulation could logically be used to fabricate multifunctional NCOF-based nanomedical agents for PDT and PTT synergistic treatment. In addition, more recent studies demonstrated that PTT can enhance the sensitivity of cells to PDT; consequently, a significantly improved therapeutic effect for tumor treatment was observed.

Herein, we report a nanoscale COF (NCOF)-based PDT/PTT dual-modal therapeutic agent obtained via stepwise BDF and guest encapsulation processes (Figure 1A). The obtained VONc@COF-Por (3) with surface-decorated porphyrin (Por) and encapsulated naphthalocyanine (VONc) species exhibited highly efficient singlet oxygen generation and photothermal conversion ability and could significantly inhibit MCF-7 breast cancer cell proliferation and metastasis in vitro and in vivo.

RESULTS AND DISCUSSION

Synthesis. The preparation of NCOFs with high crystallinity, especially under ambient conditions, is a major challenge for COF-based medical applications. The existing procedures for NCOF preparation, including the polymer-assisted solvothermal method, high-power ultrasonic exfoliation, steric hindrance-induced chemical exfoliation, and the template method, are usually tedious as well as necessitating strict synthetic conditions. In addition, the large-scale synthesis of NCOFs is another bottleneck to their application in nanomedicine. Thus, the synthesis of NCOFs under ambient conditions on a large scale is extremely vital. Imine-linked TPB-DMTP-COF (1), which was generated from 1,3,5-tris(4-aminophenyl)benzene and 2,5-dimethoxyterephthaldehyde, was prepared on a gram-scale under mild reaction conditions (CH3CN, 25 °C, 12 h) with the aid of acetic acid and polyvinylpyrrolidone (PVP). Unlike traditional solvothermal COF synthesis, this approach did not require any vigorous reaction conditions, such as solvothermal and inert atmosphere. More importantly, scaling up to a gram-scale NCOF synthesis was easy.

COF-Por (2) was prepared via Schiff-base condensation between the free end aldehyde groups on 1 and the monoamino-decorated porphyrin (5-(4-aminophenyl)-10,15,20-triphenylporphyrin, Por) in 1,4-dioxane under reflux for 12 h (Figure 1B). By soaking 2 in an N,N-dimethylacetamide (DMAC) solution of vanadyl 2,11,20,29-tetra(tert-butyl)-2,3-naphthalocyanine (VONc) at room temperature, the host–guest supramolecular system VONc@COF-Por (3) was readily obtained (Figure 1B).

Structural Characterization. The structure of 3 is shown in Figure 2A. Significant color change occurred during these stepwise free end aldehyde modification (from yellow for 1 to brown for 2) and VONc loading (from brown for 2 to black
green for 3) processes (Figure 2B). Quantitatively, the contents of Por and VONc in 3 were determined to be 0.091 ± 0.010 and 0.256 ± 0.030 μmol/mg, respectively, by the standard curve method (Figure S1).

Notably, 1 was highly crystalline and featured a 2D network with an AA stacking model, which was the same as 1 reported by Jiang et al. and obtained under solvothermal conditions (degassed and sealed Pyrex tube, o-dichlorobenzene/n-ButOH, HOAc, 120 °C, 3 days).70 For example, 1 also exhibited one intensive peak at 2.74°, along with five less intensive diffraction peaks at 2θ = 4.80, 5.56, 7.38, 9.71, and 25.2°. These observed peaks were assigned to the (100), (110), (200), (210), (220), and (001) facets, respectively (Figure 2C).

After Por surface decoration and VONc guest loading, basically no peak shift was observed for 2 (2θ = 2.76, 4.82, 5.59, 7.40, 9.73, and 24.7°) and 3 (2θ = 2.78, 4.93, 5.65, 7.45, 9.84, and 23.0°) relative to 1, implying that the structural features of 1 were well maintained during these successive modifications.71,72 Notably, no characteristic diffraction peaks for Por and VONc species were observed in the powder X-ray diffraction (PXRD) patterns of 2 and 3, suggesting that 2 and 3 were generated in a single phase instead of a simple physical mixture of 1 with Por and VONc.73,74 The PXRD pattern of a physical mixture of 2 and VONc, as a control, is shown in Figure S2, and the diffraction peak belonging to VONc was observed at 2θ = 5.24°.

The obtained 1, 2, and 3 were further characterized by Fourier transform infrared (FT-IR) spectrometry. As shown in Figure 2D, the peaks at 1682 and 3373 cm⁻¹ associated with the free —CHO and —NH₂ groups in 1 directly evidenced the existence of bonding defects in the COF.75 On the other hand, the C≡N stretching vibration at 1617 cm⁻¹ indicated that an imine linkage was present in 1, 2, and 3.76,77 In 2, the monosubstituted Ar—H in-plane vibration at 792 cm⁻¹, together with the pyrrole N—H stretching vibration at 3319 cm⁻¹, proved that the Por species was grafted on 2. Additionally, the stretching vibrations at 3641 and 3541 cm⁻¹ for the —NH₂ groups on Por disappeared, unambiguously implying that the Por moiety was attached to 1 via a covalent bonding interaction. After VONc loading, the observed characteristic vibration band of V=O at 1007 cm⁻¹ and the stretching vibrations of —CH₂ at 2955 and 2864 cm⁻¹ in 3 demonstrated that the VONc species was successfully encapsulated by the COF host framework (Figure 2D).

Owing to the 3d⁴ electronic configuration of V(IV) in VONc, electron paramagnetic resonance (EPR) measurements were performed. As indicated in Figure 2E, the solid samples of VONc and 3 exhibited similar EPR spectra, which were characterized by the 8-fold hyperfine structure resulting from the coupling between the ⁵¹V nucleus with nuclear spin I = 7/2 and an unpaired electron of V(IV) with spin S = 1/2.78,79 The EPR signals were further split due to the anisotropic components of the hyperfine coupling in the vertical and parallel directions.80 Therefore, the vanadium species in VONc and 3 was a VO⁺² cation with a C₄ᵥ symmetric ligand field. As controls, 1, Por, and 2 did not exhibit any EPR signal, which provided further evidence for the presence of VONc in 3.

The light absorption properties of the samples were also investigated by ultraviolet—visible (UV—vis) absorption spectrosopy. As shown in Figure 2F, the broad absorption band of the COF framework almost covered the whole ultraviolet and visible regions (300—600 nm), indicating the delocalization of the π electrons in the COF framework.81 In the UV—vis spectra of 2 and 3, both the B- and Q-bands (Figure 2G) from Por were observed. In comparison to the spectrum of molecular Por, the spectra of 2 and 3 exhibited a slightly red-shifted and negligibly widened band, further confirming that the Por species was covalently anchored onto 1. In addition, a broad absorption band in the range 750—900 nm for the encapsulated VONc was observed in 3, suggesting that VONc-based aggregation occurred in the COF pores. The Q-band absorption in the red range and the wide NIR absorption of 3 made it highly suitable for PDT and PTT applications.

The porosity of the NCOFs was studied by N₂ adsorption and desorption experiments at 77 K (Figures 2H and S3). The type IV isotherm of 1 was indicative of its mesoporous character. Based on the Brunauer—Emmett—Teller (BET) equation, the BET specific surface area (S_BET) of 1 was calculated as 1217 m²/g, which was significantly lower than the previously reported value (2081 m²/g).70 This difference may be because the crystallinity of the COF prepared by room-temperature synthesis was inferior to that of the COF prepared by solvothermal synthesis. Based on the nonlocal density functional theory (NLDFT) model, 1 has a narrow pore size distribution centered at 3.2 nm, suggesting the good regularity of 1, which was consistent with previous reports.70 The S_BET values of 1 and 2 were almost the same, suggesting that modification with Por occurred on the surface of 1 rather than in its pores. If Por modification had occurred in the pores of 1, the S_BET would have obviously decreased. In addition, for 2 and 3, the S_BET values correspondingly decreased from 1180 m²/g (2) to 115 m²/g (3), which was clearly caused by VONc guest loading. Furthermore, the pore size distribution of 3 showed that the mesopore size of 3.2 nm was shifted to 2.5 nm after VONc loading.

Finally, the encapsulated VONc could be released from 3 with a slow release rate in DMAC organic solvent. UV—vis spectral monitoring indicated that 43.9% of VONc was released when 3 was immersed in DMAC for 24 h, further indicating the formation of the VONc@COF host—guest system. Notably, no VONc release occurred when 3 was in PBS at 37 °C due to its extreme hydrophobic nature, so 3 was stable under physiological conditions. In contrast, no free Por species were detected during this DMAC extraction process, revealing that the Por moiety was indeed covalently attached to the COF NPs (Figure S4).

**Morphology and Surface Characterization.** Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) showed that the as-synthesized 1—3 were obtained as radial spherical NPs with diameters of ~140 nm (Figures 3A and S5), indicating that BDF and guest loading processes did not change their particle size and morphology. The particle size statistics showed that the NPs of 1, 2, and 3 were evenly distributed (Figure S6). Dynamic light scattering (DLS) and polydispersity index (PDI) measurements further supported the above conclusions (Figure 3B).

It is known that the synthesis of well-dispersed NPs, preferably in the size range 50—200 nm with appropriate surface modification, is pivotal to access to optimal pharmacokinetics in vivo, that is, enhanced accumulation at the tumor site along with prolongation of blood circulation and evasion of opsonization by serum proteins and clearance by immune sentinels, the mononuclear phagocytic system.82
Notably, the zeta potentials of 1, 2, and 3 were 25.7 ± 1.2, 15.9 ± 1.0, and 18.5 ± 1.3 mV, respectively (Figure 3C). The positive zeta potential could significantly enhance the interaction between the NPs and the negatively charged tumor cell membrane, thereby greatly promoting tumor cellular uptake. In addition, the particle sizes, PDIs, and zeta potentials of NPs in PBS (pH 6.5 and 7.4), DMEM, FBS, and normal saline were basically unchanged over 72 h, indicating their prominent colloidal stability under physiological conditions (Figures S7 and S8).

**Photodynamic and Photothermal Properties.** Based on the visible and NIR absorption displayed by 3, a low-powered red LED and an 808 nm laser were selected and used as excitation light sources to test its photodynamic and photothermal properties.

The photodynamic behavior of 3 was evaluated using 1,3-diphenylisobenzofuran (DPBF) as an 1O2 probe (Figures 4, S9, and S10). After the dispersion of 3 (40 μg/mL) was exposed to a red LED (50 mW/cm²) for 7 min, the absorbance of DPBF was reduced to 20.8% of the initial value, suggesting highly efficient 1O2 generation. In contrast, no significant decrease in DPBF absorbance was observed (92.3%) when the PBS dispersion of 3 was irradiated with the 808 nm laser (1.5 W/cm²), implying that an NIR light source could not induce effective 1O2 generation. Upon irradiation with both the red LED and 808 nm laser, no enhanced 1O2 generation efficiency compared to that obtained under only red LED irradiation was observed. Thus, the red LED was the light source responsible for 1O2 generation.

NIR light at 808 nm, however, could effectively induce the photothermal conversion of 3 under physiological conditions. The photothermal conversion results of 3 under different conditions (concentration, 0–600 μg/mL; power density, 0–2.0 W/cm²) are given in Figure 5A–D. As shown, the photothermal behavior of 3 was concentration- and laser-density-dependent. For example, when 3 (600 μg/mL) was exposed to an 808 nm laser (1.5 W/cm²) for 10 min, the system temperature increased from 23.6 to 58.1 °C (ΔT = 34.5 °C), and the photothermal conversion efficiency was calculated to be η = 55.9% (Figure S11). Moreover, only a 1.4 °C temperature increase of pure water was detected in the absence of 3 under the same conditions. Notably, the photothermal conversion efficiency displayed by 3 was universally higher than those displayed by previously reported phthalocyanine- and naphthalocyanine-based PTT systems (Table S1). This high photothermal conversion

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**Figure 3.** Morphology and surface characterization of 1, 2, and 3 NPs. (A) SEM (scale bar, 1 μm) and TEM images (scale bar, 500 nm) of 1, 2, and 3. (B) DLS size profiles and PDI of 1, 2, and 3 in PBS (pH 6.5) at 25 °C. (C) Zeta potentials of 1, 2, and 3 in PBS (pH 6.5). Data are presented as the mean ± SD (n = 3).

**Figure 4.** Photodynamic properties of 3. (A) The principle of detecting 1O2 with 1,3-diphenylisobenzofuran (DPBF) as a probe. (B) Comparison of the decay rate of DPBF induced by 3 (40 μg/mL) under red LED (50 mW/cm²) and/or 808 nm laser (1.5 W/cm²) irradiation. (C–E) UV–vis spectra of DPBF induced by 3 under a red LED (C), an 808 nm laser (D), and a red LED + 808 nm laser (E).
efficiency may benefit from the high VONc loading capacity of the COF.

In addition to high PTA loading, light stability is another important factor affecting PTT efficacy. In comparison to free VONc, 3 exhibited excellent photothermal stability even when exposed to a laser for 50 min (Figure S5E), which was in good agreement with the findings derived from the light stability examination (Figure S12). With the NCOF platform, not only was VONc nanocrystallization successfully realized, but its photostability was also significantly improved.

Laser Scanning Confocal Fluorescence Microscopic Images of Cells. As mentioned above, NCOF 3 was dual-modal with independent photodynamic and photothermal properties under light irradiation with different wavelengths; that is, visible light triggered ROS generation and NIR induced photothermal conversion. Therefore, we concluded that dual-wavelength illumination should facilitate combinatorial PDT and PTT. Accordingly, the 3-based PDT and PTT monotherapy effects and the additive effects of the PDT/PTT combination on MCF-7 cells were initially tested by laser scanning confocal fluorescence microscopy.

First, subcellular localization analysis showed that the Pearson colocalization coefficients of 3 for the mitochondria, lysosomes, and nuclei were $R_p = 0.730, 0.539,$ and 0.192, respectively (Figure S13). This result indicated that 3 was internalized by MCF-7 cells through endocytosis, was mainly localized to the cytoplasm, and did not have distinct subcellular targeting characteristics.

Second, intracellular $^{1}O_2$ levels were assessed using the $^{1}O_2$ fluorescent probe Singlet Oxygen Sensor Green (SOSG). As shown in Figure 6A, after incubation with 3 (40 $\mu$g/mL) for 2 h and exposure to a red LED (50 mW/cm$^2$) for 10 min, the intracellular $^{1}O_2$ concentration significantly increased. By contrast, NIR irradiation (808 nm laser, 1.5 W/cm$^2$, 10 min) had no effect on the intracellular $^{1}O_2$ levels. Compared with the red LED case, dual-wavelength illumination did not induce an increase in the $^{1}O_2$ level.

Figure 5. Photothermal properties of 3. (A) Temperature increase induced by 3 (0–600 $\mu$g/mL) under an 808 nm laser (1.5 W/cm$^2$). (B) Temperature increase induced by 3 (600 $\mu$g/mL) under an 808 nm laser (0–2.0 W/cm$^2$). (C) Thermal images of 3 (600 $\mu$g/mL) under red LED (50 mW/cm$^2$) and/or 808 nm laser (1.5 W/cm$^2$) irradiation for 10 min. (D) Temperature rises induced by 3 (600 $\mu$g/mL) under red LED (50 mW/cm$^2$) and/or 808 nm laser (1.5 W/cm$^2$) irradiation. (E) Temperature cycling curves of 3 (600 $\mu$g/mL) and VONc (153.6 $\mu$M) over five on–off cycles of 808 nm laser (1.5 W/cm$^2$) irradiation.

Third, the decrease in mitochondrial membrane potential ($\Delta\Psi$, as an initial indicator of cell death, was evaluated using a JC-1 fluorescent probe. As shown in Figure 6B, PTT monotherapy had little effect on $\Delta\Psi$, whereas PDT monotherapy decreased $\Delta\Psi$ to a limited extent and combination therapy resulted in a significant decrease in $\Delta\Psi$, as reflected by the enhanced green fluorescence from the JC-1 monomer and reduced red fluorescence from the JC-1 J-aggregate. These results were in stark contrast to the intracellular $^{1}O_2$ concentration results.

Fourth, the mitochondrial oxidative stress state was further confirmed by the MitoTracker Red CM-H$_2$Xros fluorescent probe, which is oxidized by mitochondrial reactive oxygen species (ROS) to a species emitting bright orange-yellow fluorescence. As shown in Figure 6C, consistent with the trend in $\Delta\Psi$, the mean fluorescence intensity (MFI) was increased more by the combination therapy than by PDT monotherapy, and PTT monotherapy did not affect MFI. This result confirmed the synergistic stimulatory effects of the combination therapy on oxidative stress.

Fifth, the effects of phototherapy on intracellular membrane structures, such as lysosomal membranes, were also investigated. As shown in Figure 6D, before treatment, acridine orange (AO) was protonated in the lysosome to emit red fluorescence; after treatment, AO that leaked from the lysosome to the cytosol due to lysosomal damage was deprotonated and emitted green fluorescence. Thus, PDT or PTT monotherapy induced an increase in lysosomal membrane permeability (LMP), and the combination therapy further enhanced this change. The enhanced LMP promoted endosomal/lysosomal escape of the NPs, avoiding premature degradation of NPs, and was beneficial for sustained therapeutic effects.

Based on the above results, we concluded that the combination therapy could improve the antitumor treatment efficacy by exacerbating mitochondrial and lysosomal damage, although it did not increase intracellular $^{1}O_2$ levels.
**In Vitro Antitumor Therapy.** The combinatorial PDT and PTT efficacy of 3 was evaluated in MCF-7 cells in vitro. As shown in Figure 7A and Table S2, a standard 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method was used to assess cell proliferation and cytotoxicity. The results showed that the cell viability remained as high as 83.8 ± 4.7% in the dark, even with a concentration as high as 400 μg/mL, indicating that 3 possessed negligible dark toxicity and excellent biocompatibility. On the other hand, light irradiation alone also caused a negligible change in cell viability. However, PDT monotherapy (3, 100 μg/mL; red LED, 50 mW/cm², 10 min) and PTT monotherapy (3, 100 μg/mL; 808 nm laser, 1.5 W/cm², 10 min) gave cell viabilities of 63.2 ± 6.5 and 47.3 ± 5.0%, respectively. Overall, the cell viability derived from the combination of PDT/PTT sharply declined to 16.5 ± 1.4%. The IC₅₀ under different treatment

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**Figure 6.** Laser scanning confocal fluorescence microscopy images. (A) Detection of intracellular ¹⁰O₂ using SOSG. (B) Detection of mitochondrial membrane potential (ΔΨ) using JC-1. (C) Detection of cellular oxidative stress using MitoTracker Red CM-H₂Xros. (D) Detection of lysosomal membrane permeability using acridine orange (AO). Experimentally, the cells preincubated with 3 (40 μg/mL, 2 h) were exposed to a red LED (50 mW/cm², 10 min) for PDT and/or an 808 nm laser (1.5 W/cm², 10 min) for PTT. The cells without any treatment were used as controls. Scale bar, 100 μm. The mean fluorescence intensity (MFI) analyzed by ImageJ software was presented as the mean ± SD (n = 5). ***p < 0.001; ns, no significance (p > 0.05).
conditions was calculated based on a logistic fit. As shown in Figure 7B, the IC₅₀ for the combination therapy was only 42 μg/mL, significantly lower than that of both types of monotherapy (131 μg/mL for PDT and 93 μg/mL for PTT). These results demonstrated that, compared with the corresponding monotherapies, the combination therapy indeed could significantly inhibit tumor cell proliferation.

To further validate the synergy between PDT and PTT, cells were treated with NaN₃ (25 mM) prior to combination therapy to consume the 1O₂ produced during PDT, and the cell viability was found to be restored to a level similar to the cell viability induced by PTT monotherapy. Similarly, combination therapy at 4 °C negated the effects of PTT and recovered the cell viability to the level induced by PDT monotherapy (Figure 7C).

As shown in Figure 7D, the outstanding therapeutic effects of the combination therapy were further confirmed by calcein-AM/PI double staining. The proportion of dead cells increased from 41.0 ± 5.9% for PDT and 75.2 ± 10.5% for PTT to 94.4 ± 3.5% for the combination therapy when a dispersion of 3 (100 μg/mL) was used for phototherapy.

In addition, the effect of the combination therapy on the migration and invasiveness of MCF-7 cells was evaluated using a scratch assay. As shown in Figure 7E, when using a dispersion of 3 (40 μg/mL) for phototherapy, compared to the control group, the group treated with PDT monotherapy showed a very limited inhibitory effect on cell migration, and the group treated with PTT monotherapy exhibited inhibited migration to a certain extent. By contrast, the combination therapy almost completely inhibited MCF-7 cell migration.
On the basis of these results, we could conclude that the combination therapy exhibited a much better curative effect than either type of monotherapy on inhibiting cell proliferation, inducing cell death and suppressing cell migration.

**In Vivo Antitumor Therapy.** The in vivo antitumor effect of 1 was evaluated by an MCF-7 xenograft model. Thirty nude mice bearing tumors were randomly divided into six groups (Figure 8A and Table S3). Group i was the control group without any treatment. Group ii was the laser group, and only light was administered. Group iii was the dark group, and only 3 was administered. Groups iv, v, and vi were the treatment groups: 4 h after the intratumor injection of 3 (Figure S14, the NPs still retained in the tumor principally at this time), light was administered to perform PDT, PTT and the combination therapy, respectively. As shown in Figures 8 and S15, for groups ii and iii, the tumor volume increased rapidly, and there was no difference from the control group i. For the treatment groups iv, v, and vi, as demonstrated by the *in vitro* experiments, 3 displayed strong photodynamic and photothermal effects. For group iv, PDT monotherapy caused limited damage to tumor tissues, and observable recurrences occurred immediately after treatment. For group v, PTT caused significant increases in the temperature at the tumor site, showing rapid shrinkage of the tumor volume on the first day after treatment and edema at the tumor site and scar formation on day 3. However, there was still some residual tumor tissue, which caused the tumor to recur on day 5 after treatment. For group vi, the combination therapy eradicated almost all of the tumor tissues, and scabs were exfoliated on days 8–12 without obvious signs of recurrence, indicating the synergy and great advantages of combination therapy over monotherapy. The histopathological analysis on the main organ tissues from mice sacrificed after therapy was also performed (Figure S16). No detectable signs of damaged tissues or inflammatory lesions were observed, indicating high histocompatibility of COF-based nanomaterials.

To further confirm the necessity and advantages of the combination therapy, we conducted an enhanced *in vivo* experiment: increasing the illumination time from 10 to 20 min and using the maximum power of the laser devices (200 mW/cm² for the red laser and 2.0 W/cm² for the 808 nm laser). As shown in Table S4 and Figure S17, the enhanced PDT (group vii) significantly inhibited tumor growth at an early stage, but the tumor gradually relapsed after the fifth day due to failure to eradicate the tumor. The enhanced PTT (group viii) almost completely eradicated the tumor, but the higher power and longer illumination time caused certain damage to normal tissues (e.g., the skin around the tumor, Figure S17F). On the 15th day, the skin damage was still not fully recovered. However, the combined treatment with the lower power and shorter illumination time (group ix) has less damage to the skin (Figure S15H). These results reflect the importance of combination therapy: to achieve effective inhibition of tumors while minimizing side effects.

**CONCLUSION**

In summary, we prepared an NCOF (1) via a facile synthetic approach under ambient conditions and used it as a dual-modal PDT/PTT therapeutic platform. VONc@COF-Por (3) was successfully fabricated by stepwise BDF and guest encapsulation processes. The covalently grafted porphyrinic PS (Por) and the noncovalently loaded naphthalocyanine PTA (VONc) are independently responsible for PDT and PTT. Under visible (red LED) and NIR (808 nm laser) irradiation, 3 displayed high ¹O₂ generation and photothermal conversion ability (55.9%), consequently providing an ideal combined PDT/PTT therapeutic effect on inhibiting MCF-7 tumor cell proliferation and metastasis, which was further evidenced by *in vitro* and *in vivo* experiments. We believe that our research can promote the development of nanomedicines based on COFs.

**EXPERIMENTAL SECTION**

**Synthesis of COF (1).** A mixture of 1,3,5-tris(4-aminophenyl)benzene (984 mg, 2.8 mmol), 2,5-dimethoxyterephthaldehyde (854 mg, 4.4 mmol), PVP (Mw = 8000, 1.0 g), and acetic acid solution (1.0 mL, 3 M) was stirred at 25 °C for 12 h. Next, benzaldehyde (40 μL, 0.4 mmol) was added to the reaction system to quench the reaction. After 1 h, the particles were isolated by centrifugation and washed with acetone three times to generate COF 1 as a yellow powder. Yield: 1.48 g. FT-IR (ATR, cm⁻¹): 3373 (w), 2998 (w), 2940 (w), 2832 (w), 1682 (m), 1617 (m), 1593 (m), 1505 (m), 1487 (m), 1465 (m), 1409 (s), 1393 (m), 1309 (m), 1182 (w), 1142 (m), 1039 (m), 1013 (w), 972 (w), 979 (w), 830 (m), 736 (w), 693 (w), 608 (w), 540 (w).

**Synthesis of COF-Por (2).** A mixture of newly prepared COF 1 (50 mg), Por (126 mg, 0.2 mmol), acetic acid solution (1.0 mL, 3 M), and 1,4-dioxane (200 mL) was heated to reflux for 12 h. The particles were isolated by centrifugation and washed with 1,4-dioxane until the supernatant liquid was colorless to generate COF-Por (2) as a brown.
The particles were washed with water three times to generate VONc.

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ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.9b06467.
Additional experimental details, including materials and instrumentations; cell culture and laboratory animals; Por and VONc contents; release experiments; photodynamic property; photothermal conversion efficiency; subcellular localization of mitochondria, lysosomes, and nuclei; intracellular single oxygen measurement; mitochondrial membrane potential; mitochondrial oxidative stress; lysosomal membrane permeabilization; calcine-AM/PI double staining; in vitro scratch assay; in vivo antitumor therapy; enhanced in vivo antitumor therapy; and additional results (Figures S1–S17 and Tables S1–S4) (PDF)

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Notes
The authors declare no competing financial interest.

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