Highly Stable Organic Photothermal Agent Based on Near-Infrared-II Fluorophores for Tumor Treatment

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Research

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Abstract

**Background:** To develop highly stable near-infrared (NIR) photo-induced tumor therapy agent has obtained considerable role for biological application. With longer wavelength, biological imaging exhibits high signal-to-background ratio, deep tissue penetration and maximum permissible light power, and photoinduced tumor therapy can reduce damage minimization to organism.

**Results:** A class of stable NIR-II fluorophores (NIR998, NIR1028, NIR980, NIR1030 and NIR1028-S) based on aza-BODIPY dyes with donor-acceptor-donor structures have been rationally designed and synthesized via utilizing steric relaxation effect and intramolecular photoinduced electron transfer (IPET). These fluorophores exhibit superior stability against photobleaching, NIR-II emission, large Stokes shift (≥ 100 nm) and excellent photothermal conversion performance. Among them, NIR998 with better near-infrared-II emission and higher temperature increase was utilized to prepare NIR998 nanoparticles (NIR998 NPs) by lipidosome encapsulation. NIR998 NPs show super stability in the presence of light, heat, reactive oxygen nitrogen species than that of ICG NPs, as well as high photothermal conversion ability (η = 50.5%). Finally, under the guidance of photothermal imaging, NIR998 NPs with negligible cytotoxicity have effectively eliminated tumors by their excellent photothermal conversion performance.

**Conclusions:** Utilizing IPET and steric relaxation effect can effectively fulfill NIR-II emission of aza-BODIPY dyes and super stable NIR998 NPs with excellent photothermal conversion performance and negligible dark cytotoxicity can act as potential photothermal agents for biological applications.

**Background**

The development of stable near-infrared (NIR) fluorophores, especially the NIR-II ones, has captured considerable attention because, with longer wavelength, biological imaging shows high signal-to-background ratio, deep tissue penetration and maximum permissible light power [1], and photoinduced tumor therapy can decrease damage minimization to organism. To date, various NIR-II fluorophores based on inorganic materials such as transition-metal sulfide/oxide, rare-earth nanoparticles, wide-bandgap semiconducting, have been explored for biological imaging and tumor therapy because of their excellent photo-stabilities and photothermal conversion performance [2]. But their poor biodegradability, potential toxicity have limited their biological application [3]. When compared to inorganic NIR-II fluorophores, organic ones, especially small molecules, show many superiorities, such as inherent chemical structure and biodegradability, flexible design and absorption wavelength, and low toxicity [4]. Nevertheless, their poor stability and photothermal conversion properties provide hinder to their promising biological applications as flexible NIR-absorbing imaging contrast agents or therapeutic agents [5]. These problems can induce misleading guidance, poor therapeutic results or even badly side effect. Therefore, it is a challenge to rationally design and accessibly synthesize stable organic NIR-II small molecular fluorophores.
Currently, there are mainly two sorts of methods to construct NIR-II organic small molecular fluorophores. They are changing heterocyclic substitutions and conjugation length to construct polymethines derivates [6], and adjusting versatile donor–acceptor–donor (D−A−D) structure to develop benzo[1,2-c:4,5-c0]bis([1,2,5]thiadiazole) (BBTD) derivates, respectively [7]. With the rational design via changing electron donating properties of substituents and bonding methods, some NIR-II fluorophores based on BBTD have been reported [8]. Although these fluorophores exhibit excellent photophysical and photochemical performance accelerates their application in biological imaging and tumor therapy, new NIR-II fluorophores with stable structure, rational design by theoretical guidance and facile synthesis are still needed.

Aza-borondipyrromethene (aza-BODIPY) dyes, as a kind of traditional dyes, show many advantages, such as much better stability than cyanine derivatives, strong absorbance, tunable photophysical properties [9]. They have been extensively explored in photoelectric device, biological imaging and sensing, and tumor therapy [10]. Aza-BODIPY mother core skeleton is a typical electron deficient structure. It can incorporate electron donor to construct a D−A−D skeleton for distinct enhanced maximal absorption and emission [11], which shows their potential as NIR-II fluorophores for biological imaging and therapy.

In this work, a series of NIR-II fluorophores (NIR998, NIR1028, NIR980,NIR1030, NIR1028-S) based on aza-BODIPY dyes have been rationally developed by steric relaxation effect and intramolecular photoinduced electron transfer (IPET) (Scheme 1a). They exhibit NIR-II emission, large Stokes shift (≥ 100 nm), excellent photostability and photothermal conversion performance. NIR998 with better fluorescence emission and photothermal conversion performance was encapsulated by lipidosome (Scheme 1b). The prepared NIR998 nanoparticles (NIR998 NPs) with excellent water solubility and biocompatibility show much better resistance to photobleaching, heat and reactive oxygen nitrogen species stability than that of ICG NPs. Concentration-dependent temperature change, excellent enhanced permeability and retention (EPR) effects of NIR998 NPs have successfully prompted them use for photothermal imaging of mice (Scheme 1b). With these superiorities, NIR998 NPs have effectively removed ovarian tumors by their produced heat under the guidance of photothermal imaging. H&E staining and immunohistochemical analyses have also demonstrated the negligible dark cytotoxicity of NIR998 NPs. The exploration displays that IPET and steric relaxation effect are effectively strategies to fulfill NIR-II emission of aza-BODIPY dyes and NIR998 NPs with super stability and high photothermal conversion capacities exhibited promising clinical applications as diagnostic reagents.

Results And Discussion

Design and synthesis of NIR-II fluorophores

To construct D−A−D system with intramolecular photoinduced electron transfer properties is an effective strategy to develop low-band gap NIR dyes for biological application in vivo [12]. Aza-BODIPY as the inherent photothermal agent is chosen as research object [13], and aza-BODIPYs with D−A−D structures, especially, alkyl aniline parts as the strong electron donor, exhibit satisfactory NIR-I absorption and
emission [14]. Besides, electron donor with small steric hindrance can also boost the red-shifted absorption and emission of aza-BODIPYs core by steric relaxation effect [15]. With above consideration, diethylamine with strong donor electronic ability are introduced at position 3 and 5 of aza-BODIPYs core to reduce energy gap for red-shifted absorption and emission due to the better effect than other positions [16]. Finally, a series of NIR-II fluorophores (NIR998, NIR1028, NIR980, NIR1030, NIR1028-S) with D-A-D’ structures were rationally developed by further introduction of other alkylaniline derivatives or aromatic rings skeleton with low steric bulk at positions 2 and 7 of aza-BODIPYs skeleton.

As shown in supporting information Scheme S1, ketene structures (X-1, X = 1, 2, 3, 4, 5) with high yields (>90%) were first synthesized by Claisen–Schmidt reaction between aldehyde derivatives and diethylaminoacetophenone under mild condition. Nitromethane anions were then introduced by addition reaction, forming X-2. Homodimer of X-2 provided dipyrromethene derivatives (X-3). Finally, NIR-II dyes (NIR998, NIR1028, NIR980, NIR1030, NIR1028-S) were obtained by treating X-3 with BF₃·OEt₂ in the presence of diisopropylethylamine. They were full demonstrated by MS and NMR.

Photophysical Characterization of NIR-II Fluorophores

The photophysical properties of the free NIR-II dyes were first explored. They showed good dispersity in organic solvents, indicating their outstanding processability (Fig. S1). Their maximal absorption peak (MAP) exhibited distinct difference with various solvents. NIR998, NIR1028, NIR980, NIR1030 and NIR1028-S with the wavelength corresponding to MAP showed obviously red shift as 123 nm, 124nm, 89 nm, 104 nm and 40 nm with the increase of solvent polarity (Fig. S1), respectively, which was due to intramolecular charge transfer (ICT) effect [17]. They also showed wide absorption in 500−1000 nm region with the wavelength corresponding to MAP as 859 nm, 853 nm, 880 nm, 913 nm and 910 nm in DMSO (Fig. 1a), respectively. NIR998, NIR980, NIR1028-S, NIR1028 and NIR1030 displayed NIR-II emission with wavelength corresponding to maximal emission peak (MEP) as 998 nm, 980 nm, 1028 nm, 1028 nm and 1030 nm (Fig. 1b), respectively, suggested that the construction of D−A−D’ system with IPET and steric relaxation effect contribute to boost the red-shifted emission of aza-BODIPY framework and fulfill NIR-II emission, and they also displayed reduced luminescence intensity in turn when they shared same absorption at excitation wavelength. They also exhibited large Stokes shift as 139 nm, 175 nm, 100 nm, 117 nm and 117 nm in DMSO (Fig. 1a and 1b), respectively, which was due to IPET [18].

Their intense NIR absorption and emission range was in accordance well with biological window, indicating their promising NIR light-regulated biological application as imaging contrast agents and therapeutic agents. The antiphotobleaching property of dyes as imaging contrast agents, probes or photosensitizers is a very important index for imaging, sensing and disease treatments [19].

Photobleaching resistance of NIR-II dyes were then investigated under irradiation (808 nm, 0.2 W cm⁻²) by recording the change of their maximal absorption. Commercial dyes (ICG and S1451) acted as control. Absorption spectra of all NIR-II dyes showed slightly change after irradiation for 10 min, by marked contrast, the maximal absorption of commercial dyes such as ICG and S1451 reduced distinctively. For example, after 10 min irradiation, the maximal absorption of ICG and S1451 decreased to 75% and 71% of original value while above 97% of NIR-II dyes left (Fig. 1c and S2), indicating the better
antiphoto bleaching of the as prepared NIR-II dyes. Photothermal conversion properties of NIR-II dyes (10 μM) in DMSO were also explored under irradiation. The temperature of **NIR998, NIR1028, NIR980, NIR1030, NIR1028-S** solutions increased with the continues irradiation, and the temperature increase of them were 12.8 °C, 10.6 °C, 9.1 °C, 8.0 °C and 8.5 °C (Fig. 1d), respectively, while DMSO exhibited negligible temperature increase under the same condition. The results indicated the excellent photothermal conversion performance of the prepared NIR-II dyes. The relative better NIR-II emission and photothermal conversion performance of **NIR-998** was chosen for the following test.

**Synthesis and Characterization of NIR998 NPs**

For biological application, water-soluble **NIR998** nanoparticles (**NPs**) with excellent biocompatibility were acquired via encapsulation for biological application. The concentration of **NIR998NPs** was calculated as 400 μM (Fig. S3). **NIR998NPs** showed uniform spherical morphology, and their hydrodynamic diameter in PBS (pH = 7.4) are 115 nm, bigger than transmission electron microscopy (TEM) results as 75 ± 5 nm due to the swell of core and liposome in PBS (Fig. S4). The size of **NIR998 NPs** in PBS contributed to their accumulation at tumor parts by the EPR effects [20]. In addition, **NIR998 NPs** solutions exhibited similar hydrodynamic diameter for half a month in refrigerator at 4 °C (Fig. S5), indicating the excellent water solubility, biocompatibility and structure stability of **NIR998 NPs**. **NIR998 NPs** in PBS (pH = 7.4) showed similar absorption shape corresponding to that of **NIR998** in DMSO solution (Fig. 2a), however, compared to the emission of **NIR998** in DMSO, their luminescence was severely quenched due to the aggregation of free **NIR998** in micelles (Fig. 2b), indicating the worse NIR-II fluorescence imaging, which, of course, help to improve the photothermal conversion capacity of **NIR998 NPs**. The results matched well with previous works [21]. The strong NIR light capture capability and negligible emission of **NIR998 NPs** endowed them promising photoacoustic imaging (PAI), photothermal imaging and tumor photothermal therapy performance because of the fact that PA signal was produced via molecular vibration induced by heat and the intense NIR absorption with negligible luminescence prompted nonradiative conversion process for improved photothermal conversion capacity [22]. The photothermal conversion performance of **NIR998 NPs** solutions was first explored in various concentrations. **NIR998 NPs** showed obvious temperature increase with the rising concentration or light power (Fig. S6a and S6b). Besides, the photothermal conversion efficiency was also obtained as 50.5% (Fig. S6c - S6f), higher than most common photothermal agents [23]. In addition, **NIR998 NPs** (20 μM) solution showed high temperature increase as 26.0 °C under irradiation (808 nm, 0.5 W cm⁻², 6 min), which helped to effectively suppress tumor growth, indicating that **NIR998 NPs** could serve as promising photothermal agent for tumor photothermal therapy. **NIR998 NPs** solutions also showed concentrations-dependent PA signal intensity with excellent linearity ($R^2 = 0.9893$) (Fig. S7), which offered a chance to quantize the distribution of **NIR998 NPs** in vitro or in vivo.

**Stability Exploration of NIR998 NPs**

The destroyed agents usually induced misleading guidance, worse therapeutic efficacy or even serious side effect [24]. The photothermal circulation stability, antiphotobleaching properties, pH or reactive
oxygen and nitrogen species (RONS) (hydrogen peroxide (ONOO−) or peroxynitrite (H₂O₂)) resistance were investigated by comparing that with ICG NPs, which has been verified by food and drug administration (FDA) for clinic application and show similar absorption to that of NIR998 NPs [25]. The photothermal circulation stability of NIR998 NPs and ICG NPs in PBS (pH = 7.4) was first explored after heating and cooling circles. The temperature increase (35.9 °C) of NIR998 NPs exhibited negligible changes during seven circles, whereas the temperature changes of ICG NPs (27.4 °C) distinctly reduced to 54% (14.8 °C) and 28% (7.6 °C) of the original number after first and second circles, respectively (Fig. 2c, S8a and S8b). The results confirmed the better photothermal conversion performance, photothermal circulation stability and antiphotobleaching of NIR998 NPs than that of ICG NPs. Their antiphotobleaching in PBS (pH = 7.4) were further explored under irradiation. As shown in Fig. 2d, S8c and S8d, NIR998 NPs solution displayed negligible changes of colors and absorption spectra even with irradiation for 15 min while ICG NPs solution exhibited obviously changed color and decreased maximal absorption. For example, the visible green color disappeared and only 22% of ICG NPs left after irradiation for 15 min. These results further confirmed the super antiphotobleaching ability of NIR998 NPs.

The RONS including ONOO− and H₂O₂ were considerable important signal molecule and they were over expressed in many diseases [26]. Therefore, it was very necessary for therapeutic agents to resist RONS. Then, the RONS-resistant stabilities of NIR998 NPs were investigated by recording the absorption spectra of them with H₂O₂, ONOO− or not, respectively. The absorption spectrum of NIR998 NPs solutions showed negligible change in the presence of H₂O₂, and the maximal absorption of NIR998 NPs solutions with ONOO− was still above 94% of the begining number (Fig. 2e, S8e and S8f). The maximal absorption of ICG NPs solutions dramatically decreased to 88% and 7% with H₂O₂ and ONOO−, respectively. These results confirmed the super resistance of NIR998 NPs to H₂O₂ and ONOO− than that of ICG NPs. Besides, the abnormal metabolism of tumor cells induced the acidic extracellular microenvironment (pH = 6.5−6.8) comparing with that of blood or normal tissue [27]. Diethylamine parts endowed NIR998 NPs with the responsive ability to acid conditions [27]. The absorption spectra of NIR998 NPs in different pH (6.4 ~ 7.4) solutions were then explored. The characteristic absorption spectra of NIR998 NPs displayed negligible change in the chosen pH range (Fig. 2f and S9), which was due to the protection of liposome. These results demonstrated the super stability of NIR998 NPs under light, heat, RONS and weak acid conditions.

In Vitro Cytotoxicity Assay of NIR998 NPs

To explore photothermal therapy capacity of NIR998 NPs in vitro, standard MTT assay was used to assess the dark cytotoxicity of NIR998 NPs to SKOV3 cells. NIR998 NPs with various concentrations (0−200 μM) were incubated with SKOV3 cells for 24 h, respectively. SKOV3 cells treated with various concentrations of NIR998 NPs showed high viability and its viability displayed slight decrease with the rising concentrations. For example, the viabilities were about 84% and 78% even with high concentrations of NIR998 NPs at 100 μM and 200 μM (Fig. 3a), respectively, manifesting the slight dark cytotoxicity of NIR998 NPs to SKOV3 cells.
To rationally choose reasonable light power for the following tests, SKOV3 cells were incubated with NIR998 NPs or PBS for 2 h, and then irradiated under various light power, respectively. As shown in Fig. 3b, with the rising light power, SKOV3 cells without NIR998 NPs displayed negligible reduced viabilities while the cells treated with NIR998 NPs exhibited rapidly decreased viabilities. For example, under irradiation (0.5 W cm\(^{-2}\)), cells viabilities were even below 25% and above 97% of original value after treated with NIR998 NPs and PBS, respectively. The results indicated the negligible phototoxicity of 808 nm light and excellent photothermal therapeutic performance of NIR998 NPs. Finally, 0.5 W cm\(^{-2}\) was chosen to launch next tests.

Flow cytometry experiment was then carried out to further prove the good photothermal effect of NIR998 NPs. SKOV3 cells were treated with NIR998 NPs (20 μM) plus light irradiation, NIR998 NPs, light irradiation or PBS, respectively. Dead cells and apoptotic cells were distinguished by propidium iodide (PI) and Annexin V-FITC, respectively. SKOV3 cells treated with NIR998 NPs, light irradiation or PBS displayed high viability as 96.5%, 94.6% and 95.1%, respectively (Fig. 3c), further confirming the negligible phototoxicity of light and dark cytotoxicity NIR998 NPs while SKOV3 cells treated with NIR998 NPs plus light irradiation showed obviously low cell viability as 12.0% (Fig. 3c), further demonstrating the excellent photothermal therapeutic performance of NIR998 NPs to SKOV3 cells. The same result was also verified by fluorescent imaging of live/dead cell launched under the same environments (Fig. S10).

**In Vivo Photothermal Imaging of NIR998 NPs**

The excellent photothermal therapy of SKOV3 cells motivated us to implement tumor therapy experiments. To explore the optimum time for tumor therapy, inspired by concentrations-dependent photothermal conversion performance of NIR998 NPs, The photothermal imaging of mice in different time after intravenous injection of NIR998 NPs (200 μM, 150 μL) samples was first investigated, due to the accessible infrared thermal imaging instruments and easy manipulation. Poor temperature increase (3.6 °C) was observed before injection of NIR998 NPs (Fig. 4a). The temperature of tumor part gradually increased with time, and tumor part showed the highest temperature (54.1 °C) after 6 h injection of NIR998 NPs (Fig. 4a), manifesting the maximal accumulation of NIR998 NPs in tumor segments, due to the excellent passive targeting tumor capacity of NIR998 NPs via EPR effect. The results suggested the excellent accumulation ability of NIR998 NPs at tumor parts after intravenous injection of NIR998 NPs for 6 h, which is selected as optimal time point for next mice tumor therapy.

**In Vivo PTT**

Motivated by the excellent photothermal therapeutic ability to SKOV3 cells and effective accumulation of NIR998 NPs in tumor segments, photothermal therapy of mice tumors was carried out on SKOV3 tumor mice model. 24 mice were randomly assigned into four groups. These mice were treated with I) NIR998 NPs plus light irradiation, II) NIR998 NPs (150 μL, 200 μM), III) PBS + light irradiation (808 nm, 0.5 W cm\(^{-2}\), 6 min), IV) PBS, respectively. I) group served as experimental group. II), III) and IV) groups worked as control. Mice weight, state, tumor volume were important indexes of tumor therapeutic results. They were
monitored at the same point in time every other day before next therapy. All mice showed good states and mice weight (~ 20 g) displayed similar change trend (Fig. 4b,4c and S11). Besides, mice tumors in control groups exhibited distinct growth with time. The tumor sizes were 10–12 times of the original value after 30 days treatment (Fig. 4b,4d, S11 and S12). Compared with the fast-growing mice tumors of control groups, mice tumors of experimental group exhibited burned traces before the second therapy, they were completely removed after the fourth treatment, and no new tumors were observed after 30 days (Fig. 4b, 4d and S12). The above results collectively demonstrated the negligible dark cytotoxicity of NIR998 NPs and phototoxicity of light irradiation to mice, and excellent photothermal therapeutic performance of NIR998 NPs to tumor, manifesting that NIR998 NPs displayed potential practical application as phototherapeutic agents.

To further assess the dark cytotoxicity of NIR998 NPs, all mice were sacrificed after 30 days treatments. The major organs were acquired for H&E staining due to that nanomaterials were inclined to enrich in reticuloendothelial parts. H&E staining results indicated that all organs exhibited same and normal morphological properties with no necrotic areas (Fig. 5a). Blood was also collected for immunohistochemical analyses by comparing the blood indexes, such as mean platelet volume (MPV), hematocrit (HCT), hemoglobin (HGB) and red blood cells (RBC) etc. As is shown in Fig. 5b and S13, these considerable important blood indexes exhibited negligible statistical difference among mice with different treatments, further demonstrating the negligible influence of NIR998 NPs or light irradiation to mice normal organs and body weight. The above results fully manifested that NIR998 NPs were highly biosafety phototherapeutic agents to living mice.

Conclusion

In summary, a class of aza-BODIPY dyes based NIR-II fluorophores (NIR998, NIR1028, NIR980, NIR1030, NIR1028-S) with D-A-D’ structures have been rationally developed via steric relaxation effect and IPET. These fluorophores exhibit superior stability against photobleaching, NIR-II emission, large Stokes shift (≥ 100 nm) and excellent photothermal conversion performance. Among them, NIR998 with better NIR-II emission and photothermal conversion capacities was used to prepare NIR998 NPs by lipidosome encapsulation. NIR998 NPs show much better stability than ICG NPs in the presence of light, heat and reactive oxygen nitrogen species, as well as high photothermal conversion performance (η = 50.5%). The excellent photothermal conversion ability and concentrations-dependent photoacoustic signal make NIR998 NPs as excellent photothermal imaging contrast agent and photothermal agent for guiding effectively tumor elimination under mild conditions. The highly efficient tumor photothermal therapeutic performance by NIR998 NPs, H&E staining and immunohistochemical analyses have fully demonstrated the negligible cytotoxicity of NIR998 NPs and phototoxicity of light irradiation, making NIR998 NPs as potential diagnostic reagents for clinical applications. Unfortunately, NIR998 NPs showed worse NIR-II emission, which shows disadvantages to NIR-II fluorescence imaging. Then, we will explore more reasonable strategies to design NPs based on NIR-II fluorophores with strong emission or better methods to construct NIR-II fluorophores NPs with better NIR-II emission in the following works.
Materials And Methods

Materials and characterization

Aldehydes and ketone derivatives were got from Bidepharm. Diethylamine, nitromethane, ethanol, ammonium acetate, diisopropyl ethylamine were acquired from Macklin. Aldehydes and ketone derivatives were used directly. Other solvent were used without further purification. The nuclear paramagnetic resonance (Bruker Ultra Shield Plus) and Mass spectra (Bruker) were used for demonstrating the chemical structures of compounds. Transmission electron microscopy and dynamic light scattering (Nano ZS90) were used for confirming morphology and particle size of nanoparticles, respectively. Ultraviolet–visible light (UV-Vis) spectrophotometer (Cintra 2020) and spectrofluorometer (Horiba Fluoromax-4) are used to record the absorption emission spectra of samples. Confocal luminescence imaging was conducted by an Olympus IX81 laser scanning confocal microscope. Photothermal images were measured by NIR thermal imager (FLIR E40). The power density meter was obtained by VLP-2000 laser power meter.

Photothermal effect of samples

The temperature change of samples solution was monitored by infrared thermal imaging system. The temperature change of samples (300 μL) solution in different concentrations or light power was obtained. The temperature increase and decrease process of samples (20 μM) solution with irradiation (808 nm, 0.5 W cm⁻²) or not was obtained. Finally, samples photothermal conversion efficiency was got referring to previous work.¹⁹

PA signal of NIR998 NPs

Concentrations-dependent PA signal of NIR998 NPs was got via a point-to-point method. The PA signal of NIR998 NPs in different concentrations was got by monitoring region of interest, respectively, λ_{Ex} = 808 nm.

Cytotoxicity assay

The dark toxicity assay of NIR998 NPs to SKOV3 cells was launched by the standared methyl thiazolyl tetrazolium experiments. SKOV3 cells were treated with NIR998 NPs in different concentrations for 24 h, respectively. Then, MTT (10 μL/well, 5 mg/mL) was added for further 4 h incubation. Finally, 150 μL of DMSO was added, and OD570 was measured by an enzyme-linked immunosorbent assay reader. Then cell viability was obtained referring to previous report.¹⁹ In addition, SKOV3 cells were treated with PBS and NIR998 NPs (20 μM), respectively. They were irradiated (808 nm) in various light power for 6 min. Then, they were treated following above operation.

In vitro Photothermal toxicity of NIR998 NPs
SKOV3 cells were treated with PBS (pH = 7.4), light irradiation (808 nm, 0.5 W cm\(^{-2}\), 6 min), **NIR998 NPs** (20 μM), **NIR998 NPs** plus irradiation, respectively. The redundant **NIR998 NPs** were washed with fresh complete medium. Propidium iodide (PI) and annexin V-FITC were used for differentiating dead cells and apoptotic cells for the following confocal microscopy and flow cytometry.

**Animals and tumor model**

Female mice were purchased from Medical Animal Laboratory Center of Guangdong (Permit number: 44007200079864). All in vivo experiments were performed with the approval of the Medical Department of Shenzhen University.

**In vivo photothermal imaging exploration**

All imaging and therapy exploration of mice are launched in line with standard principles and guidelines. SKOV3 tumor bearing nude mice with tumor volume about 100 mm\(^3\) were used for photothermal imaging of mice under irradiation (808 nm, 0.5 W cm\(^{-2}\), 6 min) before and after intravenous injection of **NIR998 NPs** (150 μL, 200 μM) with time.

**In vivo photothermal efficacy**

SKOV3 tumor-bearing mice with tumor volume about 100 mm\(^3\) were assigned into four groups. They were treated with **NIR998 NPs** (150 μL, 200 μM) + light irradiation (808 nm, 0.5 W cm\(^{-2}\), 6 min), **NIR998 NPs**, light irradiation, PBS, respectively. The first group served as experimental groups, and the rest groups worked as control groups. The tumors were irradiated after intravenous injection of **NIR998 NPs** for 6 h. The size of SKOV3 was obtained by \(V = \frac{LW^2}{2}\), L and W are length and width of tumors, respectively. Body weight and tumor size of mice were collected every two days for 30 days. At day 30, major organs and blood of mice were collected for hematoxylin-eosin staining and immunohistochemical analyses, respectively.

**Synthesis of NIR-II fluorophores**

Synthesis and Characterization of **NIR998. 1-3** (0.31 g, 0.40 mmol) dissolved into the mixture of diisopropylethylamine (4 mL) and dry CH\(_2\)Cl\(_2\) (20 mL) at 0 °C. Then, BF\(_3\)-OEt\(_2\) (6.40 mmol) was dropped. They reacted for 8 h at 0 °C. The final reaction solution was quenched by methanol. The precipitate was obtained by vacuum filter. The final blue solid **NIR998** (0.29 g, Yield: 91 %) was acquired by column chromatography. \(^1\)H NMR (400 MHz, CDCl\(_3\)) δ(ppm) = 8.08 (d, J = 7.2 Hz, 4H), 7.96 (d, J = 8.4 Hz, 2H), 7.80 (s, 2H), 6.85 (s, 2H), 6.72 (d, J = 7.6 Hz, 4H), 6.65 (d, J = 8.4 Hz, 2H), 3.50 – 3.31 (m, 16H), 2.85 (t, J = 5.2 Hz, 4H), 2.01 (t, J = 4.8 Hz, 4H), 1.23 – 1.18 (m, 18H). \(^13\)C NMR (100 MHz, CDCl\(_3\)) δ(ppm) = 154.34, 147.83, 144.40, 139.94, 130.34, 128.90, 127.87, 121.05, 120.12, 118.23, 113.02, 110.03, 109.23, 47.67, 44.38, 43.40, 27.36, 21.21, 11.75, 10.08. MALDI-TOF-MS m/z: 804.992.
Synthesis and Characterization of **NIR1028. 2-3** (0.31 g, 0.40 mmol) dissolved into the mixture of diisopropylethylamine (4 mL) and dry CH$_2$Cl$_2$ (20 mL) at 0 °C. Then BF$_3$·OEt$_2$ (6.40 mmol) was dropped. They reacted for 8 h at 0 °C. The final reaction solution was quenched by methanol. The precipitate was obtained by vacuum filter. The final blue solid **NIR1028** (0.31 g, Yield: 93 %) was acquired by column chromatography. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) = 8.05 (d, $J$ = 9.0 Hz, 4H), 7.59 (s, 4H), 6.81 (s, 2H), 6.71 (d, $J$ = 9.0 Hz, 4H), 3.43 (q, $J$ = 6.6 Hz, 8H), 3.24 (t, $J$ = 5.4 Hz, 8H), 2.78 (t, $J$ = 6.6 Hz, 8H), 2.02 – 1.97 (m, 8H), 1.21 (t, $J$ = 7.2 Hz, 12H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ (ppm) = 155.15, 148.81, 144.87, 143.31, 141.31, 131.36, 128.18, 121.23, 121.02, 119.35, 114.37, 111.03, 50.12, 44.42, 28.01, 21.96, 12.78. MALDI-TOF-MS m/z: 829.173.

Synthesis and Characterization of **NIR980. 3-3** (0.29 g, 0.40 mmol) dissolved into the mixture of diisopropylethylamine (4 mL) and dry CH$_2$Cl$_2$ (20 mL) at 0 °C. Then BF$_3$·OEt$_2$ (6.40 mmol) was dropped. They reacted for 8 h at 0 °C. The final reaction solution was quenched by methanol. The precipitate was obtained by vacuum filter. The final blue solid **NIR980** (0.28 g, Yield: 90 %) was acquired by column chromatography. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) = 8.19 – 8.09 (m, 4H), 7.68 – 7.60 (m, 2H), 7.44 – 7.33 (m, 4H), 7.18 – 7.13 (m, 4H), 7.00 – 6.96 (m, 2H), 6.83 – 6.73 (m, 4H), 4.44 (q, $J$ = 13.2, 4H), 3.48 (q, $J$ = 15.6, 8H), 1.43 (t, $J$ = 6.4 Hz, 6H), 1.26 (t, $J$ = 7.2 Hz, 12H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ (ppm) = 155.08, 154.11, 150.05, 148.64, 131.08, 128.47, 122.19, 120.154, 115.21, 116.23, 115.21, 110.13, 107.36, 47.61, 44.60, 15.60, 12.81. MALDI-TOF-MS m/z: 773.271.

Synthesis and Characterization of **NIR1030. 4-3** (0.27 g, 0.40 mmol) dissolved into the mixture of diisopropylethylamine (4 mL) and dry CH$_2$Cl$_2$ (20 mL) at 0 °C. Then BF$_3$·OEt$_2$ (6.40 mmol) was dropped. They reacted for 8 h at 0 °C. The final reaction solution was quenched by methanol. The precipitate was obtained by vacuum filter. The final blue solid **NIR1030** (0.28 g, Yield: 96 %) was acquired by column chromatography. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) = 8.18 (d, $J$ = 3.6 Hz, 4H), 7.77 – 7.66 (m, 4H), 7.57 (d, $J$ = 8.0 Hz, 2H), 7.38 – 7.28 (m, 6H), 6.76 (s, 4H), 3.48 (q, $J$ = 12.8Hz, 8H), 1.26 (t, $J$ = 7.2 Hz, 12H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ (ppm) = 156.08, 154.11, 150.05, 149.64, 131.08, 128.47, 122.19, 120.154, 115.21, 116.23, 115.21, 110.13, 107.36, 47.61, 44.60, 15.60, 12.81. MALDI-TOF-MS m/z: 719.242.

Synthesis and Characterization of **NIR1028-S. 5-3** (0.31 g, 0.40 mmol) dissolved into the mixture of diisopropylethylamine (4 mL) and dry CH$_2$Cl$_2$ (20 mL) at 0 °C. Then BF$_3$·OEt$_2$ (6.40 mmol) was dropped. They reacted for 8 h at 0 °C. The final reaction solution was quenched by methanol. The precipitate was obtained by vacuum filter. The final blue solid **NIR1028-S** (0.31 g, Yield: 93 %) was acquired by column chromatography. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ (ppm) = 8.32 (s, 2H), 8.16 – 8.12 (m, 4H), 7.89 – 7.85 (m, 4H), 7.43 – 7.35 (m, 4H), 7.10 (s, 2H), 6.77 – 6.73 (m, 4H), 3.46 (q, $J$ = 7.2 Hz, 8H), 1.24 (t, $J$ = 7.2 Hz, 12H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ (ppm) = 156.00, 149.68, 145.01, 141.01, 140.48, 135.49, 134.43, 132.02, 125.45, 125.05, 124.64, 124.08, 122.21, 118.28, 117.07, 111.43, 44.61, 12.80. MALDI-TOF-MS m/z: 750.264.
The preparation of **NIR998 NPs** (ICG NPs). 20 mg DSPE−mPEG$_{5000}$ dissolved into 8 mL deionized water under sonication (120 W, 2 min). 1.5 mg **NIR998 (ICG)** in 4 mL THF was dropped in above mixture quickly under sonication (120 W, 2 min). The prepared solution was stirred by blowing its surface with argon at 50 °C overnight. The blue solution was washed with PBS (pH = 7.4) by a centrifugal-filter for three times. The final concentrated blue solution was used for next experiments.

Concentrations-dependent absorption of **NIR998** in DMSO was acquired. The linear relationship between concentration and the absorption of **NIR998** at 859 nm (maximal absorption) in DMSO was then obtained. **NIR998 NPs** solution (150 μL) was dried. Then, their absorption in DMSO was got. Finally, according to above linear relationship, the concentration of **NIR998 NPs** solution was obtained as about 400 μM.

### Abbreviations

ICG: Indole cyanide green; DMSO: Dimethyl sulfoxide; MTT: methyl thiazolyl tetrazolium; Photothermal therapy; FDA: food and drug administration; NIR: Near-infrared; MS: Mass spectrum; NMR: nuclear magnetic resonance.

### Declarations

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#### Authors’ contributions

YX performed the design, materials preparation and coordination of this work. SW, ZC, SL and YZ participated in the in vitro/vivo experiments. YX wrote the manuscript. RH, LL and JL offered suggestion, financial support and supervision. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

Not applicable.

#### Competing interests

The authors declare no competing financial interest.

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