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Selenium-Integrated Cyanine Dye With Enhanced Photophysical Properties for Optoacoustic-Guided Cancer Imaging and Therapy

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ABSTRACT

Near-infrared (NIR) cyanine dyes demonstrate considerable promise in medical applications, notably in cancer diagnosis, due to their favourable fluorescence properties and biocompatibility. However, these dyes generally have low photostability and tend to aggregate in aqueous solution, which can constrain their usefulness for prolonged in vivo imaging and phototherapy applications. Here, we report the synthesis of a selenium-integrated NIR cyanine dye, Se-Cyn, designed to modulate its electronic and photophysical properties. Se-Cyn demonstrates high photostability, molar absorptivity, photothermal conversion efficiency (PCE), and sustained optoacoustic (OptA) contrast under prolonged irradiation. Se-Cyn nanoparticles (NPs) outperform the indocyanine green (ICG) NPs, exhibiting PCE of 59% and a 1.36-fold higher OptA generation efficiency. Additionally, nano-formulated Se-Cyn NPs show excellent aqueous stability and improved bioavailability, supporting its suitability for biological applications. Furthermore, Se-Cyn NPs accumulate in tumors following systemic administration, enabling effective OptA tumor imaging. Together with their strong photodynamic and photothermal properties, these NPs show considerable potential for cancer theranostics.

1 | Introduction

The development of cyanine-based compounds that can serve as both near-infrared (NIR) contrast agents and therapeutic

agents remains one of the major challenges in modern cancer research. Conventional cyanine dyes have been widely studied, but they exhibit several major drawbacks, including poor photostability, low photothermal conversion efficiency (PCE), weak

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optoacoustic (OptA) signal generation, and limited reactive oxygen species (ROS) production [1, 2]. Solving the challenge of developing multifunctional cyanine agents with strong OptA imaging capabilities in the NIR window could facilitate deeper tissue imaging and effective phototherapy within a single platform. In contrast to conventional fluorescence methods, NIR light penetrates tissue more effectively than light at visible wavelengths, enabling clinicians to observe internal structures or tumors with higher clarity and less interference from background [3–6].

The improved imaging depth of NIR agents addresses key spatial limitations found using traditional agents, allowing more accurate tumor delineation, precise localization, and real-time monitoring of therapeutic responses [7, 8]. Incorporating therapeutic functionalities, such as photothermal and photodynamic properties, into NIR-active agents enables both imaging and treatment. Upon NIR light activation, these agents can directly eliminate tumor cells either by generating heat (photothermal therapy) or by producing cytotoxic ROS (photodynamic therapy) [9]. This dual functionality enables precise image-guided interventions and synergistic combination therapies that effectively suppress tumor growth and reduce the risk of metastasis and recurrence. Integrating these multiple functions into a single platform represents a significant advancement in real-time image-guided cancer therapy, offering targeted treatment with enhanced safety and therapeutic efficacy [10].

The solution to the aforementioned limitations lies in developing improved cyanine-based agents capable of achieving deep-tissue imaging, while simultaneously serving as effective cancer therapeutics [11]. Such a solution would pave the way for more precise and efficient OptA-guided cancer therapies. Photostable cyanine dyes, mainly those modified with benzo[cd]indolonyl and azobenzene groups, have been developed and utilized in longitudinal, optical, and OptA imaging due to their unique NIR optical characteristics and potential for deep-tissue imaging [12–14]. Despite these synthetic advantages, one notable obstacle remains: low aqueous stability, which severely restricts their utility in in vivo imaging applications. Although iodinated xanthene-cyanine dyes have been reported for photodynamic therapy (PDT) and exhibit good PDT properties, they tend to aggregate in aqueous solutions [15]. This limitation makes them unsuitable for combined imaging and phototherapy applications. The resulting aggregation leads to reduced fluorescence and diminished therapeutic performance in in vivo settings [16, 17]. Thus, their instability and propensity for aggregation render them inadequate for multifunctional biomedical techniques until further advancements are achieved.

As such, the current work focuses on developing a multifunctional selenium-integrated cyanine dye for OptA imaging in the NIR range while concurrently integrating photothermal performance and photosensitive oxidative activity to enable both cancer treatment and imaging. Designed specifically for in-vivo use, this dye is aimed at addressing key limitations such as poor photostability and aggregation. Ultimately, this approach offers a promising molecular platform for integrated cancer diagnosis and therapy. We hypothesize that integrating an organoselenium unit into the cyanine backbone could modulate the electronic and photophysical properties of the dye, thereby improving its

performance as an NIR theranostic agent. Furthermore, the nanoformulated Se-Cyn nanoparticles (Se-Cyn NPs), owing to their enhanced stability in biological solvents, are anticipated to enable superior in vivo OptA imaging and phototherapeutic performance by minimising aggregation and degradation. Together, these properties make Se-Cyn NPs a favourable candidate for multifunctional cancer therapy and longitudinal imaging.

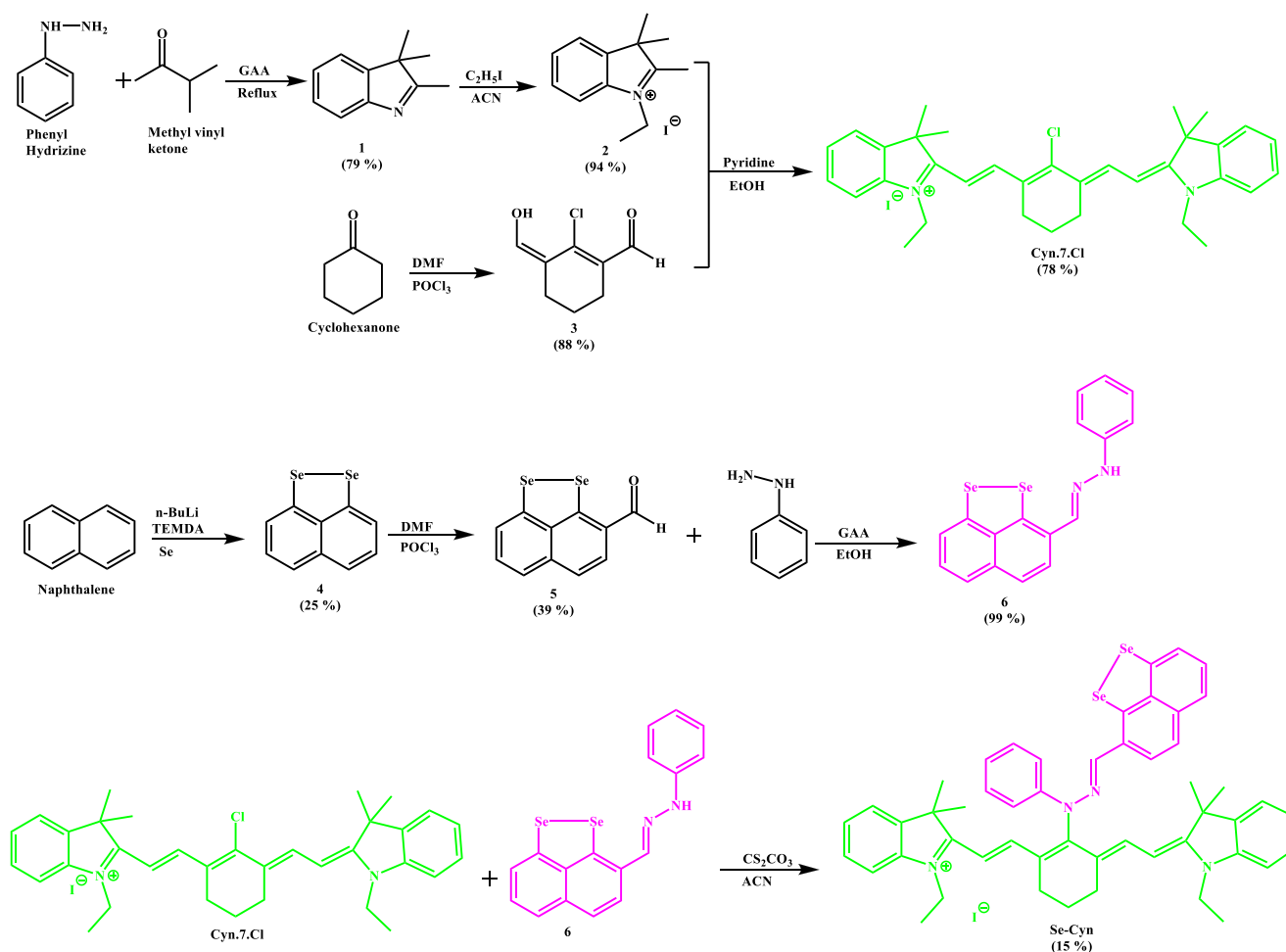
Here, we report the synthesis of a NIR cyanine dye with an organoselenium moiety integrated into its backbone. The resulting Se-Cyn dye shows significantly improved photostability, higher molar absorptivity, and enhanced PCE in the NIR window. This dye is optimized for efficient OptA signal generation and sustained performance, supporting its potential for deep-tissue and longitudinal NIR imaging. As a next step, we developed a PEGylated lipid-based nano-formulation of Se-Cyn, which shows improved in vivo biocompatibility and enhanced therapeutic potential compared to existing cyanine formulations. Following systemic injection, Se-Cyn NPs preferentially accumulate in tumors through passive targeting. This accumulation enables OptA tumor imaging and localized therapeutic intervention under NIR light irradiation, with the experimental findings supporting contributions from both photothermal heating and photosensitive oxidative activity.

2 | Results

2.1 | Reaction Scheme of Se-Cyanine (Se-Cyn)

To obtain the Se-Cyn dye, we employed a donor–acceptor integration strategy in which a selenium-containing donor unit was incorporated into the cyanine scaffold to modulate its electronic structure and achieve red-shifted NIR absorption through intramolecular charge-transfer character. The synthesis of cyclic organoselenium-based cyanine compound (Se-Cyn) is presented in Scheme 1. Intermediates **1**, **2**, **3**, **4**, **5**, and **Cyn.7.Cl** were synthesized according to previously published methods (Figures S1–S6) [18–23]. The compound 1-(naphtho[1,8-cd][1,2]diselenol-3-ylmethylene)-2-phenylhydrazine (**6**) was synthesized by condensation of naphtho[1,8-cd][1,2]diselenole-3-carbaldehyde (**5**) and phenyl hydrazine in the presence of a catalytic amount of glacial acetic acid in ethanol. The reaction mixture was refluxed for 12 h under an inert atmosphere. Compound **6** was recrystallized using n-hexane, resulting in orange-yellow crystals with a yield of 99.05% (0.20 g), and characterised using ¹H, ¹³C NMR spectroscopy and mass spectrometry (Figures S7–S10). The ¹H NMR spectrum of compound **6** showed a singlet at δ 8.11 ppm, which is a characteristic peak of the imine proton (–N=CH–), while a broad peak at δ 7.82 ppm indicated the –NH (heteroatomic proton). Aromatic protons appeared in the aromatic range between δ 7.60–6.94 ppm (Figure S7). In the ¹³C NMR spectrum, the imine carbon (–CH=N–) of compound **6** appeared at δ 143.4 ppm, whereas the nitrogen-attached phenyl carbon (–N–C=) appeared at δ 142.2 ppm (Figure S8). The ES-MS spectrum of compound **6** indicated a [C₁₇H₁₂N₂Se₂]⁺ ion at m/z 402, consistent with an isotopic selenium pattern (Figures S9 and S10).

1-ethyl-2-((E)-2-((E)-3-(2-((E)-1-ethyl-3,3-dimethylindolin-2-ylidene)ethylidene)-2-((E)-naphtho[1,8-cd][1,2]diselenol-3-ylm-



SCHEME 1 | Schematic depicting the synthesis of the Selenium-cyanine dye (Se-Cyn). GAA: Glacial acetic acid. ACN: Acetonitrile. DMF: N, N-Dimethylformamide. EtOH: Ethanol. Cyn.7.Cl: 2-[2-[2-chloro-3-[2-(1-ethyl-1,3-dihydro-3,3-dimethyl-2H-indol-2-ylidene)ethylidene]-1-cyclohexen-1-yl]ethenyl]-1-ethyl-3,3-dimethyl-3H-indolium iodide. TEMDA: Tetramethyl ethylenediamine.

ethylene)-1-phenylhydrazineyl)cyclohex-1-en-1-yl)viny]-3,3-dimethyl-3H-indol-1-ium (Se-Cyn) was synthesized by reacting 1-(naphtho[1,8-cd][1,2]diselenol-3-ylmethylene)-2-phenylhydrazine (**6**) and Cyn.7.Cl with cesium carbonate (Cs_2CO_3) in anhydrous acetonitrile at -10°C . The resulting product, Se-Cyn, was purified by silica gel column chromatography, affording a yield of 14.45% (0.072 g). This modest overall yield is primarily attributable to the sensitivity of the final selenium-containing coupling step and to material losses incurred during purification. Accordingly, while the current synthetic route is suitable for proof-of-concept studies, further optimization would be required before scale-up. Se-Cyn was characterized by ^1H , ^{13}C and ^{77}Se NMR spectroscopy and mass spectrometry (Figures S11–S16). The ^1H NMR spectrum of Se-Cyn confirmed the absence of the atomic $-\text{NH}$ proton from reactant **6** at δ 7.82 ppm and the appearance of an imine proton in Se-Cyn at δ 8.33 ppm. All aliphatic protons were observed in the range of δ 4.17–0.85 ppm (Figure S11). The ^{77}Se NMR spectrum of the Se-Cyn exhibited selenium peaks at δ 534 ppm and δ 391 ppm, indicating the presence of two non-equivalent selenium atoms in the compound (Figure S13). ES-MS showed a $[\text{C}_{51}\text{H}_{51}\text{N}_4\text{Se}_2]^+$ ion peak at m/z 877.4 with the expected isotopic selenium pattern (Figures S14 and S15). Collectively, the data from the NMR and

mass spectrometric analyses confirm the successful synthesis of compounds **6** and Se-Cyn.

2.2 | Development of Se-Cyn Nanoparticles and Their Characterization

Water-soluble Se-Cyn NPs were developed to improve the in vivo bioavailability of Se-Cyn. These NPs were synthesized via nanoprecipitation using DSPE-PEG5000 (Figure 1a). Transmission electron microscopy (TEM) and dynamic light scattering (DLS) confirmed spherical nanoparticle morphology, with diameters predominantly in the 70–110 nm range (Figure 1b) and a polydispersity index (PDI) of 0.151, indicating a fairly uniform nanoparticle population (Figure S17). This size range is well within the <250 nm threshold required for penetration through tumor vasculature [24, 25]. The colloidal stability of Se-Cyn NPs was assessed over 14 days in water and 10% fetal bovine serum (FBS) (Figure 1c). As shown in Figure S18, Se-Cyn NPs exhibited a zeta potential of -5.4 mV on day 0, with only minimal variation over 14 days (Figure S19), indicating good colloidal stability. These findings demonstrate excellent nanoparticle stability, with no significant aggregation or degradation, confirming their suitability

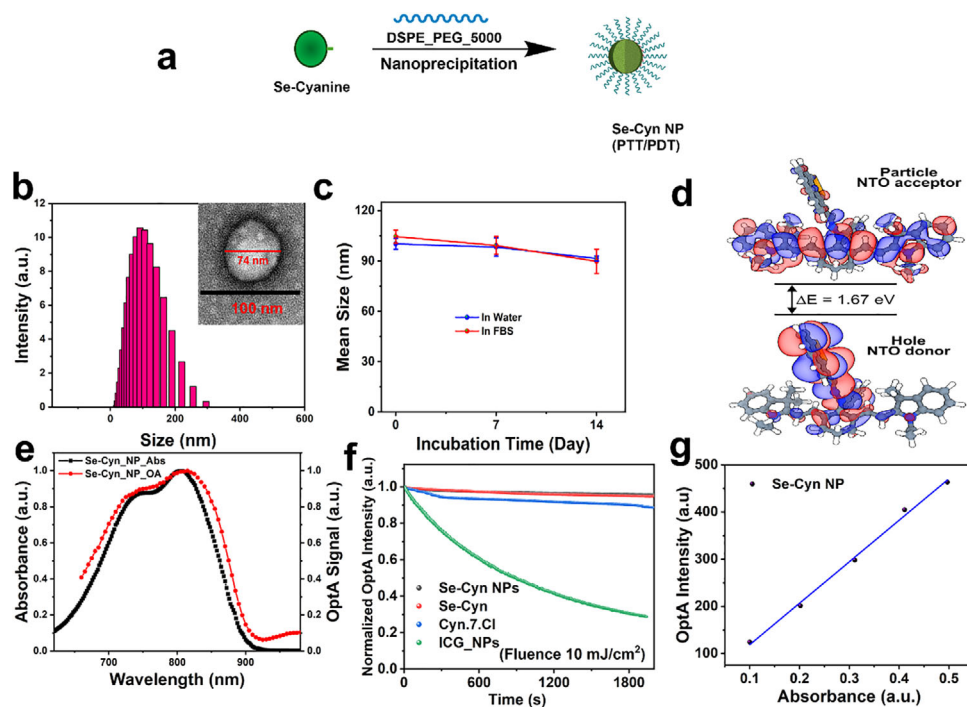


FIGURE 1 | Nanoformulation development and characterization of Selenium-Cyanine (Se-Cyn) nanoparticles (NPs) (a) Synthesis scheme of Se-Cyn NPs using a nanoprecipitation method (DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, PEG_5000: Polyethylene glycol with a molecular weight of 5000). (b) Transmission electron microscopy (TEM) image and dynamic light scattering (DLS) profile of Se-Cyn NPs. (c) Average size distribution of Se-Cyn NPs in water and in fetal bovine serum (FBS) over a 14-day incubation period. (d) Natural transition orbitals (NTOs) corresponding to the lowest-energy electronic excitation (S_1), calculated using time-dependent density functional theory (TD-DFT) at the B3LYP/def2-TZVP level with implicit water solvation (SMD). The dominant NTO pair is shown, where the “hole” represents the electron density removed upon excitation (donor orbital) and the “particle” represents the electron density gained upon excitation (acceptor orbital). (e) Absorbance and optoacoustic (OptA) spectra of Se-Cyn NPs in water. (f) OptA signal stability of Se-Cyn NPs, Se-Cyn, Cyn.7.Cl, and Indocyanine Green (ICG) NPs during 30 min of pulsed laser irradiation (fluence: 10 mJ/cm²). (g) Linear relationship between OptA signal intensities and absorbances of Se-Cyn-NPs. a.u.: arbitrary units. Cyn.7.Cl: 2-[2-[2-chloro-3-[2-(1-ethyl-1,3-dihydro-3,3-dimethyl-2H-indol-2-ylidene)ethylidene]-1-cyclohexen-1-yl]ethenyl]-1-ethyl-3,3-dimethyl-3H-indolium iodide. PTT: Photothermal Therapy. PDT: Photodynamic Therapy. Schematic illustration of the nanoparticle system used in this study, created with BioRender.com.

for both in vitro and in vivo applications. The encapsulation efficacy (%EE) of 59.5% indicated the good efficiency of this nanoprecipitation method, which retained up to 30.1% of the Se-Cyn dye within the NPs (Figure S20–S22, Equations 3 and 4) [26, 27].

Density functional theory (DFT) calculations followed by time-dependent density functional theory (TD-DFT) analyses were carried out using the ORCA software package at the B3LYP/def2-TZVP level with implicit water solvation (SMD) for Se-Cyn, Cyn.7.Cl, and ICG as a reference compound (Figures 1d and S23) [28]. Incorporation of selenium markedly reduces the low-energy excitation energy compared with the chloro analogue, consistent with the experimentally observed bathochromic shift. For Se-Cyn, the lowest-energy singlet excitation ($S_0 \rightarrow S_1$) was calculated at 1.67 eV (741 nm, $f = 0.026$) showing reasonable agreement with the experimental absorption maximum at 806 nm, whereas the corresponding excitation energies for Cyn.7.Cl and ICG were 2.20 eV (564 nm) and 2.13 eV (582 nm), respectively. The strongest low-energy allowed transition for Se-Cyn was predicted at 1.94 eV (639 nm, $f = 2.21$), indicating that the experimentally observed NIR absorption arises from the red-edge intramolecular charge transfer (ICT) character of the S_1 state rather than from a single strongly allowed transition. To elucidate the electronic nature

of the red-shifted S_1 excitation, natural transition orbital (NTO) analysis was performed. This excitation was dominated by a single NTO pair ($n = 0.9996$), indicating a highly localized and well-defined one-electron transition. The hole (NTO donor) was primarily localized on the selenium-containing donor fragment, whereas the particle (NTO acceptor) was distributed over the polymethine cyanine backbone. This orbital redistribution demonstrates ICT from the selenium donor unit to the conjugated cyanine scaffold upon excitation, consistent with the experimentally observed bathochromic shift and enhanced donor–acceptor electronic coupling introduced by selenium incorporation. Both S_1 and S_2 excitations collapsed to essentially the same dominant donor–acceptor pair in the NTO representation, indicating a common underlying charge-transfer excitation. Consistent with expectations, Se-Cyn NPs showed negligible fluorescence in solution, with quantum yields <0.2% (Equation 1).

Following the synthesis and characterization of Se-Cyn NPs, their optical properties were evaluated. As depicted in Figure 1e, the absorbance and OptA spectra of Se-Cyn NPs in PBS exhibited precise overlap within the region of interest, indicating that the NPs function as ideal linear absorbers suitable for OptA applications, assuming they exhibit high nonradiative efficiency. This overlap is important because only absorbed energy converted into

heat can induce thermoelastic expansion and generate acoustic waves.¹³ Photostability was further assessed by irradiating Se-Cyn NPs with a continuous pulsed laser (fluence: 10 mJ.cm⁻²) for 30 min in 10% FBS. As shown in Figure 1f, the OptA signal generated by Se-Cyn NPs remained stable throughout irradiation, whereas signals from ICG NPs in 10% FBS progressively decreased, indicating the superior photostability of Se-Cyn NPs.

The optoacoustic signal generation efficiency (OGE) was evaluated by plotting a linear correlation between the OptA signal intensity of Se-Cyn NPs and their absorbance (Figure 1g). The calculated OGE of Se-Cyn NPs was 1.36 times higher than that of ICG NPs evaluated under same conditions (Figures S24–S27, Table S1). This increased OGE supports improved deep tissue imaging and enables more accurate quantification of contrast agents for in vivo applications.

2.3 | In Vitro Photothermal, Photodynamic Properties, and Cytotoxicity

In the NIR window, biological tissues exhibit reduced absorption and scattering; therefore, the absorption maxima of Se-Cyn NPs at 806 nm closely match the excitation wavelength (808 nm) utilized in photothermal therapy [29]. Although Se-Cyn NPs exhibit an absorption maximum at ~806 nm, we used 780 nm for irradiation because it falls within their strong NIR absorption band and provides effective photothermal excitation. These findings encouraged us to further investigate the heat-generating ability and PTT potential of Se-Cyn NPs. To evaluate concentration-dependent temperature changes, Se-Cyn NPs were irradiated with a continuous-wave (CW) laser (780 nm, 0.8 W/cm²), and the temperature increase was monitored using a thermal camera. As shown in Figure 2a, PBS solutions containing 50 µg/mL and 100 µg/mL of Se-Cyn NPs exhibited a marked temperature increase of approximately 15°C, whereas PBS alone showed no considerable temperature change. Such an increase in intracellular temperature is sufficient to trigger cell death, demonstrating the suitability of Se-Cyn NPs for PTT applications [30, 31]. To further assess the effect of laser power, temperature changes due to Se-Cyn NPs (100 µg/mL) were examined under 780 nm CW laser irradiation at different power settings. As shown in Figure 2b, the observed temperature increase was correlated with laser power, confirming that a power density of 0.8 W/cm² was sufficient to induce a temperature shift capable of causing cancer cell death.

To evaluate photostability, a solution of Se-Cyn NPs (100 µg/mL) in PBS was subjected to four repeated heating and cooling cycles, comprising 10 min of irradiation (780 nm, CW laser, 0.8 W/cm²) followed by 10 min without laser irradiation. These repeated irradiation cycles demonstrated the sustained stability of Se-Cyn NPs (Figure 2c). The TD-DFT/NTD analysis supports the intramolecular charge-transfer character of Se-Cyn. Experimentally, Se-Cyn/Se-Cyn NPs showed good photostability, photosensitive oxidative activity, and efficient photothermal performance. Although selenium substitution can promote intersystem crossing through the heavy-atom effect in related cyanine systems, these properties are discussed here based on the experimental observations without assigning them to a specific excited-state mechanism [32–34]. The PCE of Se-Cyn NPs was subsequently

calculated. As shown in Figure 2d, Se-Cyn NPs (100 µg/mL, PBS) exhibited a linear relationship between the cooling time and the negative logarithm of temperature. Based on this observation and Figure 2c (Equation 5), the PCE was calculated to be 59%, which exceeded reported values for other cyanine derivatives [1, 35, 36]. Together, these results demonstrate that Se-Cyn exhibits strong photothermal properties, making it well-suited for theranostic applications.

To evaluate the phototherapeutic efficacy of Se-Cyn NPs, an MTT cell viability assay was conducted to assess their in vitro cytotoxicity in three cancer cell lines (4T1, HCT116, and U87MG) (Figure 2e; Figures S28 and S29). As shown in Figure 2e, 4T1 cells treated with Se-Cyn NPs (0–25 µg/mL in PBS) and laser irradiation (780 nm, 0.8 W/cm², 5 min) showed a significant reduction (~3.5-fold) in cell viability compared to the non-irradiated cells, demonstrating the strong phototherapeutic potential of Se-Cyn NPs.

Additionally, the photosensitive oxidative activity of Se-Cyn NPs were assessed using a ROS sensor, 1,3-Diphenylisobenzofuran (DPBF). As shown in Figure 2f, the absorption spectra of DPBF changed after treatment with Se-Cyn NPs and irradiation with a 780 nm CW laser (0.8 W/cm²) at different time intervals. A progressive decrease in absorbance at the characteristic peak (~410 nm) was observed, indicating light-induced oxidative activity within the solution. Subsequently, we evaluated the in vitro photodynamic efficacy of Se-Cyn NPs on 4T1 cells. As depicted in Figure 2g, representative images show increased DCF fluorescence in 4T1 cells only after incubation with Se-Cyn NPs and laser irradiation, but not in controls, indicating increased intracellular oxidative activity.

To further assess the phototherapeutic efficiency of Se-Cyn NPs, a live/dead cell staining assay was performed on 4T1 cancer cells. Cells were co-stained with Calcein-AM (live cells, green fluorescence) and EthD-1 (dead cells, red fluorescence). As shown in Figure 2h, strong green fluorescence was observed in cells treated with PBS (with or without laser irradiation) and in cells treated with Se-Cyn NPs without irradiation, indicating high cell viability under these conditions. In contrast, cells treated with Se-Cyn NPs (100 µg/mL in PBS) followed by laser irradiation (780 nm, 0.8 W/cm², 5 min) displayed prominent red fluorescence, corresponding to cell death. The DPBF-based apparent singlet oxygen quantum yield of Se-Cyn NPs was estimated to be 17.33% using ICG NPs as the reference standard, indicating appreciable photosensitizing activity (Figure S30, Equation 2). Although DPBF is widely used for evaluating singlet oxygen generation, its response is not completely specific to ¹O₂; therefore, the calculated value is considered as an indirect estimate of photooxidative activity consistent with, but not definitive evidence for ¹O₂ generation. Time-dependent UV–vis photobleaching analysis showed only a slight decrease in the characteristic absorption band during 10 min of irradiation, indicating good photostability of the compound (Figure S31). To differentiate the individual contributions of PDT and PTT, Se-Cyn NPs were further evaluated under an ice-water bath to suppress heat generation and in the presence of glutathione (GSH) to quench ROS. As shown in Figure S32, each monotherapy produced only partial inhibition of cancer cell growth, whereas the combined phototherapies caused

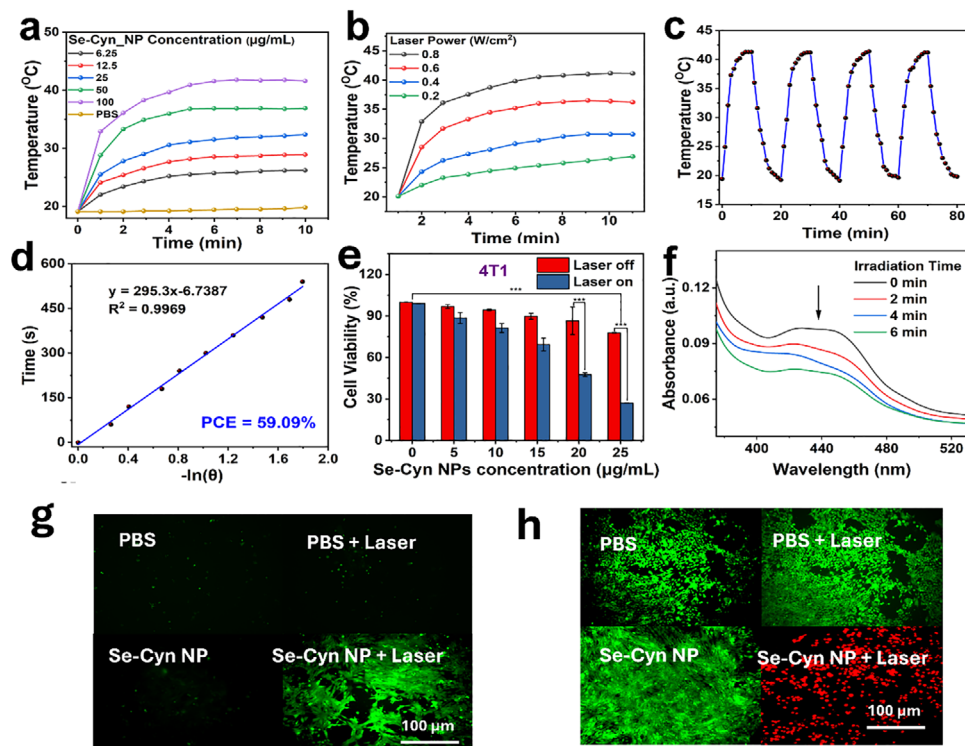


FIGURE 2 | Photothermal characteristics of Selenium-Cyanine (Se-Cyn) nanoparticles (NPs) and their effects on 4T1 cell viability in vitro. (a) Temperature change curves for 1 mL of PBS containing various concentrations of Se-Cyn NPs subjected to 780 nm continuous wave (CW) laser irradiation (0.8 W/cm²). (b) Temperature change curves for 1 mL of PBS containing 100 µg of Se-Cyn NPs subjected to 780 nm CW laser irradiation at different laser power levels. (c) Temperature changes recorded in a Se-Cyn NP solution (100 µg/mL, PBS) over four cycles of heating (10 min, 780 nm CW laser, 0.8 W/cm²) and subsequent cooling (10 min without laser irradiation). (d) The cooling time of a Se-Cyn NP solution (100 µg/mL, PBS) demonstrates a linear relationship with the negative logarithm of temperature. (e) Relative cell viabilities of 4T1 cells incubated with Se-Cyn NPs (0–25 µg/mL, PBS) with or without laser irradiation (780 nm excitation, 0.8 W/cm² for 5 min). ****p* < 0.001. (f) Absorption spectra for a solution of 1,3-Diphenylisobenzofuran (DPBF) and Se-Cyn NPs after 780 nm CW laser irradiation (0.8 W/cm²) at different time points. The arrow indicates a decrease in DPBF absorbance at its characteristic peak (~410 nm). (g) Representative images showing reactive oxygen species (ROS) staining (green) of 4T1 cells incubated with Se-Cyn NPs with or without laser irradiation (780 nm CW laser, 0.8 W/cm² for 5 min, *n* = 3). (h) Live/dead cell staining images of 4T1 cells after incubation with PBS or Se-Cyn NPs with or without laser irradiation. Calcein-AM (green color) or EthD-1 (red) staining indicates live or dead cells, respectively. (*n* = 3). PCE: Photothermal conversion efficiency.

markedly enhanced cell killing [37]. These findings demonstrate that the phototherapeutic effect of Se-Cyn NPs is primarily due to the combined photothermal and photosensitive oxidative activities.

2.4 | In Vivo OptA Imaging

The 4T1 subcutaneous mouse tumor model was used to evaluate the in vivo OptA imaging performance of Se-Cyn NPs, given their promising optoacoustic properties. Figure 3a presents representative unmixed multispectral optoacoustic tomography (MSOT) images of tumors acquired before and at 1, 6, and 24 h after intravenous injection of Se-Cyn NPs (0.2 mg in 100 µL PBS). The images reveal peak Se-Cyn NP accumulation in a tumor between 1 and 6 h post-injection, most likely attributable to passive targeting. These results indicate that a 6 h interval is sufficient for effective tumor uptake and accumulation of Se-Cyn NPs for in vivo OptA imaging. Overall, the findings clearly indicate that Se-Cyn NPs generate strong in vivo OptA contrast and represent effective contrast agents for longitudinal OptA imaging.

Additionally, imaging data suggest that photothermal treatment can be initiated at any point between 1 and 6 h following intravenous administration of Se-Cyn NPs. The biodistribution of Se-Cyn NPs was evaluated in isolated major organs (heart, liver, kidney, spleen, and tumor) by MSOT imaging 24 h after Se-Cyn NP injection (Figure S33, *n* = 3). As shown in Figure 3b, signal intensities from isolated organs and tumor 24 h post-injection of Se-Cyn NPs (*n* = 3) demonstrate that Se-Cyn NPs are present in the tumor even at 24 h post-injection.

2.5 | In Vivo Phototherapy

Motivated by the in vitro findings and in vivo imaging results, we proceeded to evaluate the phototherapeutic potential of Se-Cyn NPs in 4T1 tumor-bearing mice, focusing on their demonstrated photothermal properties. The tumor-bearing mice were divided into four groups to evaluate the in vivo phototherapeutic effect: PBS, PBS + laser, Se-Cyn NPs, and Se-Cyn NPs + laser (*n* = 5 each). Temperature analysis curves (Figure 3c) and thermal camera images (Figure 3d) show that 4T1 tumors in mice injected with Se-Cyn NPs (0.2 mg in 100 µL PBS) 6 h prior to irradiation

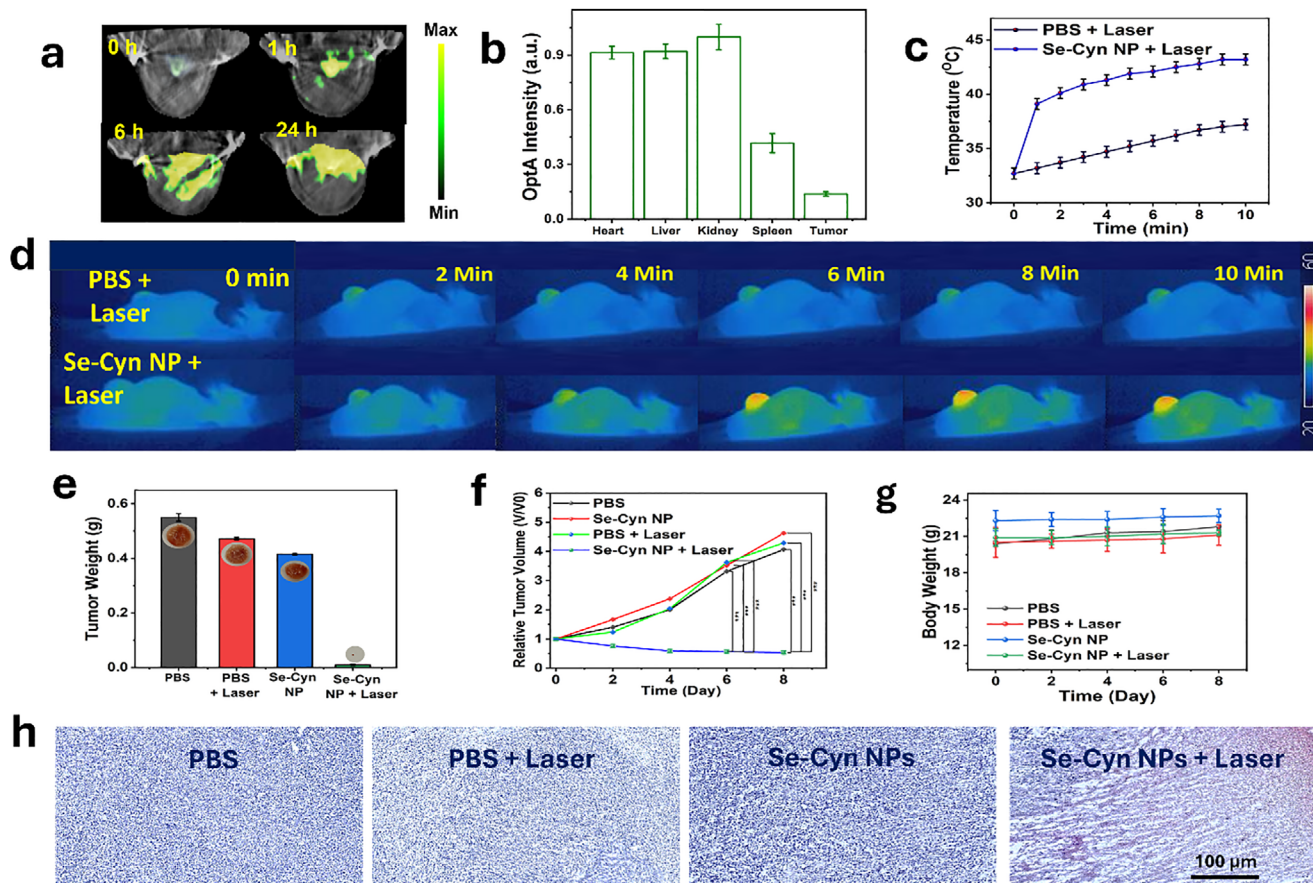


FIGURE 3 | In vivo optoacoustic (OptA) imaging and photothermal therapy of tumors labelled with Selenium-Cyanine (Se-Cyn) nanoparticles (NPs). (a) Representative unmixed multispectral optoacoustic tomography (MSOT) images of a 4T1 subcutaneous tumor in a mouse after intravenous injection of 0.2 mg Se-Cyn NPs in 100 μ L of PBS. Imaging data are shown from a single independent in vivo imaging experiment with $n = 3$ biologically independent mice per group. Longitudinal imaging measurements from the same mouse were analyzed as repeated measures. (b) OptA signal intensities of isolated major organs and tumor 24 h post-injection of Se-Cyn NPs ($n = 3$). a.u.: arbitrary units. (c) Temperature changes in 4T1 tumors in mice during CW laser irradiation (780 nm excitation, 0.8 W/cm²) 6 h after intravenous injection of PBS or Se-Cyn NPs (0.2 mg in 100 μ L of PBS). (d) Representative thermal images of 4T1 tumor-bearing mice before and during a 10 min irradiation period with a 780 nm continuous wave (CW) laser (0.8 W/cm²). Mice received intravenous injections of either PBS or Se-Cyn NPs 6 h before irradiation. Data are shown from one independent in vivo experiment with $n = 5$ biologically independent mice per group, with each mouse receiving the assigned treatment once. (e–g) Comparison of tumor weights (e), relative tumor volumes (f), and mouse body weights (g) at different time points after treatment with Se-Cyn NPs or PBS with or without laser irradiation. V: Tumor volume at different time points, V₀: Initial tumor volume. (h) Representative images of hematoxylin and eosin (H&E)-stained tumor tissue sections isolated from mice on day 8 post-treatment. A two-tailed Student's *t* test was performed for two-group comparisons, and a one-way analysis of variance (ANOVA) was conducted for multiple comparisons (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$); data are presented as the means $\pm$ SD.

with a 780 nm CW laser (0.8 W/cm²) reached a significantly increased tumor-surface temperature of $\sim 43.5^\circ\text{C}$ compared to control mice (PBS + irradiation). This temperature increase achieved by Se-Cyn NPs in combination with laser irradiation is sufficient to induce tumour cell death [38]. As intratumoral temperature distribution could not be assessed using a thermal camera, the therapeutic efficacy was therefore evaluated on the basis of tumor volume, weight, and histological findings.

Tumor volume and body weight were measured daily for each treatment group to monitor the phototherapeutic effect. Figure 3e,f show the changes in tumor weight and relative tumor volumes at various time points after treatment with Se-Cyn NPs or PBS alone, with or without laser irradiation. The results indicate that mice treated with both Se-Cyn NPs and laser irradiation exhibited significant retardation of tumor growth

compared to the other groups. During treatment, body weights were recorded to assess the a general indicator of tolerability, following administration of Se-Cyn NPs. Figure 3g shows changes in body weight over time in mice treated with either Se-Cyn NPs or PBS alone, with or without laser irradiation ($n = 3$ per group). No significant weight loss was observed over the 8-day observation period, indicating that the photothermal therapy was well-tolerated.

Furthermore, histological examination of key organs with H&E indicated minimal toxicity, proving that Se-Cyn NPs do not adversely affect vital organs (Figure S34). To assess the therapeutic effects of Se-Cyn NPs on tumors, H&E staining was carried out on tumors excised during an 8-day treatment period. Tumors treated with Se-Cyn NPs combined with irradiation show clear signs of necrosis and cell shrinkage, while these effects are not

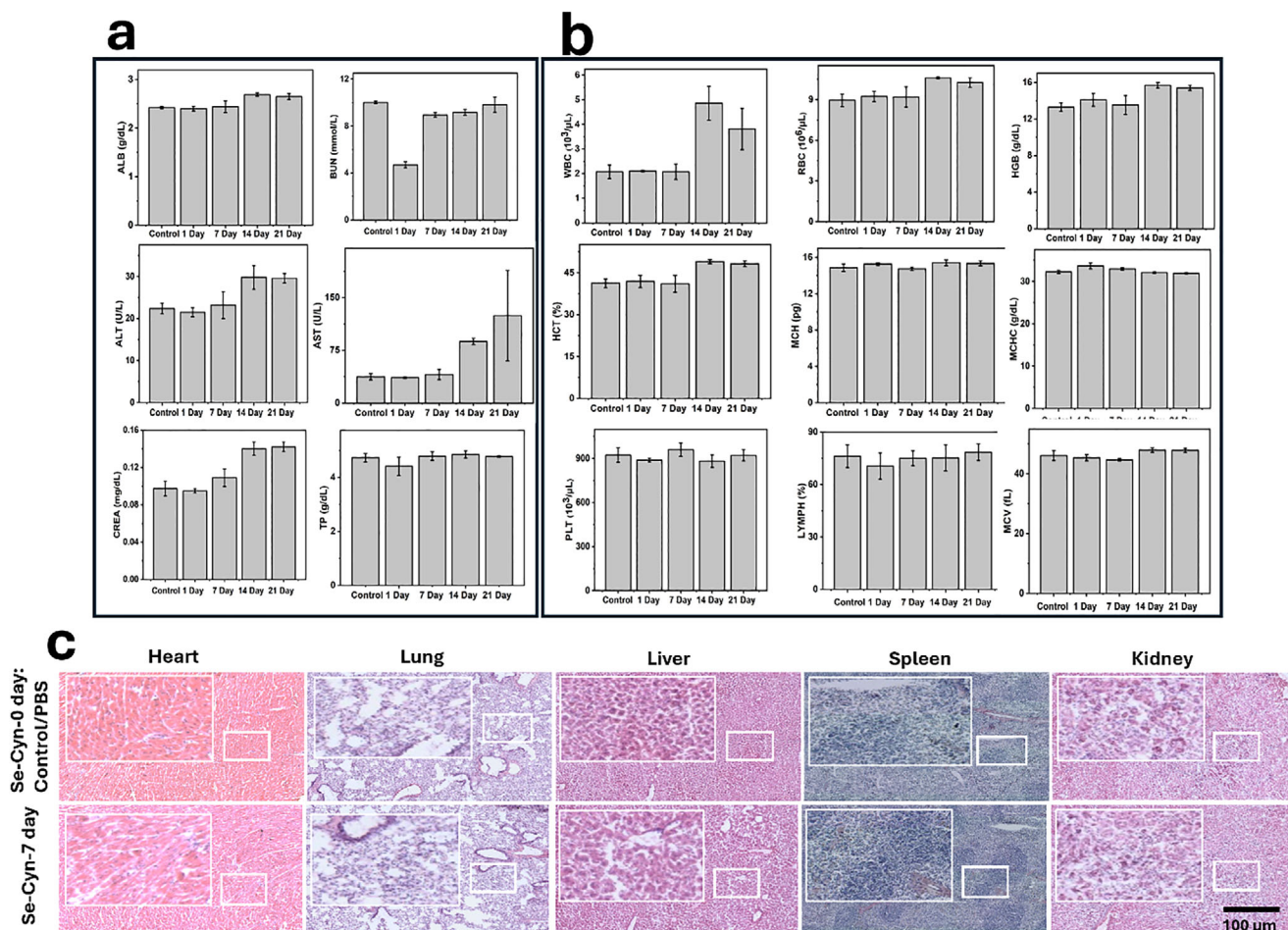


FIGURE 4 | Preliminary tolerability assessment of Selenium-Cyanine nanoparticles (Se-Cyn NPs). Blood samples from healthy mice injected with Se-Cyn NPs or PBS (control) were analyzed for (a) blood biochemistry, and (b) hematological parameters. ALB, albumin; ALT, alanine transferase; AST, aspartate transferase; BUN, blood urea nitrogen; CREA, creatinine; HCT, hematocrit; HGB, hemoglobin; LYMPH, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cells; TP, total protein; WBC, white blood cells; (c) Images of hematoxylin and eosin (H&E)-stained sections of major organs from mice treated with PBS (controls) or Se-Cyn NPs. Data are presented as mean $\pm$ SD ($n = 3$ mice per group). Comparisons between groups were performed using the two-tailed non-parametric Mann–Whitney U test.

observed in the other groups (Figure 3b). Overall, the study showed the photothermal therapeutic efficacy of Se-Cyn NPs and provided preliminary evidence of their in vivo tolerability.

2.6 | In Vivo Preliminary Tolerability

The preliminary tolerability of Se-Cyn NPs was assessed through blood biochemical analyses, organ function tests, and H&E staining of major organs. Hematological and biochemical results from healthy C57BL/6 mice, injected with 100 μ L of 3 mg/mL Se-Cyn NPs and analyzed at 1, 7, 14, and 21 days after injection, or with PBS as the control, are shown in Figure 4a–c, respectively. Overall, no overt treatment-related abnormalities were observed. Minor variations in plasma protein, serum urea and creatinine, and selected hematological parameters were observed at individual time points, with most values trending toward control levels at later time points. A modest increase in aspartate aminotransferase activity was observed, which may reflect pre-analytical variability associated with sample collection. Similarly,

total white blood cell count increased on days 14 and 21; however, values remained within the range routinely observed in untreated wild-type animals. Importantly, histopathological examination revealed no treatment-related tissue abnormalities or overt evidence of organ injury (Figures 4 and S35). Given the limited sample size ($n = 3$ per group), these findings are interpreted as preliminary tolerability data.

3 | Discussion

This study presents a selenium-incorporated cyanine dye, Se-Cyn with enhanced OptA contrast in the NIR window for efficient deep-tissue imaging. Se-Cyn NPs exhibit superior OptA performance compared to ICG NPs, making them strong candidates for non-invasive in vivo NIR imaging. In addition, Se-Cyn NPs produces substantial heat and shows photosensitive oxidative activity, enabling synergistic photothermal and photodynamic cancer cell ablation following nanoparticle-mediated delivery and light irradiation. These combined imaging and therapeutic

capabilities establish Se-Cyn as a promising platform for integrated cancer diagnosis and treatment. Beyond oncology, the unique properties of selenium also highlight Se-Cyn's potential for diverse in vivo applications, including reducing liver injury and lipid peroxidation, serving as an antichagasic agent, and contributing to antimicrobial therapy [39–42].

We successfully synthesized and characterized Se-Cyn and its nanoformulation (Se-Cyn NPs) using spectroscopic and spectrometric techniques. Both forms display peak absorbance in the red/NIR region at 806 nm, along with high OGE, strong PCE, and excellent photostability. Se-Cyn NPs exhibit stable particle size, strong contrast, and good bioavailability, making them highly suitable for in vivo NIR imaging applications. Compared to previously reported selenium-based dyes, Se-Cyn exhibits superior photostability and higher PCE [1, 14, 29, 32, 33]. The comparative analysis presented in Table S2 further underscores the advantages of Se-Cyn_NPs over various reported agents. Importantly, Se-Cyn NPs provide markedly better OptA properties (OGE and PCE) than the ICG NPs evaluated under the conditions used in this study. In addition, their robust photothermal and photodynamic effects enable effective phototherapy.

Since Se is sensitive to moisture, its handling presents substantial challenges and requires inert conditions during reactions [43, 44]. These conditions are essential to prevent compound degradation or unwanted reactions. Therefore, the synthesis of Se-Cyn dye is quite a complex process. Nonetheless, this limitation can be addressed, as selenium possesses favorable redox properties, high environmental and industrial relevance, and potential utility for various biomedical applications. These factors collectively justify the effort required for the synthesis of the dye [45–47]. Furthermore, the FDA approval of certain selenium-containing formulations underscores its potential in developing next-generation cyanine-based theranostic agents with improved contrast in the NIR window [48].

We found that Se-Cyn NPs induce cytotoxicity in 4T1, HCT116, and U87MG cancer cells under laser irradiation, attributable to synergistic photothermal and photosensitive oxidative activities. In vivo phototherapy experiments further showed that Se-Cyn NPs administered with laser irradiation significantly suppressed tumor growth in mice at rates comparable to those of established phototherapy agents [49–51]. These results highlight the potential of Se-Cyn NPs as an effective multimodal platform, enabling both OptA imaging and therapeutic intervention in diverse tumors. Nevertheless, a key challenge for broader clinical application is establishing dosage regimens that balance safety and therapeutic efficacy [52, 53]. Addressing this challenge will require further studies aimed at optimizing the dosing parameters of Se-Cyn dyes in large animals.

As an initial step, we examined tolerance to Se-Cyn NPs in healthy C57BL/6 mice and observed good tolerance. Se-Cyn NP administration induced only slight changes in blood biochemical and hematological values, supporting overall good tolerability. Histopathological analysis revealed no abnormalities in the vital organs examined, including the heart, liver, spleen, and kidneys. Although these findings suggest good short-term tolerability at the administered dose, more comprehensive safety and toxicity assessments in small and larger animal models remain necessary.

This study provides an initial in vivo evaluation of selenium-based cyanine NPs. These findings suggest that Se-Cyn NPs could be employed as phototherapy and imaging agents in theranostic applications. Nonetheless, it is important to acknowledge that current findings in murine models may not fully extrapolate to human clinical settings due to limited sample size, interspecies differences and the narrow therapeutic window of selenium. Further studies using large cohort, longer follow-up periods, and appropriate large-animal studies are still required to establish biosafety and clinical translation [54, 55].

4 | Conclusion

We synthesized Se integrated Cyn contrast agent that exhibits strong optical absorption in the NIR wavelength range and high photothermal conversion efficiency, thus allowing for prolonged OptA imaging with high contrast. The key advantage of this study lies in the use of the NIR Se-Cyn dye, which enables non-invasive, real-time tumor visualization with deep tissue penetration and low background autofluorescence in a murine model. The dye also showed good biocompatibility in both cells and animals, underscoring its potential for clinical translation. The combined imaging and therapeutic capabilities of Se-Cyn support precise localization and treatment of pathological tissue. Furthermore, Se-Cyn NPs offer promise for clinical image-guided interventions by facilitating monitoring of biodistributions, targeting of tumors, and evaluation of therapeutic efficacy. A current limitation of Se-Cyn NPs is the absence of an active cancer-targeting moiety. To achieve active tumor targeting, conjugation with a specific ligand will be necessary [56, 57]. In this study, our focus was on evaluating passive tumor accumulation; therefore, targeting ligands were not incorporated. Nevertheless, the Se-Cyn dye structure is amenable to chemical modification, and a cancer-targeting group can be readily attached to the dye using standard conjugation chemistry. Future work will focus on modifying Se-Cyn dyes for specific applications, such as sensing components of the tumor microenvironment, thereby expanding their potential for clinical use. In summary, we have developed a Se-Cyn dye and its nanoformulation that exhibits outstanding optoacoustic properties and strong phototherapeutic efficacy, both of which have been validated in vivo. Beyond introducing a novel NIR Se-Cyn derivative for OptA imaging, this study lays the foundation for future research exploring the photopharmacological applications of NIR Se-Cyn NPs.

5 | Experimental Section

5.1 | Materials

The chemicals used were of analytical grade, purchased from commercially available sources (SDFCL, Spectrochem, Sigma-Aldrich, and Aura Chemicals), and used without further purification. Column chromatography was done by using silica gel with a mesh size of 60–120. HPLC-grade solvents were used for spectrophotometric studies. Reactions were carried out in an inert atmosphere. The thin-layer chromatography (TLC) was done on UV-active (254 nm) aluminum-coated silica plates (60, F-254) from Merck. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay kit) and Indocyanine

green (ICG) were purchased from Sigma-Aldrich (Munich, Germany). Calcein acetoxymethyl (AM) and ethidium homodimer-1 (EthD1) were obtained from Thermo Fisher Scientific. Nanocs Inc. (USA) was the source of DSPE-PEG5000.

5.2 | Synthesis of Se-Cyn and Se-Cyn NPs

5.2.1 | Synthesis of 1-(Naphtho[1,8-cd][1,2]Diselenol-3-Ylmethylene)-2-Phenylhydrazine (6)

Naphtho[1,8-cd][1,2]diselenole-3-carbaldehyde (5) (0.150 g, 0.48 mmol) and phenyl hydrazine (0.06 g, 0.58 mmol) were combined in 10 mL of ethanol, and the mixture was refluxed for 12 h under an inert atmosphere in the presence of catalytic amount of glacial acetic acid (GAA). Upon completion of the reaction, the reaction mixture was cooled to room temperature, and the solvent was evaporated under vacuum. Further, the crude compound was recrystallized using n-hexane to yield orange-yellow crystals of compound (6) (0.20 g, 99.05%). The compound was characterized by NMR and mass spectroscopy. Spectroscopic Data: ^1H NMR (300 MHz, CDCl_3): δ (ppm) 8.11 (s, 1H), 7.83 (bs, 1H), 7.61–7.58 (m, 2H), 7.53–7.50 (d, 1H), 7.39–7.26 (m, 6H), 6.99 (t, 1H); ^{13}C (75 MHz, CDCl_3): δ (ppm) 143.45, 142.16, 140.64, 138.27, 136.46, 129.46, 127.71, 127.42, 127.11, 127.14, 122.77, 122.59, 122.48, 120.76, 112.79; ESI-MS: calculated Mass for $[\text{C}_{17}\text{H}_{12}\text{N}_2\text{Se}_2]^+$ (m/z):- 402.21 and observed (m/z):- 402.1.

5.2.2 | Synthesis of 1-Ethyl-2-((E)-2-((E)-3-((E)-1-Ethyl-3,3-Dimethylindolin-2-Ylidene)Ethylidene)-2-((E)-Naphtho[1,8-cd][1,2]Diselenol-3-Ylmethylene)-1-Phenylhydrazineyl)Cyclohex-1-en-1-yl)Vinyl)-3,3-Dimethyl-3H-Indol-1-Ium (Se-Cyn) (7)

As shown in Scheme 1, to a solution of Cesium carbonate (0.21 g, 0.65 mmol) in 5 mL of anhydrous acetonitrile, 1-(naphtho[1,8-cd][1,2]diselenol-3-ylmethylene)-2-phenylhydrazine (6) (0.24 g, 0.60 mmol) dissolved in 5 mL of dry dichloromethane was added at -10°C under an inert atmosphere. The reaction mixture was stirred for 45 min at -10°C . Dissolved Cyn.7.Cl (0.32 g, 0.80 mmol) in 5 mL of acetonitrile was added dropwise to the reaction mixture at -10°C for 15 min, and the reaction mixture was stirred for 3 h at RT. The solvent was evaporated under vacuum. The solid was purified by silica gel column chromatography using ethyl acetate/methanol (95:5 v/v) as mobile phase. The bluish solid was obtained in a yield of 14.45% (0.072 g). Spectroscopic Data: ^1H NMR (300 MHz, CDCl_3) δ (ppm):- 7.96 (s, 1H), 7.68–7.53 (m, 5H), 7.48–7.39 (m, 5H), 7.28 (m, 4H), 7.15 (m, 3H), 7.08–7.06 (d, 2H), 7.02 (s, 1H), 6.24–6.20 (d, 1H), 4.18 (m, 4H), 3–2.93 (d, 2H), 2.23 (m, 2H), 1.73 (s, 2H), 1.39 (s, 6H), 1.25 (s, 6H), 1.09 (s, 3H), 0.88–0.86 (d, 3H); ^{13}C (75 MHz, CDCl_3): δ (ppm):- 172.8, 153.9, 146.1, 145.3, 143.5, 142.3, 142.0, 141.9, 141.7, 141.5, 140.0, 139.1, 138.3, 137.1, 130.1, 129.3, 129.1, 128.2, 127.8, 127.1, 126.8, 125.9, 124.6, 123.1, 122.8, 122.6, 122.2, 114.3, 111.0, 101.2, 100.8, 39.9, 31.9, 30.0, 28.2, 28.0, 27.8, 26.3, 25.0, 23.0, 21.3, 14.2, 12.5, 12.4; ^{77}Se NMR (76.34 MHz, CDCl_3): δ (ppm) 534, 391. ESI-MS: calculated mass for $[\text{C}_{51}\text{H}_{51}\text{N}_4\text{Se}_2]^+$ (m/z):- 877.88 and observed (m/z):- 877.4.

5.2.3 | Synthesis of Se-Cyn NPs

Se-Cyn and DSPE-PEG5000 were each dissolved in tetrahydrofuran (0.5 mL) in separate vials. After dissolution, the solutions were mixed and rapidly injected into deionized water (9 mL). The solution was sonicated with a microtip probe sonicator (12 W) for 5 min. Residual tetrahydrofuran was removed using a rotary evaporator. The resulting Se-Cyn NPs were filtered through a 200 nm filter and then concentrated using a centrifugal filter (MWCO = 100 kDa).

5.3 | Time-Dependent Density-Functional Theory (TD-DFT)

All quantum chemical calculations were performed using the ORCA 6.0.1 (Win64) software package [28]. Geometry optimizations of the dye molecules Cyn-7-Cl, Se-Cyn and ICG were carried out using density functional theory (DFT) with the B3LYP exchange–correlation functional and the def2-TZVP basis set. Implicit solvation effects were modelled using the SMD (Solvation Model based on Density) approach, with water and dimethyl sulfoxide (DMSO) as solvents. Standard integration grids and tight self-consistent field (SCF) convergence criteria were applied. Canonical molecular orbitals were printed to enable electronic structure analysis. Vertical excitation energies were computed using time-dependent DFT (TD-DFT) at the same level of theory (B3LYP/def2-TZVP), incorporating solvent effects via the SMD model with water and DMSO as dielectric media. Excited-state calculations were performed using the DRACO module, with 30 singlet excited states requested (NROOTS = 30). The DONTOK keyword was enabled to include contributions from double excitations within the linear-response framework. Solvent-dependent differences (water vs. DMSO) in excitation energies and oscillator strengths were found to be negligible. Molecular structures and electronic transitions were visualized and analyzed using UCSF ChimeraX version 1.8 [58].

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5.4 | Characterization of Se-Cyn and Se-Cyn NPs

Nuclear magnetic resonance (NMR) characterization was done on a Bruker Avance 300 and 400. The ^1H , and ^{13}C NMR spectra were recorded in deuterated solvents (CDCl_3 and DMSO-d_6) on Bruker Avance 300 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. The ^{77}Se NMR spectrum was recorded on Bruker Avance 400 MHz spectrometer using diphenyl diselenide as an external standard. Mass spectra were recorded on AB SCIEX 3200 Q TRAP LC/MS/MS spectrometer. HPLC-UV/MS analyses were performed on a Dionex UltiMate 3000 HPLC system coupled with a Thermo Scientific ISQ EC Single Quadrupole Mass Spectrometer. Analyses were performed using a Thermo Scientific Accucore RP-MS LC-column (2.1×50 mm, $2.6 \mu\text{m}$) with a gradient (5% to 95% of acetonitrile + 0.1% formic acid v/v in water + 0.1% formic acid v/v) over 5 min at a flow rate of 0.6 mL/min, and UV detection at 254 nm. Transmission electron microscopy (Jeol JEM-1400 plus) was used for morphological analysis of the nanoformulations, while nanoparticle size distributions, polydispersity index and zeta potential were quantified using a Malvern Zetasizer. UV-visible spectra were recorded using a Tecan i-control and Shimadzu UV 2450, while OptA spectra were recorded using MSOT inVision 256-TF (iThera Medical). Corrections were done with India ink, Brilliant Black BN (BBN) [59]. Infrared thermal images were captured using an infrared camera (FLIR I60).

5.4.1 | Determination of Quantum Yield

The quantum yield of Se-Cyn was calculated using the following equation.

$$\phi_1 = \phi_2 \frac{A_2}{A_1} \times \frac{S_1}{S_2} \times \left(\frac{\eta_1}{\eta_2} \right)^2 \quad (1)$$

where, ϕ_2 is the quantum yield of standard,

A is the absorbance at an excited wavelength,

S is the integrated emission band area at an excitation wavelength,

η is the refractive index of the solvents,

The subscripts 1 and 2 refer to the sample and the standard, respectively.

Indocyanine Green (ICG) was used as a standard [60].

5.4.2 | Apparent Singlet Oxygen Quantum Yields (Φ_{Δ})

Singlet oxygen quantum yields were assessed spectroscopically in a cuvette. Se-Cyn NP solutions containing DPBF were irradiated using a laser (780 nm, 0.8 W/cm^2), and absorption spectra were captured at 2-min intervals during laser irradiation in PBS buffer (pH 7.4) at room temperature, with ICG used as a standard. Following laser irradiation, the absorbance of DPBF at 410 nm was measured over 0–6 min, and absorbance was plotted as a

function of irradiation time. By using the following equation, the apparent singlet oxygen quantum yield was estimated.

$$\text{Singlet oxygen quantum yield} = \Phi_{\text{std}} \frac{\text{grad}_{\text{Sample}}}{\text{grad}_{\text{Std}}} \times \frac{F_{\text{Sample}}}{F_{\text{Std}}} \quad (2)$$

where, ϕ_{std} is the singlet oxygen quantum yield of ICG (0.077 in DMSO) [61],

grad is the rate of reaction,

F is the absorption correction factor ($F = 1 - 10^{-\text{Abs}}$)

5.4.3 | Determination of Encapsulation Efficiency (%EE)

Encapsulation efficiency (%EE) was defined as the percentage of Se-Cyn dye successfully encapsulated within the NPs relative to the total amount of dye feed. To determine %EE, calibration curves were first constructed by plotting the absorbance of Se-Cyn dye in Tetrahydrofuran solution at 794 nm across different concentrations. Absorbance values were recorded by a UV-Vis spectrophotometer, and the amount of Se-Cyn encapsulated in the NPs was determined from the standard calibration curve. % EE was calculated using Equation 3 [62].

$$\% \text{ EE} = \left(\frac{\text{Amount of dye encapsulated in NPs}}{\text{Amount of dye feed}} \right) \times 100 \quad (3)$$

5.4.4 | Determination of Dye-Loading Percentage

Dye-loading percentages were determined as the ratio of the encapsulated dye mass to the total mass of NPs. To determine dye-loading percentages in the NPs, freeze-dried Se-Cyn NPs were first weighed to obtain the total mass of the NPs prior to dissolution in THF. The mass of Se-Cyn encapsulated in the NPs was subsequently determined from the standard calibration curve. The drug loading percentage was calculated using Equation 4 [62].

$$\% \text{ Drug Loading} = \left(\frac{\text{Mass of dye encapsulated in NPs}}{\text{Total mass of dried NPs}} \right) \times 100 \quad (4)$$

5.5 | In Vitro Photothermal and Optoacoustic Generation Properties of Se-Cyn NPs

The photothermal properties of Se-Cyn NPs were examined by monitoring temperature changes in solutions containing varying concentrations of Se-Cyn NPs during continuous laser irradiation at 780 nm and varying power settings. Temperature was measured with an infrared thermal camera.

Photothermal conversion efficiency (PCE): PCE of Se-Cyn NPs was calculated using the following equation:

$$\eta = \left\{ \frac{[hs(T - T_{\text{surr}}) - Q_{\text{Dis}}]}{I} \left[1 - 10^{\left(\frac{-A}{780} \right)} \right] \right\} \quad (5)$$

where, η stands for photothermal conversion efficiency,

h is the heat transfer coefficient,
 s is the surface area of the cuvette,
 T and T_{sur} are the reached thermal equilibrium state temperature and the surrounding temperature, respectively,
 Q_{Dis} is the energy input from deionized water in the detected solution,
 I is the power of the continuous wave laser (0.8 W/cm^2),
 A_{780} refers to the absorbance of Se-Cyn NPs at 780 nm (0.45) [63].

The photothermal conversion capability of Se-Cyn NP solutions was calculated over several cycles of laser irradiation on and off.

Optoacoustic generation efficiency (OGE): For OGE determination, each dye or nanoparticle solution was diluted in deionized water to obtain optical absorbance values of 0.1, 0.2, 0.3, 0.4, and 0.5 at the respective excitation wavelength. Samples were imaged using MSOT under identical acquisition and reconstruction conditions. Nanosecond-pulsed light generated by a tunable optical parametric oscillator laser was delivered to the sample through a ring-shaped fiber bundle. Measurements were performed within the 680–900 nm wavelength range, with the excitation wavelength selected based on the absorption maximum of each sample. Absorption of the excitation light induced thermoelastic expansion and generated acoustic waves, which were detected using a cylindrically focused 256-element transducer array with a central frequency of 5 MHz, a radius of curvature of 40 mm, and an angular coverage of 270° . OGE was defined as the slope of the linear regression between optoacoustic signal intensity and optical absorbance.

5.6 | In Vitro Cytotoxicity Assessment of Se-Cyn NPs

The cytotoxic effects of Se-Cyn NPs were assessed across several cell lines, including 4T1, HCT116, and U87, using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). For this study, cells were suspended in a suitable medium supplemented with 10% FBS and 1% PS and incubated in a 5% CO_2 atmosphere at 37°C . The cells were cultured in a 96-well plate overnight at a density of 1×10^3 cells/well. Afterward, the cells were treated with various concentrations of NPs for 12 h. After 12 h, the cells were washed, and fresh media was given. Cells were then irradiated using a laser (780 nm, 0.8 W/cm^2 , 5 min), and cell viability was then evaluated using the MTT assay.

5.7 | In Vitro ROS Generation

Prior to testing the cells, ROS generation was assessed spectroscopically in a cuvette. Se-Cyn NPs solutions containing DPBF were irradiated using a laser (780 nm, 0.8 W/cm^2) with absorption spectra captured every two minutes of laser irradiation to determine photosensitive oxidative activity [64].

The cell-based in vitro photosensitive oxidative activity Se-Cyn NPs was identified by 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Briefly, the cells were incubated for 12 h with Se-Cyn NPs and with DCFH-DA for an additional 1 h, and the cells

were washed and replaced with fresh medium and exposed to laser irradiation for 5 min. Leica DMI3000 B inverted microscope (Wetzlar, Germany) was used to visualize the photosensitive oxidative activity of Se-Cyn NPs at the cellular level.

5.8 | In Vitro Live/Dead Cell Staining

A live-dead cell staining study was performed on 4T1 cells. Cells at a density of 1×10^6 were cultured in a 6-well plate overnight. The cells were then treated with PBS or Se-Cyn NPs for 12 h. After 12 h, the cells underwent laser irradiation (780 nm, 0.8 W/cm^2 , 5 mins) or no irradiation as control. After laser irradiation, cells were washed with PBS and incubated with the Calcein-AM and EthD-1 for 30 min. Finally, cells were washed with PBS, and fluorescence imaging was carried out using a Leica DMI3000 B inverted microscope (Wetzlar, Germany).

5.9 | In Vivo OptA Imaging of 4T1 Tumor-Bearing Mice

The Government of Upper Bavaria approved the protocols that were used for animal experiments. The 4T1 subcutaneous tumor model was prepared by implanting 4T1 cells (1×10^6 cells in $30 \mu\text{L}$ PBS) subcutaneously on the back of 6-week-old nude mice ($n = 3$). OptA imaging was carried out once tumors reached around 100 mm^3 . Tumor-bearing mice were injected with Se-Cyn NPs (3 mg/mL), and the MSOT inVision 256-TF was used to obtain OptA images at pre-injection, 1, 6, and 24 h post-injection. Images were reconstructed using ViewMSOT 4.0 software's (iThera Medical, Munich) "model linear" technique [65].

5.10 | In Vivo PTT of 4T1 Tumor-Bearing Mice

To assess the in vivo anti-tumor efficacy of Se-Cyn NPs, 4T1 tumor-bearing mice were split up into four groups ($n = 5$ each) and treated with: PBS (i.v. injection, $100 \mu\text{L}$), PBS + laser (i.v. injection, $100 \mu\text{L}$), Se-Cyn NPs (i.v. injection, 3 mg/mL , $100 \mu\text{L}$), Se-Cyn NPs + laser (i.v. injection, 3 mg/mL , $100 \mu\text{L}$). The tumors of laser-treated groups were irradiated (780 nm, 0.8 W/cm^2 , 10 min) 6 h after injection of PBS or Se-Cyn NPs. Tumor temperatures were recorded by using a thermal camera. Tumor volumes and the weights of the mice in all the groups were measured over 8 days after treatments. On the final day of therapy, all the mice were sacrificed, and the major organs and tumors were isolated and subjected to H&E staining.

5.11 | In Vivo Tolerability Evaluation of Se-Cyn NPs

The preliminary tolerability of Se-Cyn NPs was assessed using C57BL/6 mice (3 groups, $n = 3$) [66]. Blood from Se-Cyn NP-treated groups (i.v. injection, 3 mg/mL , $100 \mu\text{L}$) was collected on days 1, 7, 14, and 21. Hematological analyses were performed using a Sysmex XN1000V hematology analyzer applying pre-set mouse settings and the pre-dilution measurement mode applied to blood samples diluted 1:7 with Cellpack DCL buffer (Sysmex). Blood biochemistry parameters were determined using a AU480 Clini-

cal Chemistry Analyzer (Beckman-Coulter Germany, Germany) with adapted kits provided by Beckman-Coulter as described earlier) [67]. The isolated vital organs of these 3 groups were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

5.12 | Statistical Analysis

Experimental data were analysed using OriginPro 2023 (Origin-Lab Corporation, Northampton, MA, USA) with built-in statistical analysis and curve-fitting tools. Graphs were plotted using the software's high-resolution plotting features. The sample size was selected before the experiments based on practical feasibility, prior experience with comparable MSOT imaging and phototherapy studies [5, 66] and ethical considerations to minimize animal use. For studies with 5 animals per group, two-tailed Student's t tests were performed for two-group comparisons, whereas a one-way analysis of variance (ANOVA) was applied for multiple comparisons. Data are presented as the means $\pm$ SD, with statistically significant differences denoted by asterisks ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$). For studies with 3 animals per group, group comparisons were performed using the two-tailed nonparametric Mann-Whitney U test.

Author Contributions

Divyesh Shelar: methodology, conceptualization, data curation, formal analysis, investigation, validation, visualization, writing – review and editing, writing – original draft. **Snehal Salunkhe:** methodology, formal analysis. **Tony Fröhlich:** formal analysis, data curation, writing – review and editing. **Uwe Klemm:** data curation, investigation. **Pia Anzenhofer:** data curation, investigation. **Birgit Rathkolb:** investigation, writing – review and editing. **Martin Hrabě de Angelis:** investigation, writing – review and editing. **Oliver Plettenburg:** supervision, data curation. **Michael Sattler:** supervision, writing – review and editing, data curation. **Sudesh T. Manjare:** methodology, formal analysis, supervision, writing – review and editing. **Vipul Gujrati:** methodology, data curation, formal analysis, supervision, visualization, writing – original draft, writing – review and editing. **Vasilis Ntziachristos:** funding acquisition, resources, formal analysis, supervision, visualization, writing – original draft, writing – review and editing.

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Ethics Approval Statement

All animal experimental procedures were conducted in accordance with protocols approved by the Helmholtz Zentrum München Animal Care and Handling Office and the Government of Upper Bavaria.

Conflicts of Interest

V.N. is a founder and equity owner of iTheraNEXT Imaging GmbH, Maurus OY, sThesis GmbH, Spear UG, Biosense Innovations P.C. and I3 Inc. The remaining authors declare no conflicts of interest.

Data Availability Statement

All data presented in the paper are available from the authors upon reasonable request.

References

1. K. Bilici, S. Cetin, E. Celikbas, H. Yagci Acar, and S. Kolemen, "Recent Advances in Cyanine-Based Phototherapy Agents," *Frontiers in Chemistry* 9 (2021): 707876, <https://doi.org/10.3389/fchem.2021.707876>.
2. N. Lange, W. Szlasa, J. Saczko, and A. Chwilkowska, "Potential of Cyanine Derived Dyes in Photodynamic Therapy," *Pharmaceutics* 13 (2021): 818, <https://doi.org/10.3390/pharmaceutics13060818>.
3. F. Knieling, S. Lee, and V. Ntziachristos, "A Primer on Current Status and Future Opportunities of Clinical Optoacoustic Imaging," *Npj Imaging* 3 (2025): 4, <https://doi.org/10.1038/s44303-024-00065-9>.
4. Z. Kejik, J. Hajdich, N. Abramenko, et al., "Cyanine Dyes in the Mitochondria-Targeting Photodynamic and Photothermal Therapy," *Communications Chemistry* 7 (2024): 180, <https://doi.org/10.1038/s42004-024-01256-6>.
5. N. Liu, P. O'Connor, V. Gujrati, et al., "Tuning the Photophysical Properties of Cyanine by Barbiturate Functionalization and Nanoformulation for Efficient Optoacoustics- Guided Phototherapy," *Journal of Controlled Release* 372 (2024): 522–530, <https://doi.org/10.1016/j.jconrel.2024.06.037>.
6. V. Ntziachristos, "Addressing Unmet Clinical Need With Optoacoustic Imaging," *Nature Reviews Bioengineering* 3 (2025): 182–184, <https://doi.org/10.1038/s44222-024-00242-w>.
7. E. I. Shramova, A. B. Kotlyar, E. N. Lebedenko, S. M. Deyev, and G. M. Proshkina, "Near-Infrared Activated Cyanine Dyes as Agents for Photothermal Therapy and Diagnosis of Tumors," *Acta Naturae* 12 (2020): 102–113, <https://doi.org/10.32607/actanaturae.11028>.
8. M. D. Laramie, B. L. Fouts, W. M. MacCuaig, et al., "Improved Pentamethine Cyanine Nanosensors for Optoacoustic Imaging of Pancreatic Cancer," *Scientific Reports* 11 (2021): 4366, <https://doi.org/10.1038/s41598-021-83658-3>.
9. Y. Zhang, Y. Jia, and S. Zhu, "NIR-II Cyanine@ Albumin Fluorophore for Deep Tissue Imaging and Imaging-Guided Surgery," *SmartMat* 5 (2024): 1245.
10. G. Fei, S. Ma, C. Wang, et al., "Imaging Strategies Using Cyanine Probes and Materials for Biomedical Visualization of Live Animals," *Coordination Chemistry Reviews* 447 (2021): 214134, <https://doi.org/10.1016/j.ccr.2021.214134>.
11. A. Y. Sonay, "Deep Tissue Imaging of Cancer in the Infrared," *Nature Reviews Cancer* 25 (2025): 570, <https://doi.org/10.1038/s41568-025-00823-w>.
12. K. Funabiki, R. Yanagawa, Y. Kubota, and T. Inuzuka, "Thermo- and Photo-Stable Symmetrical Benzo[cd]Indolenyl-Substituted Hep- tamethine Cyanine Dye Carrying a Tetrakis(Pentafluorophenyl)Borate

- that Absorbs Only Near-Infrared Light Over 1000 nm,” *New Journal of Chemistry* 43 (2019): 7491–7501, <https://doi.org/10.1039/C9NJ00867E>.
13. M. Müller, N. Liu, V. Gujrati, et al., “Merged Molecular Switches Excel as Optoacoustic Dyes: Azobenzene–Cyanines Are Loud and Photostable NIR Imaging Agents,” *Angewandte Chemie International Edition* 63 (2024): 202405636, <https://doi.org/10.1002/anie.202405636>.
 14. A. V. Prakash, F. Yazabak, I. Hovor, et al., “Highly Efficient Near-IR Cyclohexene Cyanine Photosensitizers for Antibacterial Photodynamic Therapy,” *Dyes and Pigments* 211 (2023): 111053, <https://doi.org/10.1016/j.dyepig.2022.111053>.
 15. T. M. Ebaston, F. Nakonechny, E. Talalai, G. Gellerman, and L. Patsenker, “Iodinated Xanthene-Cyanine NIR Dyes as Potential Photosensitizers for Antimicrobial Photodynamic Therapy,” *Dyes and Pigments* 184 (2021): 108854, <https://doi.org/10.1016/j.dyepig.2020.108854>.
 16. F. Bertocchi, A. Delledonne, G. Vargas-Nadal, F. Terenziani, A. Painelli, and C. Sissa, “Aggregates of Cyanine Dyes: When Molecular Vibrations and Electrostatic Screening Make the Difference,” *The Journal of Physical Chemistry C* 127 (2023): 10185–10196, <https://doi.org/10.1021/acs.jpcc.3c01253>.
 17. J. Zhang, W. Wang, J. Shao, J. Chen, and X. Dong, “Small Molecular Cyanine Dyes for Phototheranostics,” *Coordination Chemistry Reviews* 516 (2024): 215986, <https://doi.org/10.1016/j.ccr.2024.215986>.
 18. A. J. Ashe, J. W. Kampf, and P. M. Savla, “The Reaction of sulfur with dilithio compounds. The syntheses and Structures of Phenanthro [1, 10-cd]-1,2-Dithiole and Phenanthro[4,5-cde] [1,2]Dithiin,” *Heteroatom Chemistry* 5 (1994): 113–119, <https://doi.org/10.1002/hc.520050206>.
 19. L. Wu, X. Li, C. Huang, and N. Jia, “Dual-Modal Colorimetric/Fluorescence Molecular Probe for Ratiometric Sensing of pH and Its Application,” *Analytical Chemistry* 88 (2016): 8332–8338, <https://doi.org/10.1021/acs.analchem.6b02398>.
 20. S. Pascal, A. Haefele, C. Monnereau, et al., “Expanding the Polymethine Paradigm: Evidence for the Contribution of a Bis-Dipolar Electronic Structure,” *The Journal of Physical Chemistry A* 118 (2014): 4038–4047, <https://doi.org/10.1021/jp501358q>.
 21. L. Wang, J. Jin, X. Chen, et al., “A Cyanine Based Fluorophore Emitting Both Single Photon Near-Infrared Fluorescence and Two-Photon Deep Red Fluorescence in Aqueous Solution,” *Organic & Biomolecular Chemistry* 10 (2012): 5366, <https://doi.org/10.1039/c2ob25619c>.
 22. D. Manna and G. Magesh, “Regioselective Deiodination of Thyroxine by Iodothyronine Deiodinase Mimics: An Unusual Mechanistic Pathway Involving Cooperative Chalcogen and Halogen Bonding,” *Journal of the American Chemical Society* 134 (2012): 4269–4279, <https://doi.org/10.1021/ja210478k>.
 23. J. Wang, W.-F. Cao, J.-H. Su, H. Tian, Y.-H. Huang, and Z.-R. Sun, “Syntheses and Nonlinear Absorption of Novel Unsymmetrical Cyanines,” *Dyes and Pigments* 57 (2003): 171–179, [https://doi.org/10.1016/S0143-7208\(03\)00034-2](https://doi.org/10.1016/S0143-7208(03)00034-2).
 24. F. Yuan, M. Dellian, D. Fukumura, et al., “Vascular Permeability in a Human Tumor Xenograft: Molecular Size Dependence and Cutoff Size,” *Cancer Research* 55 (1995): 3752.
 25. M. U. Munir, “Nanomedicine Penetration to Tumor: Challenges, and Advanced Strategies to Tackle This Issue,” *Cancers* 14 (2022): 2904, <https://doi.org/10.3390/cancers14122904>.
 26. K. Chansaenpak, G. Y. Yong, A. Prajit, et al., “Aza-BODIPY-Based Polymeric Nanoparticles for Photothermal Cancer Therapy in a Chicken Egg Tumor Model,” *Nanoscale Advances* 6 (2024): 406–417, <https://doi.org/10.1039/D3NA00718A>.
 27. Y. Dong, H. Wang, X. Zhang, et al., “Croconaine-Based NIR-II Fluorescence Imaging-Guided Tumor Photothermal Therapy Induces Long-Term Antitumor Immune Memory,” *Journal of Nanobiotechnology* 22 (2024): 481, <https://doi.org/10.1186/s12951-024-02695-y>.
 28. F. Neese, F. Wennmohs, U. Becker, and C. Riplinger, “The ORCA Quantum Chemistry Program Package,” *The Journal of Chemical Physics* 152 (2020): 224108, <https://doi.org/10.1063/5.0004608>.
 29. L. Yang, Y. Gao, J. Wei, et al., “Selenium-Integrated Conjugated Oligomer Nanoparticles With High Photothermal Conversion Efficiency for NIR-II Imaging-Guided Cancer Phototheranostics In Vivo,” *Journal of Nanobiotechnology* 21 (2023): 314, <https://doi.org/10.1186/s12951-023-02080-1>.
 30. D. Kim and H. Kim, “Induction of Apoptotic Temperature in Photothermal Therapy Under Various Heating Conditions in Multi-Layered Skin Structure,” *International Journal of Molecular Sciences* 22 (2021): 11091, <https://doi.org/10.3390/ijms222011091>.
 31. Y. Zhang, X. Zhan, J. Xiong, et al., “Temperature-Dependent Cell Death Patterns Induced by Functionalized Gold Nanoparticle Photothermal Therapy in Melanoma Cells,” *Scientific Reports* 8 (2018): 8720, <https://doi.org/10.1038/s41598-018-26978-1>.
 32. Selenium-Substituted BOIMPY for Enhanced Photodynamic Therapy | ACS Medicinal Chemistry Letters.
 33. S. Bölke, T. Keller, F. Trilling, et al., “The Influence of the Side Chain Structure on the Photostability of Low Band Gap Polymers,” *Molecules* 28 (2023): 3858, <https://doi.org/10.3390/molecules28093858>.
 34. Y. Hei, B. Wang, Y. He, W. Wang, Y. Xue, and C. Chen, “Photostability of Organic Fluorophore Influenced by Adjacent Amino Acid Residues,” *Communications Chemistry* 8 (2025): 262, <https://doi.org/10.1038/s42004-025-01661-5>.
 35. J. Xia, H. Quan, Y. Huang, Z. Zhang, Y. Zhang, and B. Lu, “Side Chain Programming Synchronously Enhances the Photothermal Conversion Efficiency and Photodynamic Activity of A–D–A Photosensitizers,” *ACS Macro Letters* 13 (2024): 489–494, <https://doi.org/10.1021/acsmacrolett.4c00031>.
 36. Y. Lian, C. Wang, Y. Meng, et al., “Selenide Heterostructure Nanosheets With Efficient Near-Infrared Photothermal Conversion for Therapy,” *ACS Omega* 8 (2023): 9371–9378, <https://doi.org/10.1021/acsomega.2c07964>.
 37. Y. Zou, S. Long, T. Xiong, et al., “Single-Molecule Förster Resonance Energy Transfer-Based Photosensitizer for Synergistic Photodynamic/Photothermal Therapy,” *ACS Central Science* 7 (2021): 327–334, <https://doi.org/10.1021/acscentsci.0c01551>.
 38. D. Yoo, H. Jeong, C. Preihs, et al., “Double-Effector Nanoparticles: A Synergistic Approach to Apoptotic Hyperthermia,” *Angewandte Chemie International Edition* 51 (2012): 12482–12485, <https://doi.org/10.1002/anie.201206400>.
 39. Q. Luo, X. Bai, X. Li, and C. Liu, “The Role and Mechanism of Selenium in the Prevention and Progression of Hepatocellular Carcinoma,” *Frontiers in Oncology* 15 (2025): 1557233, <https://doi.org/10.3389/fonc.2025.1557233>.
 40. A. Grudniak, J. Folcik, J. Szmytko, and A. Sentkowska, “Mechanism of Antioxidant Activity of Selenium Nanoparticles Obtained by Green and Chemical Synthesis,” *International Journal of Nanomedicine* 20 (2025): 2797–2811, <https://doi.org/10.2147/IJN.S507712>.
 41. N. Filipović, D. Ušjak, M. T. Milenković, et al., “Comparative Study of the Antimicrobial Activity of Selenium Nanoparticles With Different Surface Chemistry and Structure,” *Frontiers in Bioengineering and Biotechnology* 8 (2021): 624621, <https://doi.org/10.3389/fbioe.2020.624621>.
 42. M. V. Golyaev and E. G. Varlamova, “The Role of Selenium Nanoparticles in the Treatment of Liver Pathologies of Various Natures,” *International Journal of Molecular Sciences* 24 (2023): 10547, <https://doi.org/10.3390/ijms241310547>.
 43. J. A. Ansari, J. A. Malik, S. Ahmed, M. Manzoor, N. Ahemad, and S. Anwar, “Recent advances in the therapeutic applications of selenium nanoparticles,” *Molecular Biology Reports* 51 (2024): 688, <https://doi.org/10.1007/s11033-024-09598-z>.

44. S. Venugopal, "Therapeutic Potential of Selenium Nanoparticles," *Frontiers in Nanotechnology* 4 (2022): 1042338.
45. D. S. Shelar, G. S. Malankar, P. R. Singh, S. P. Vaidya, R. V. Pinjari, and S. T. Manjare, "Superoxide Targeted "Turn-On" Fluorescence Sensing Enabled by a Diselenide Based Quinoline Probe and Its In Vitro Anticancer Activity in Cancer Cells," *New Journal of Chemistry* 47 (2023): 6653–6660, <https://doi.org/10.1039/D2NJ06241K>.
46. D. S. Shelar, G. S. Malankar, S. S. Salunkhe, et al., "Synthesis and Characterization of Organoselenium Based BODIPY and Its Application in Living Cells," *Bioorganic Chemistry* 150 (2024): 107568, <https://doi.org/10.1016/j.bioorg.2024.107568>.
47. D. S. Shelar, P. P. Dhavan, P. R. Singh, B. L. Jadhav, S. P. Vaidya, and S. T. Manjare, "Synthesis and Study of Organoselenium Compound: DNA/Protein Interactions, In Vitro Antibacterial, Antioxidant, Anti-Inflammatory Activities and Anticancer Activity Against Carcinoma Cells," *Journal of Molecular Structure* 1244 (2021): 130914, <https://doi.org/10.1016/j.molstruc.2021.130914>.
48. I. Fabijanić, A. Kurutos, A. Tomašić Paić, et al., "Selenium-Substituted Monomethine Cyanine Dyes as Selective G-Quadruplex Spectroscopic Probes With Theranostic Potential," *Biomolecules* 13 (2023): 128, <https://doi.org/10.3390/biom13010128>.
49. O. C. Omiyale, M. Musa, A. I. Otuyalo, et al., "A Review on Selenium and Gold Nanoparticles Combined Photodynamic and Photothermal Prostate Cancer Tumors Ablation," *Discover Nano* 18 (2023): 150, <https://doi.org/10.1186/s11671-023-03936-z>.
50. E. G. Varlamova, S. V. Gudkov, E. V. Blinova, D. S. Blinov, and E. A. Turovsky, "Anticancer Signal Transduction Pathways of Selenium Nanoparticles in Mouse Colorectal Cancer Model," *Biochemical and Biophysical Research Communications* 769 (2025): 151962, <https://doi.org/10.1016/j.bbrc.2025.151962>.
51. S. E. Abo-Neima, A. A. Ahmed, M. El-Sheekh, and M. E. M. Makhlof, "Polycladial Myrica-Based Delivery of Selenium Nanoparticles in Combination With Radiotherapy Induces Potent In Vitro Antiviral and In Vivo Anticancer Activities Against Ehrlich Ascites Tumor," *Frontiers in Molecular Biosciences* 10 (2023): 1120422, <https://doi.org/10.3389/fmolb.2023.1120422>.
52. W. Liu, Y. Hou, W. Liu, et al., "Se-Substituted Pentamethine Cyanine for Anticancer Photodynamic Therapy Mediated Using the Hot Band Absorption Process," *Chinese Chemical Letters* 35 (2024): 109631, <https://doi.org/10.1016/j.ccllet.2024.109631>.
53. X. Zhao, J. Du, W. Sun, J. Fan, and X. Peng, "Regulating Charge Transfer in Cyanine Dyes: A Universal Methodology for Enhancing Cancer Phototherapeutic Efficacy," *Accounts of Chemical Research* 57 (2024): 2582–2593, <https://doi.org/10.1021/acs.accounts.4c00399>.
54. C. Ferro, H. F. Florindo, and H. A. Santos, "Selenium Nanoparticles for Biomedical Applications: From Development and Characterization to Therapeutics," *Advanced Healthcare Materials* 10 (2021): 2100598, <https://doi.org/10.1002/adhm.202100598>.
55. W. Li, X. Lu, L. Jiang, and X. Wang, "Biosafety and Pharmacokinetic Characteristics of Polyethylene Pyrrolidone Modified Nano Selenium in Rats," *BMC Biotechnology* 24 (2024): 98, <https://doi.org/10.1186/s12896-024-00915-9>.
56. S. Mrdenovic, "Development of Small Molecule Fluorescent Dye Drug Conjugates in Targeted Cancer Therapy," *American Journal of Clinical and Experimental Urology* 9 (2021): 261.
57. Y. Xu, H. Wu, J. Huang, et al., "Probing and Enhancing Ligand-Mediated Active Targeting of Tumors Using Sub-5 nm Ultrafine Iron Oxide Nanoparticles," *Theranostics* 10 (2020): 2479–2494, <https://doi.org/10.7150/thno.39560>.
58. E. F. Pettersen, T. D. Goddard, C. C. Huang, et al., "UCSF ChimeraX: Structure Visualization for Researchers, Educators, and Developers," *Protein Science* 30 (2021): 70–82, <https://doi.org/10.1002/pro.3943>.
59. J. P. Fuenzalida Werner, Y. Huang, K. Mishra, et al., "Challenging a Preconception: Optoacoustic Spectrum Differs From the Optical Absorption Spectrum of Proteins and Dyes for Molecular Imaging," *Analytical Chemistry* 92 (2020): 10717–10724, <https://doi.org/10.1021/acs.analchem.0c01902>.
60. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy* (Springer, 2006), <https://doi.org/10.1007/978-0-387-46312-4>.
61. Y. Liu, L. Yuan, W. Chi, et al., "Cairo Pentagon Tessellated Covalent Organic Frameworks With Mcm Topology for Near-Infrared Phototherapy," *Nature Communications* 15 (2024): 7150.
62. Z. Jiang, C. Zhang, X. Wang, et al., "A Borondifluoride-Complex-Based Photothermal Agent with an 80% Photothermal Conversion Efficiency for Photothermal Therapy in the NIR-II Window," *Angewandte Chemie International Edition* 60 (2021): 22376–22384.
63. D. K. Roper, W. Ahn, and M. Hoepfner, "Microscale Heat Transfer Transduced by Surface Plasmon Resonant Gold Nanoparticles," *The Journal of Physical Chemistry C* 111 (2007): 3636–3641, <https://doi.org/10.1021/jp064341w>.
64. T. Yang, L. Liu, Y. Deng, et al., "Ultrastable Near-Infrared Conjugated-Polymer Nanoparticles for Dually Photoactive Tumor Inhibition," *Advanced Materials* 29 (2017): 1700487, <https://doi.org/10.1002/adma.201700487>.
65. S. Huang, H. He, R. Z. Tom, et al., "Non-Invasive Optoacoustic Imaging of Dermal Microcirculatory Revascularization in Diet-Induced Obese Mice Undergoing Exercise Intervention," *Photoacoustics* 38 (2024): 100628, <https://doi.org/10.1016/j.pacs.2024.100628>.
66. N. Liu, V. Gujrati, J. Malekzadeh-Najafabadi, et al., "Croconaine-Based Nanoparticles Enable Efficient Optoacoustic Imaging of Murine Brain Tumors," *Photoacoustics* 22 (2021): 100263, <https://doi.org/10.1016/j.pacs.2021.100263>.
67. B. Rathkolb, W. Hans, C. Prehn, et al., "Clinical Chemistry and Other Laboratory Tests on Mouse Plasma or Serum," *Current Protocols in Mouse Biology* 3 (2013): 69–100, <https://doi.org/10.1002/9780470942390.mol30043>.

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