ORIGINAL RESEARCH ARTICLE

Fluorescence imaging-guided photothermal therapy of asymmetric water-soluble pentamethine cyanine for colorectal cancer

Kun Tang1†, Shuangshuang Jia1†, Yaxin Zou1†, Jiaheng Dong1, Fangyan Liu2, Kunli Cui1, Xiao Shi3, and Lei Zhang1*

1Henan International Joint Laboratory for Nuclear Protein Regulation, School of Basic Medical Sciences, Henan University, Kaifeng 475004, P. R. China
2Department of Academician Shengshou Hu Workstation, Fuwai Central China Cardiovascular Hospital, Zhengzhou 450046, P. R. China
3Department of Pharmacy, The First Affiliated Hospital of Henan University, Henan University, Kaifeng 475004, P. R. China

Abstract

Fluorescence imaging analysis showed that CY5-664 preferentially accumulated in the tumor as time elapsed, and the maximum fluorescence intensity was obtained at 24 h. Cell viability and animal xenograft model experiments showed that CY5-664 significantly inhibited tumor growth in vivo with 0.8 W/cm² of 660 nm red laser pointer for 2 min, and in vitro with 0.5 W/cm² of 660 nm red laser pointer for 2 min. In vitro and in vivo photothermal conversion experiments showed that CY5-664 exhibited an anti-CRC activity mainly through the PTT effect. Therefore, this work provides experimental evidence that CY5-664 is a drug candidate for CRC treatment mainly through the PTT effect and an alternative treatment strategy to develop a pentamethine cyanine-based CRC theranostic photosensitizer for synergistic CRC targeting, imaging, and therapy.

Keywords: Pentamethine cyanine; Photothermal therapy; Fluorescence imaging; Near-infrared; Colorectal cancer

1. Introduction

Colorectal cancer (CRC), a common clinical malignant tumor of the digestive tract, is seriously threatening human health due to the high incidence and mortality rates[1]. The occurrence and development of CRC are usually related to diet changes and environmental factors[2]. According to the statistics on cancer in 185 countries in 2018,
CRC incidence rate and mortality rate are among the top three in the world\(^2\). Surgical treatment, radiotherapy, and chemotherapy are the principal clinical treatment methods of CRC\(^3\). However, the common chemotherapeutic drugs, such as fluorouracil and oxaliplatin, have predictable hepatotoxicity\(^4\). Therefore, it is urgent to develop an effective and low-toxicity drug for CRC treatment.

The combination of advanced optical technology and tumor treatment provides non-invasive in vivo imaging and treatment (optical diagnosis and therapy) employing non-ionizing radiation, which brings new understanding and technological innovation to biomedicine, and is expected to achieve precise treatment of tumors\(^5\). Tumor phototherapy is a method of treating cancer with specific wavelength excitation photosensitizer, including photothermal therapy (PTT) and photodynamic therapy (PDT)\(^6\). PTT, based on the theory concerning the absorbed light for heat conversion, has tremendous potential for efficacious cancer therapy, compared with traditional treatment modalities such as surgery, radiation therapy, and chemical therapy, due to the non-invasive therapeutic intervention and precise spatial-temporal selectivity\(^7\). PDT is another new strategy for cancer therapy, accompanied by photosensitizers and laser activation. The laser energy is transferred to the surrounding oxygen by photosensitizer and highly active singlet oxygen is produced to kill cancer cells\(^8\). However, the low oxygen content of the tumor microenvironment limits the extensive application of oxygen-dependent PDT\(^9\). Compared with oxygen-dependent PDT, PTT is oxygen-independent and, therefore, may serve as a more promising cancer therapy approach. PTT photosensitizer effectively converts the laser energy into thermal energy through absorbing energy in the near-infrared (NIR) region and taking advantage of the sensitivity of cancer cells toward heat to induce hyperthermia and promote cancer cell necrosis and apoptosis\(^10\).

Tremendous efforts have been made to obtain high efficiency and low-toxicity PTT materials. Although a large number of polymeric and inorganic nanomaterials have been studied in the application of PTT, such as gold nanoparticles\(^11\), metal sulfide nanoparticles\(^12\), and carbon nanomaterials\(^13\), there are still tremendous challenges to clinically apply these PTT materials due to the complex synthetic process, the raised concerns regarding biocompatibility, and the unsolved biosafety problem\(^14,15\). In contrast, NIR heptamethine cyanine fluorophore such as indocyanine green (ICG) recently received increasing attention in PTT because of excellent biocompatibility, non-immunogenicity, high fluorescence and photothermal conversion efficiency, photostability, tumor-homing, and commercial-scale production\(^16,17\). Up to the present, ICG was the only contrast medium authorized by the U.S. Food and Drug Administration. Therefore, the development of new photosensitizers based on the intrinsic multifunctional characteristics of their chemical structure, which do not rely on nanopolymer materials and do not need complex chemical connections, significantly improved the potential of clinical transformation and application, and promoted the prospects of tumor optical therapy.

In the present study, recognizing the potential of cyanine fluorophore, a unique water-soluble pentamethine cyanine-derivative, 1-(4-carboxybenzyl)-2,3,3-trimethylindolenium-5-sulfonate bromide (CY5-664), was designed and synthesized to investigate the photosensitive activity and to explore its potential applications as NIR photosensitizer for CRC diagnosis and therapy. An excellent molar extinction coefficient, a relatively higher quantum yield, and good photostability are exhibited in CY5-664 aqueous solutions. CY5-664 inhibits cell viability and exhibits excellent PTT activity in two types of CRC cell lines in a dose-dependent manner under NIR laser irradiation. Moreover, it is retained preferentially in tumors and significantly inhibits tumor growth in the BALB/c nude mice xenografts bearing DLD-1 cells under NIR laser irradiation with negligible organ toxicity. Therefore, for the 1st time, we found the novel small molecular derivative of cyanine CY5-664 against CRC, which may be high efficiency and low-toxicity PTT material for image-guided CRC therapy in clinical application.

2. Materials and methods

2.1. Chemicals and reagents

CY5-664 was synthesized according to the predetermined synthetic route, and the synthetic routes were shown in Figure 1A. \(^1\)H NMR spectra were recorded on a Bruker AMX-400 spectrometer (400 MHz; Bruker, U.S.A.). Mass spectral analysis was performed using an MALDI-TOF mass spectrometer (Applied Biosystems/MDS Sciei, Foster City, CA, U.S.A.). Fluorescence measurement was performed using a Cary Eclipse spectrofluorimeter (Agilent Technologies, U.S.A.) with 5 nm excitation and emission slit widths. Absorption spectra were measured using a Jasco V-550 spectrophotometer (JASCO International Co., Japan). All reagents used for the experiments were of analytical grade. HSP70 and β-actin antibodies were purchased from Servicebio Co. Ltd. (Wuhan, China). MTT assay kit was purchased from Solarbio Co. Ltd. (Beijing, China). Singlet oxygen sensor green (SOSG) detection kit was purchased from Maokangbio Co. Ltd. (Shanghai, China).

2.2. Cell lines and cell culture

As previously described\(^18\), human CRC cell lines such as HCT-116 and SW480 were obtained from the cell culture
collection committee of the Chinese Academy of Sciences library (Shanghai, China). The cells were cultured in RPMI 1640 (Gibco, 11875119) medium with 10% heat-inactivated fetal bovine serum (Gibco, 16000-044), 100 U/mL penicillin and 100 μg/mL streptomycin (Solarbio, P1400), and maintained in 5% CO₂ at 37°C.

2.3. Cell viability assay
As previously described[24], HCT-116 and SW480 cells were seeded in 96-well plates at a density of 5 × 10³ cells per well and cultured for 12 h. CY5-664 was added into culture plates according to the different predetermined concentrations, and the cells were cultured continuously for 2 h. Cells were irradiated for 2 min (660 nm, 0.5 W/cm²), and the cells were cultured continuously for 24 h. The cell viability was determined by the MTT method.

2.4. In vivo experiments
In vivo experiments of this study were approved by the Medical and Scientific Research Ethics Committee of Henan University School of Basic Medical Sciences. As previously described[25], the mice used in this study were 5-week-old female BALB/c nude mice, which were purchased from Beijing Weitonglihua Experimental Animal Technical Co., Ltd (Beijing, China). Human CRC cell HCT-116 was harvested and suspended in normal saline (NS), and was injected subcutaneously into nude mice at a concentration of 10⁶ cells per nude mouse. The tumor volume was estimated according to the following formula: L (the long diameter) × W (the short diameter) × W × 1/2. Nude mice were randomly divided into three groups: (1) NS + Laser, (2) CY5-664 treated only, and (3) CY5-664 + Laser, under laser irradiation (660 nm, 0.5 W/cm²) for 2 min, until the tumor size reached approximately 50 mm³. The CY5-664 treatment group mice were injected with CY5-664 at a concentration of 1 mg/kg, and NS control group mice were injected with an equal volume of NS through tail vein.

2.5. In vivo NIR fluorescence imaging
In vivo NIR fluorescence imaging was observed using an optical imaging system for small animals (IVIS lumina III, Perkinelmer, U.S.A.) at different predetermined time points after injection of CY5-664 through tail vein. Nude mice were dissected and pivotal organs and tissues, such as heart, liver, spleen, lung, kidney, and tumor, were obtained at 24 h after injection with CY5-664 through tail vein for further ex vivo biodistribution assessment. The excitation wavelength is 650 nm and the emission wavelength is 670 nm in NIR fluorescence imaging analysis.
2.6. Western blot analysis

The expression of HSP70 was detected by Western blotting according to the standard experimental schemes. As previously described[26], human CRC cells HCT-116 and SW480 were collected and lysed with RIPA buffer in the presence of protease inhibitors. The protein was quantified and electrophoresed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. After electrophoresis, the protein was transferred to the polyvinylidene fluoride (PVDF) membrane from the gel using the electrospinning method. The PVDF membrane was sealed with bovine serum albumin and incubated with the primary antibody and the secondary antibody. Finally, the blot determination was detected using a chemiluminescence analyzer (Amerisham Biosciences, Boston, MA, U.S.A.).

2.7. Singlet oxygen detection analysis

The singlet oxygen generation from CY5-664 was determined by the fluorescent probe SOSG. In brief, 1.0 × 10^{-5} M CY5-664 and 1.5 × 10^{-6} M SOSG were mixed in an aqueous solution containing 2% methanol and were irradiated with 0.5 W of 660 nm red laser pointer for 2 min at room temperature. The mixed solution was quickly transferred to the cuvette after laser irradiation and the fluorescence intensity was determined using a steady-state transient fluorescence spectrometer. The excitation wavelength is 494 nm and the emission peak at 530 nm was used to evaluate singlet oxygen generation.

2.8. In vitro assessment of photothermal effect

The photothermal effect of CY5-664 was determined using a precise thermal imaging instrument in a culture medium. In brief, different concentrations CY5-664 solutions were prepared according to the predetermined concentrations and were irradiated with 0.5 W of 660 nm red laser pointer for 2 min. The temperature of CY5-664 solutions was monitored by a precise thermal imaging instrument (Fotric 225S#L24, Shanghai, China) every 30 s.

2.9. In vivo photothermal analysis

BALB/c nude mice bearing human CRC cell HCT-116 were injected with NS or CY5-664 through tail vein. At 24 h after the injection of CY5-664, the tumor was irradiated by laser (660 nm, 0.5 W/cm²) for 2 min, and the local temperature of the tumor was monitored by a precise thermal imaging instrument (Fotric 225S#L24, Shanghai, China) every 30 s.

2.10. Hematoxylin & eosin (H&E) staining analysis

As previously described[27,28], the tissues from the animal experiments were fixed in 4% paraformaldehyde fixative and embedded in paraffin. Formalin-fixed paraffin-embedded samples were sliced and stained with hematoxylin for 5 min and eosin for 1 min. Morphological changes of the tumor tissues were observed with an electronic light microscope (Olympus, Tokyo, Japan).

2.11. Cell uptake assay

As previously described[29], HCT-116 and SW480 cells were seeded in 24-well plates at a density of 5 × 10^4 cells per well and cultured for 24 h. Different concentrations of CY5-664 were added into culture plates, and the cells were cultured continuously for 2 h. The cells were washed 3 times with phosphate-buffered solution and detected using a fluorescence inverted microscope (DMI8, Leica, Germany).

2.12. PTT by CY5-664

HCT-116 and SW480 cells were seeded in 96-well plates at a density of 5 × 10^4 cells per well and cultured for 12 h. Different concentrations of CY5-664 were added to culture plates, and the cells were cultured continuously for 2 h. Cells were irradiated for 2 min (660 nm, 0.5 W/cm²) with or without ice incubation. The cell viability was determined by MTT method.

2.13. Statistical analysis

All statistical analyses were completed by SPSS16.0. The statistical difference between the treatment and control groups of IMCA was analyzed by Student's t-test.

3. Results and discussion

3.1. Synthesis and characterization of CY5-664

The synthesis routes are shown in Figure 1A. 2,3,3-trimethylindoleninium-5-sulfoacid (1), 1-bromomethyl-3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)benzene (3), and 1-(4-carboxybenzyl)-2,3,3-trimethylindoleninium-5-sulfonate bromide (5) were synthesized according to the literature.[30,31]

A mixture of 2,3,3-trimethylindoleninium-5-sulfoacid 1 (0.8 g, 3.34 mmol) and potassium acetate (0.36 g, 3.67 mmol) was stirred in 30 mL of methanol at room temperature for 0.5 h. After removal of methanol under vacuum, the resulting potassium salt 2 was heated with 1-romomethyl-3,5-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)ethoxy)benzene 3 (1.65 g, 3.33 mmol) in acetonitrile at 90°C for 24 h under argon. The mixture was cooled to room temperature and the solvent was evaporated. The pure product 4 (1.11 g) was obtained in 51% yield by the purification on silica gel column with CHCl₃/CH₃OH (15:1, V/V) as eluent and the ‘H NMR spectrum is shown in Figure S1 (in Supplementary File).

The intermediate 4 (374.2 mg, 0.57 mmol) and malonaldehyde dianilide hydrochloride
(296.2 mg, 1.14 mmol) in a mixture of acetic anhydride (4 mL) and acetic acid (4 mL) were heated at 110°C for 1 h under argon. The intermediate 5 (638.8 mg, 1.71 mmol) was added, and then the mixture was heated at 110°C for another 3 h under argon. The blue solution was poured into 100 mL of ethyl acetate. The precipitate was filtered and washed with ethyl acetate. The pure product (181.4 mg) was obtained in 30% yield by the purification using reversed-phase (C18) chromatography with CH₃CN/CH₃OH (3:1, V/V) as eluent. The structural spectrum of CY5-664 is shown in Figures S2-S4.

Photofading experiments were carried out in the quartz cuvette (3.5 mL volume) containing a 5×10⁻⁶ M solution of the dye in ultrapure water, where the sample solution was irradiated with 0.5 W of 660 nm red laser pointer at room temperature. UV-vis spectra were recorded at 2-min intervals for 20 min. The irreversible bleaching of the dye at the absorption peak was monitored as a function of time. The highest absorption spectrum of CY5-664 was 646 nm and 652 nm, and the fluorescence peak was 664 nm and 670 nm in phosphate-buffered solution and dimethyl sulfoxide (DMSO) solution, respectively (Figure 1B). CY5-664 has a substantial absorption at 660 nm, which leads to photothermal conversion and fluorescence imaging. The absorption intensity decreases with the increase of laser irradiation (660 nm, 0.5 W/cm²) time, but the light absorption was not reduced significantly after 2 min of irradiation (Figure 1C). These results showed that CY5-664 remained stable after irradiation for 2 min.

3.2. Photoinduced cytotoxicity of CY5-664

Low toxicity under dark conditions and high toxicity under laser irradiation are the noteworthy characteristics of phototherapy[32]. To evaluate the photoinduced cytotoxicity cancer specificity of CY5-664, cell viability was determined with or without CY5-664 treatment and 660 nm laser irradiation on HCT116 and SW480 cells. MTT was used as an indicator of cell viability. The results showed that different concentrations of CY5-664 did not significantly inhibit the viability of HCT116 and SW480 cells without laser irradiation, and CY5-664 significantly inhibited the viability of the CRC cells in a dose-dependent manner. However, under laser irradiation, the viability of HCT116 and SW480 cells was significantly decreased with the increased concentration of CY5-664 (0–25 μM) (Figure 2). These results confirmed the photoinduced cytotoxicity of CY5-664 in a dose-dependent manner.

3.3. Imaging, phototherapy, and in vivo toxicity evaluation

To demonstrate the tumor-targeting properties and the tumor imaging capability of CY5-664 in vivo, BALB/c nude mice bearing human CRC cell HCT-116 xenografts model were established as described previously[33]. CY5-664 was injected into the nude mice bearing HCT-116 tumor xenografts at a dose of 1 mg/kg through the tail vein and the NIR fluorescent imaging was carried out from 30 min to 96 h after injection using a non-invasive imaging system[34]. The results of fluorescence imaging analysis showed that CY5-664 preferentially accumulated in the tumor as time elapsed, and the maximum fluorescence intensity was obtained at 24 h (Figure 3A). To further confirm the tumor-targeted accumulation of the compounds, nude mice were dissected and pivotal organs and tissues, such as heart, liver, spleen, lung, kidney, and tumor, and were obtained at 24 h after injection with CY5-664 through tail vein[34]. The obtained organs and tissues were analyzed by a fluorescence imaging system. The results of fluorescence imaging analysis showed that CY5-664 was confirmed with significant tumor preferential accumulation by ex vivo imaging of the dissected organs and tissues (Figure 3B).

To evaluate the antitumor activity of CY5-664 in vivo, HCT116 subcutaneous tumor xenograft models were established, and the tumor-bearing nude mice were randomly divided into three groups: (1) NS + Laser, (2) CY5-664 treated only, and (3) CY5-664 + Laser. HCT116 subcutaneous tumor xenograft models were established on both shoulders of each mouse in the CY5-664 treatment group. The tumor on one shoulder was irradiated by laser, while the tumor on the other shoulder was not irradiated by laser. The tumor in NS group mice was irradiated by laser. According to the results of NIR fluorescence imaging described above, tumors in laser irradiation group were irradiated with 660 nm laser for 2 min (0.8 W/cm²) at 24 h after injection. The results are shown in Figure 3C-E. Compared with the NS group, tumor growth was significantly retarded in the CY5-664 + Laser irradiation group. Similar results were further observed by means of tumor imaging and tumor weight analysis in the CY5-664 + Laser irradiation group. The antitumor effect of CY5-664 was further evaluated using H&E staining of tumor tissue sections. As shown in Figure 3F-H, all of the tumor cells in the NS-treated group with laser irradiation and CY5-664-treated group without laser irradiation displayed deeper hematoxylin staining, larger nuclei, complete morphology, and vigorous division. In contrast, the tumor cells in the CY5-664 + Laser irradiation group showed lighter hematoxylin staining, damaged nuclear integrity, and shrunk chromatin. These results showed that CY5-664 significantly inhibits tumor growth with laser irradiation in vivo.

To evaluate the toxic side effects of CY5-664 in vivo, the weight of nude mice was monitored in real time during the treatment. As displayed in Figure 3I, compared with the
control group, there was no significant difference in body weight during the whole observation period. Liver and kidney functions were further determined by biochemical tests of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cre), and blood urea nitrogen (BUN). As shown in Figure 3J-M, there was no significant difference in these indicators between the CY5-664 + Laser irradiation group and the control group. To further evaluate the effects of CY5-664 on the main organs, we carried out the organ index and H&E staining analysis of the main organ sections, such as the heart, liver, spleen, lung, and kidney. The results showed that no significant changes in organ index and abnormal histomorphology were observed (Figure 3N and S). Overall, the above results indicated that tumor-targeted CY5-664 and imaging-guided phototherapy were highly biocompatible and low in toxic side effect.

3.4. The mechanism of CY5-664 phototherapy

Photodependent cancer therapy, including PTT and PDT, has emerged as a local and non-invasive therapeutic method for cancer therapy in recent years[35]. For PTT, light energy is absorbed and converted into hyperthermia by a photosensitizer, achieving cancer therapeutic activity[36]. The photothermal activities of CY5-664 were evaluated at three levels: Medium, intracellular, and nude mice. Excitedly, the temperature of the media containing different concentrations of CY5-664 increased rapidly, and the highest temperature reached 68.9°C in the media containing 50 μM CY5-664 under laser irradiation (660 nm, 0.5 W/cm², 2 min) (Figure 4A). To further evaluate the effect of photothermal treatment of CY5-664 on cell viability, cell viability was determined by the MTT method with or without ice incubation for different concentrations of CY5-664 under laser irradiation (660 nm, 0.5 W/cm², 2 min). The results showed that ice significantly reversed the inhibition of CY5-664 on cell viability under laser irradiation (Figure 4B and C). Heat shock protein 70 (Hsp70) is a member of the most ancient and universal defense systems, which can be synthesized under heat stress to achieve the purpose of cell protection[37]. To study the photothermal effect of CY5-664, the expression of HSP70 was determined using Western blotting in HCT116 and SW480 cell lines. The results showed that the expression of HSP70 had been significantly upregulated (Figure 4D). Based on the phototherapeutic efficacy of CY5-664 in vitro, in vivo phototherapeutic activity of CY5-664 was initially evaluated in HCT-116 subcutaneous
Figure 3. The biodistribution, tumor inhibition and side effects of CY5-664 in vivo. (A) The fluorescence imaging at different times after injection of CY5-664. (B) The fluorescence imaging of main organs and tumor at 24 h after injection. (C) The change of tumor volume during the observation period. (D) Photos of the tumor after treatment. (E) Tumor quality after treatment. (F–H) The morphological characteristics of tumor tissue, NS + Laser (F), CY5-664 (G), and CY5-664 + Laser (H). (I) The change of body weight during the observation period. (J) The change of ALT in the liver. (K) The change of AST in the liver. (L) The change of Cre in the kidney. (M) The change of BUN in the kidney. (N) The morphological characteristics of main organs with or without CY5-664 treatment. (O–S) The change of main organs. *P < 0.05; **P < 0.01; ***P < 0.001.
Photothermal therapy of pentamethine cyanine tumor-bearing xenograft models under the guidance of photothermal imaging. CY5-664 was injected into the tumor-bearing nude mice through the tail vein at 24 h before the laser and then the tumor was irradiated for 2 min using a 660 nm laser (0.8 W/cm$^2$). Meanwhile, the surface temperature of the tumor is monitored every 30 s using a thermal camera. As shown in Figure 4F and G, the surface temperature of the tumor rose rapidly and reached 60°C at 2 min after laser irradiation in CY5-664 treatment group mice. However, the surface temperature of the tumor only slightly increased to 43°C in NS groups mice. In summary, the antitumor activities of CY5-664 were closely related to the strong photothermal activities.

For PDT, light energy is absorbed and converted into reactive oxygen species by a photosensitizer, achieving cancer therapeutic activity$^{[38-40]}$. Based on the photothermal property of CY5-664 in vitro and in vivo, the photodynamic activities of CY5-664 were further determined by measuring the singlet oxygen production with SOSG. The results showed that singlet oxygen production was significantly induced by CY5-664 under laser irradiation (Figure 4H). To further evaluate the intracellular photodynamic property of CY5-664, cell viability was determined by the MTT method with or without antioxidant N-acetyl-L-cysteine (NAC) for different concentrations of CY5-664 under laser irradiation (660 nm, 0.5 W/cm$^2$, 2 min). However, as shown in Figure 4I and J, the inhibition of HCT-116 and SW480 cell viability induced by CY5-664 under laser irradiation was not rescued by NAC. The cellular uptake of CY5-664 was further determined by fluorescence microscope analysis at 2 h post-treatment of CY5-664. As shown in Figure S5, CY5-664 was not uptaken by HCT116 and SW480 cells. These results showed that CY5-664 did...
not induce colorectal cell death through the photodynamic effect.

4. Conclusions
In summary, an asymmetric, water-soluble pentamethine cyanine derivative CY5-664 with integrated imaging, targeting, and therapeutic properties has been found in this work. Significant anti-CRC activity of CY5-664 was observed by the multifunctional photosensitizer through cell viability and animal xenograft model experiments. This multifunctional photosensitizer CY5-664 may have great application prospects as a safe and effective reagent for treating CRC because of the following advantages. First, CY5-664 preferentially accumulates in the tumor as time elapsed, and the maximum fluorescence intensity was observed at 24 h. Second, CY5-664 has good water solubility and can be dissolved in a cell culture medium or NS without DMSO. Third, CY5-664 can be imaged \textit{in vivo}, which is conducive to its application in imaging-guided photosensitve therapy. Finally, CY5-664 exhibits significant PTT effects for CRC therapy without significant side effects. In brief, this study not only found a multifunctional photosensitizer CY5-664, but also provided an alternative treatment strategy to develop pentamethine cyanine-based CRC theranostic photosensitizer for synergistic CRC targeting, imaging, and therapy.

Acknowledgments
None.

Funding
This work was financially supported by the National Natural Science Foundation of China (No. 81803573), China Postdoctoral Science Foundation (No. 2018M640672), and Key R&D and Promotion Projects in Henan Province (No. 222102310013).

Conflict of interest
The authors declare no conflict of interest.

Author contributions
Conceptualization: Kun Tang, Shuangshuang Jia, Yaxin Zou, Jiaheng Dong, and Lei Zhang
Formal analysis: Kun Tang
Investigation: Shuangshuang Jia, Yaxin Zou, Jiaheng Dong, Fangyan Liu, Kunli Cui, and Xiao Shi
Methodology: Kun Tang, Shuangshuang Jia, Yaxin Zou, Jiaheng Dong, and Lei Zhang
Writing – original draft: Kun Tang
Writing – review & editing: Lei Zhang

All the authors read and agreed to publish the version of this study.

Availability of data and materials
Samples of the compounds are available from the authors.

References
   \url{https://doi.org/10.1200/JCO.2014.59.7633}
   \url{https://doi.org/10.1038/nrdp.2015.65}
   \url{https://doi.org/10.3322/caac.21492}
   \url{https://doi.org/10.1016/S0140-6736(19)32319-0}
   \url{https://doi.org/10.1001/jamaoncol.2017.0278}
   \url{https://doi.org/10.1093/annonc/mds074}
   \url{https://doi.org/10.1021/acs.chemrev.7b00258}
   \url{https://doi.org/10.1002/bjs.9713}
   \url{https://doi.org/10.1039/c6cs00458j}
   \url{https://doi.org/10.1002/smll.201804105}
   \url{https://doi.org/10.1039/c7cs00522a}


https://doi.org/10.1111/jipb.12654

https://doi.org/10.1111/nph.12565

https://doi.org/10.3390/molecules23030565


https://doi.org/10.1016/j.fsi.2018.06.004

https://doi.org/10.1111/j.1439-0264.2010.01033.x


https://doi.org/10.1038/srep44743