Supporting Information

A photo-activated aptamer-drug conjugate for targeted drug delivery

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Materials and instruments

L-propargylglycine was purchased from Shanghai Minrell Chemical Reagent Co., Ltd. Sodium ascorbate was purchased from J&K Scientific Ltd. Tris-(benzyltriazolylmethyl) amine (TBTA) was purchased from Tokyo Chemical Industry Ltd. Column chromatography silica gel powder (100-200 mesh) was purchased from Qingdao Ocean Chemical Factory. Lysosomal probes (LysTracker Blue, LysBlue) was purchased from Shanghai Biyuntian Biotechnology Ltd. Hoechst 33342 and Counting Kit-8 (CCK-8) were purchased from Jiangsu KeyGEN BioTECH Corp., Ltd. RPMI-1640 medium, fetal bovine serum (FBS), penicillin/streptomycin (PS), and phosphate buffered saline (PBS) were purchased from Gibco. Other common reagents were purchased from Beijing Chemical Plant. HeLa (cervical cancer), PC-3 (prostate cancer), and LoVo (colon cancer) cell lines were purchased from Typical Culture Preservation Commission Cell Bank, Chinese Academy of Sciences (Shanghai, China). A549T (Taxol-resistant A549 subline) cell line was purchased from Shanghai Aiyan Biological Technology Ltd. (Shanghai, China).

Electrosprayionization-mass spectrometry (ESI-MS) was recorded on an LC-MS 2010A system (Shimadzu). ESI-MS for oligonucleotides was recorded on a subsequent linear ion-trap MS (LITMS, Thermo). Fluorescence spectra were collected on a F-4600 fluorescent spectrophotometer (Hitachi). Absorption spectra were recorded on a UH5300 spectrophotometer (Hitachi). High performance liquid chromatography (HPLC) were recorded on a LC-20A system (Shimadzu). Fluorescence images were recorded on an FV3000-IX83 confocal microscope (Olympus). The absorbance for Cell Counting Kit-8 (CCK-8) analysis were recorded on a SpectraMax M2e Reader (Molecular Devices). Flow cytometry analysis and cell apoptosis experiments were completed on the flow cytometer (FACScalibur, Becton Dickinson, USA).

Oligonucleotide sequences

The oligonucleotides used in this study were purchased from Sangon Biotech (Shanghai) Ltd., and the sequences were shown in Table S1.

Table S1 Oligonucleotide sequences used in the experiment

Name	Sequence (from 5’ to 3’)
HG1-9	TGGATAGGGATTCTGTTGGTCGGCTGGTTGGTATCC
ctr sq	AGAGCAGCGTGGAGGATAGTTGGGGTTTGGCAAGTATTG

Experimental section

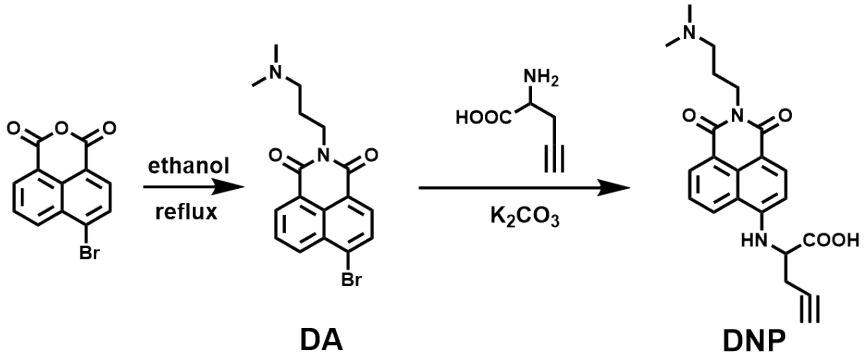
Synthesis of DNP (Scheme S1)

3.5 g (12.6 mmol) of 4-bromo-1,8 naphthalene dianhydride was mixed with 300 mL of ethanol and heated to reflux. After refluxing for 10 min, cool to 50°C, and slowly add 1.7 mL (13.3 mmol) of dimethyl-1,3-diaminopropane. The mixed solution was heated to reflux for 1 h, then cooled to room temperature, and a large amount of water was added. The obtained precipitate was filtered and

washed with water and ethanol for 3 times each, and then dried under vacuum to obtain a white solid (**DA**). MS (ESI): m/z calcd for $C_{17}H_{17}BrN_2O_2$ $[M+H]^+$: 361.05; found: 361.10.

250 mg (0.7 mmol) of **DA** and 242 mg (1.75 mmol) of K_2CO_3 were mixed in 5 mL of DMSO. The mixture was stirred magnetically at 50 ℃ for 30 min, then 200 mg (1.75 mmol) of L-propargylglycine added. After reacted at 85 ℃ for 8 h, the reaction solution was cooled to room temperature, and the solvent was removed by vacuum spin-drying. The product was purified by column chromatography using dichloromethane/methanol/triethylamine (V/V/V, 10:1:0.1) to obtain product **DNP**. MS (ESI, Fig. S19): m/z calcd for $C_{22}H_{23}N_3O_4$ [M]: 393.4; found$[M-H]^-$: 392.2.

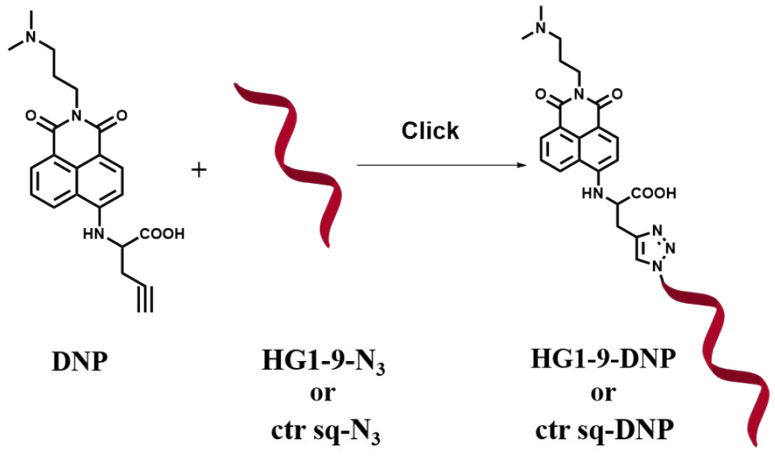
Scheme S1 Synthesis route of DNP.



Preparation of HG1-9-DNP and ctr sq-DNP by click reaction

The click reaction of DNP with HG1-9-N_3 or ctr sq-N_3 were shown in Scheme S2. TBTA-Cu^+ mixture was prepared by mixed 8 μL of TBTA (58 mg/mL) and 4 μL of CuCl (15 mg/mL) firstly. 2.0 OD of HG1-9-N_3 or ctr sq-N_3 dissolved in 30 μL H_2O, and then added 30 μL of DNP solution (10 mM), 11 μL of TBTA-Cu^+ mixture, and 11 μL of VcNa solution (20 mg/mL). The above mixture was shaken and reacted at room temperature for 30 min to get the HG1-9-DNP or ctr sq-DNP. Then the product was purified by HPLC (yield, 63.7%). MS (ESI): m/z calcd. for HG1-G-DNP: 11884.0, found: 11885.2 (Fig. S1); ctr sq-DNP: 12945.5, found: 12948.0 (Fig. S2).

Scheme S2 Schematic diagram of the click reaction of HG1-9-N_3 or ctr sq-N_3 with DNP



HPLC analysis of photolysis process

The photolysis of HG1-9-DNP under visible light irradiation was studied by HPLC. A blue LED light (465- 470 nm, 7.96 mW/cm^2) was used to irradiate the HG1-9-DNP solution for 10 min, and then the soulutions before and after light irradiation were analyzed by HPLC. The stationary phase used was a C18 reversed phase chromatography column (Promosil C18, 5 μm, 4.6×250 mm, Agela Technologies). Mobile phase A: acetonitrile; mobile phase B: 0.1% (V/V) trifluoroacetic acid (TFA) in water. The products were separated by gradient elution at a flow rate of 1.0 mL/min.

Cell culture and imaging

The cells were routinely cultured in a humidified sterile incubator with a volume fraction of CO_2 of 5% at 37°C. The medium used was RPMI-1640 medium, supplemented with 10% FBS and 1% PS. Before using, HG1-9-DNP and ctr sq-DNP solutions were denatured at 95°C for 5 min, cooled on ice for 10 min, and renatured at room temperature for 30 min. Before imaging, the cells were seeded in confocal culture dishes with a diameter of 15 mm at a density of 3×10^5 cells/dish and cultured for 24 h. Then the cells were incubated with 500 nM HG1-9-DNP or ctr sq-DNP in binding buffer (PBS solution containing 4.5 g/L glucose, 5 mM $MgCl_2$, 1 mg/mL BSA, 0.1 mg/mL herring sperm DNA) at 4 °C for 60 min, and incubated in RPMI-1640 medium at 37 °C for 60 min, respectively. After washed 3 times with PBS (pH=7.4), the confocal images were performed (E_x: 445 nm, E_m: 490-540 nm).

In order to study the effect of photolysis, the cells seeded in confocal culture dishes were incubated with 500 nM HG1-9-DNP in fresh medium and irradiated for 60 min (465-470 nm, 11.15 mW/cm^2), and then incubated for different time. After irradiation, cells were directly cultured in the dark without washing. Before imaging, the cells were washed 3 times with PBS.

In order to study the co-localization of HG1-9-DNP in cells, the cells were incubated with HG1-9-DNP (500 nM) in a fresh medium without FBS for 60 min firstly. After washed 3 times with PBS, the cells were irradiated under blue LED (11.15 mW/cm^2) for 60 min, and then further incubation for 60 min or 150 min. Then the cells were washed 3 times with PBS, and incubated with different commercial dyes LysBlue or Hoechst 33342 for 30 min. After washing 3 times with PBS, fluorescence images were collected immediately. The fluorescence image of LysBlue and Hoechst 33342 was collected in the range of 420-470 nm under excitation of 405 nm. The excitation and emission wavelengths of HG1-9-DNP were set as same as in the live cell imaging conditions.

Flow cytometry analysis

HeLa cells were seeded in a 6-well plate with approximately 5×10^5 cells per well. After 24 h of culture in the incubator, the cells were incubated with 500 nM HG1-9-DNP. As the experimental group, the cells were exposed to a blue LED (465-470 nm, 11.15 mW/cm^2) for 60 min. The cells in control group do not need this step. After all cells were further cultured for different time (30, 60, 90, 120, 150, 180 min), the cells of each group were trypsinized into a 1.5 mL centrifuge tube and washed with PBS for 3 times, then the cells were dispersed in 300 μL PBS, and analyzed by flow cytometry immediately.

Cytotoxicity assay

Different kind of cells were seeded into 96-well plates with 100 μL per well (approximately 5×10^3 cells/well), respectively. After incubating overnight, HG1-9-DNP or ctr sq-DNP solutions with different concentration were added to the corresponding wells. In order to ensure the credibility of the data, three samples were made in parallel for each concentration. For dark cytotoxicity, the cells were further incubated in the incubator for 48 h. For photo-induced cytotoxicity, the cells were irradiated under a blue LED (465-470 nm, 11.15 mW/cm^2) for 60 min, and then further incubated for 48 h. For cytotoxicity with wash step, the cells were washed after incubation with HG1-9-DNP or ctr sq-DNP for 60 min, and then the cells were irradiated under a blue LED (465-470 nm, 11.15 mW/cm^2) for 60 min, and then further incubated for 48 h. Then the cytotoxicity was detected using a CCK-8 method.

Apoptosis assay

HeLa cells were seeded in culture dishes at a density of 1×10^6 cells/dish and incubated for 24 h. Then 50 nM of HG1-9-DNP or ctr sq-DNP were incubated with cells for 1 h. To investigate the effect of internalized HG1-9-DNP or ctr sq-DNP on cell apoptosis before and after irradiated, cells were washed with fresh medium to remove the free HG1-9-DNP or ctr sq-DNP in medium. Then the cells of the experimental group were irradiated under a blue LED (11.15 mW/cm^2) for 60 min. After cells were further cultured for 24 and 48 h, the cells were digested with trypsin (EDTA-free), then collected in 1.5 mL centrifuge tubes, and washed 3 times with PBS. The treated cells were dispersed in 200 μL of binding buffer and stained with 5 μL of V-Alexa Fluor 647 and 10 μL of PI for 10 min at room temperature in the dark. After double staining, cells were analyzed by flow cytometry.

Cell cycle assay

HeLa cells were seeded into eight culture dishes at a seeding density of 1×10^6 cells/dish, and DNNH solutions of 15.63, 31.25, and 62.50 nM were added after 24 h of culture. Compared with control, the cells in the experimental group were irradiated (11.15 mW/cm2) for 60 min. After 24 h of incubation, the cells were washed, and resuspended in 500 μL of 70% pre-cooled ethanol overnight. Then the cells were washed with PBS, and 100 μL of RNase A solution was added. After incubation at 37°C for 30 min, 400 μL of PI solution was added, and the assay was performed on FACSCalibur flow cytometer after incubation at 4°C in the dark for 30 min.

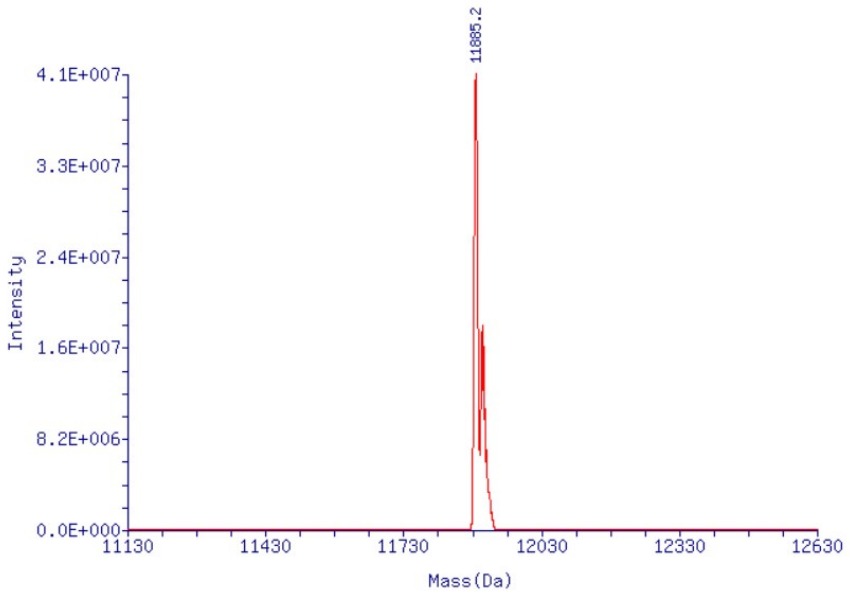


Fig. S1 MS (ESI) of HG1-9-DNP.

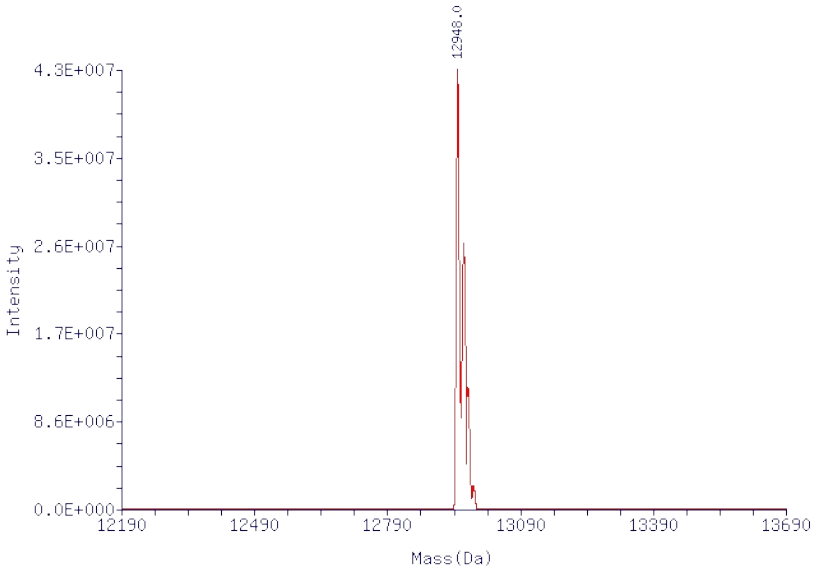


Fig. S2 MS (ESI) of ctr sq-DNP.

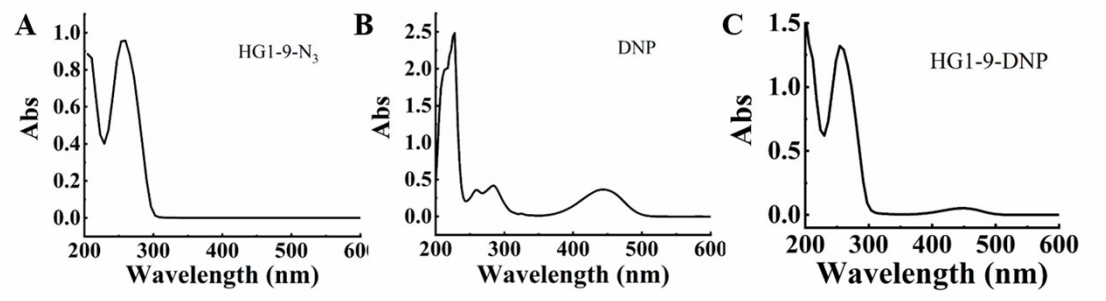


Fig. S3 UV-vis spectra of HG1-9-N_3 (A), DNP (B), and HG1-9-DNP (C).

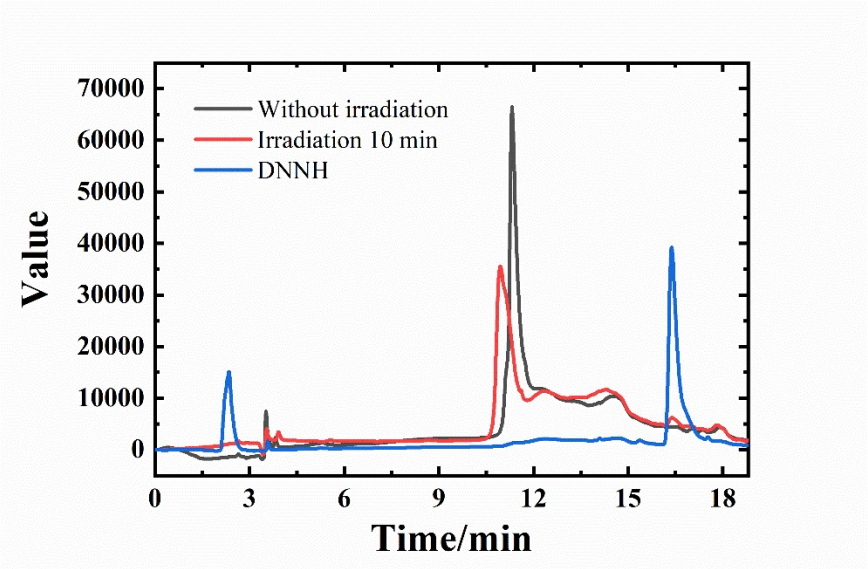


Fig. S4 HPLC chromatogram of HG1-9-DNP before and after irradiation under a blue LED (465 - 470 nm), detection wavelength, 260 nm.

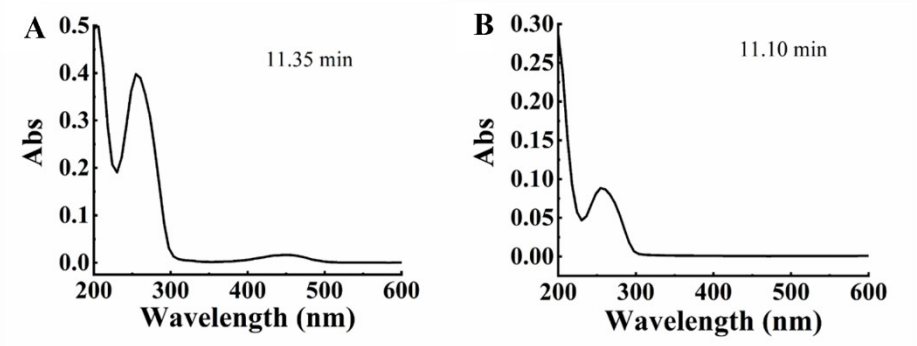


Fig. S5 Corresponding UV-vis spectra to the retention time in HPLC at 11.35 min (A), and at 11.10 min (B).

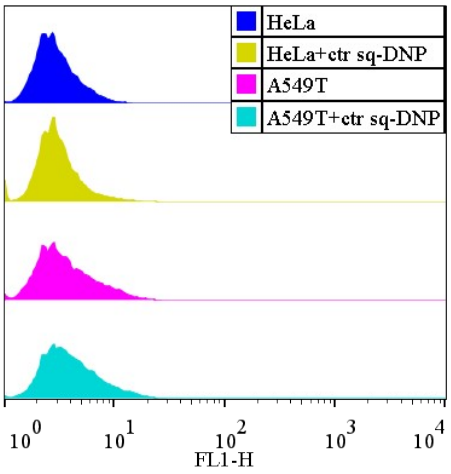


Fig. S6 Flow cytometry assay of different cells after incubated with ctr sq-DNP (500 nM).

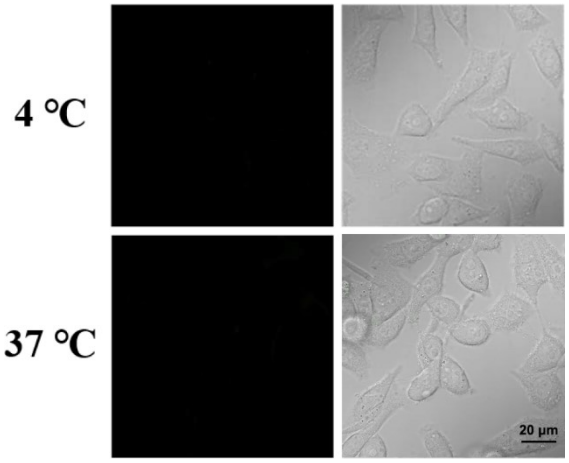


Fig. S7 Confocal images of A549T cells after incubated with HG1-9-DNP (500 nM) at 4 °C and 37 °C (E_x 445 nm, E_m 490 - 540 nm).

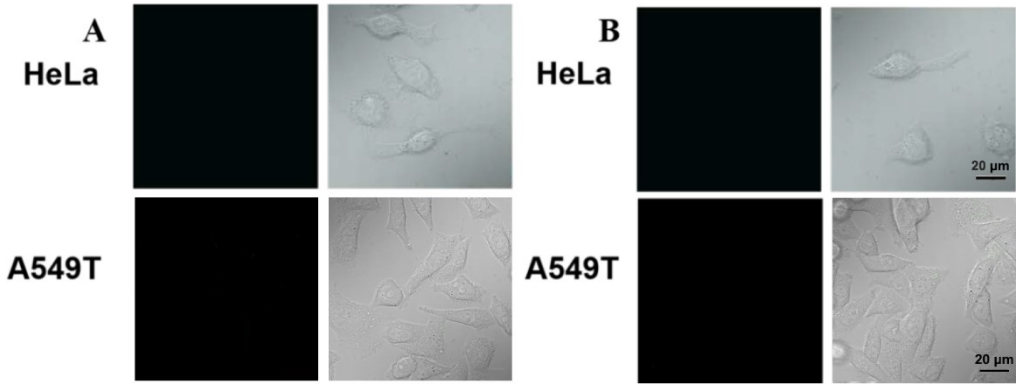


Fig. S8 Confocal images of different cells after incubated with ctr sq-DNP (500 nM) at 4 °C (A) and 37 °C (B).

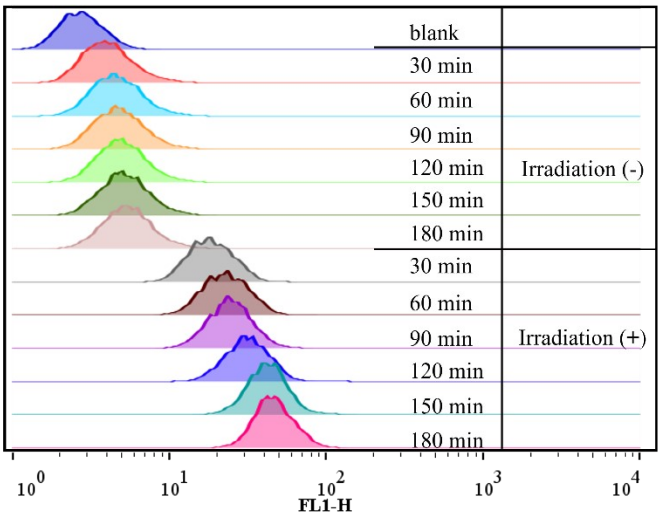


Fig. S9 Flow cytometry assay of HeLa cells after incubated with HG1-9-DNP (500 nM) for different time without or with irradiation.

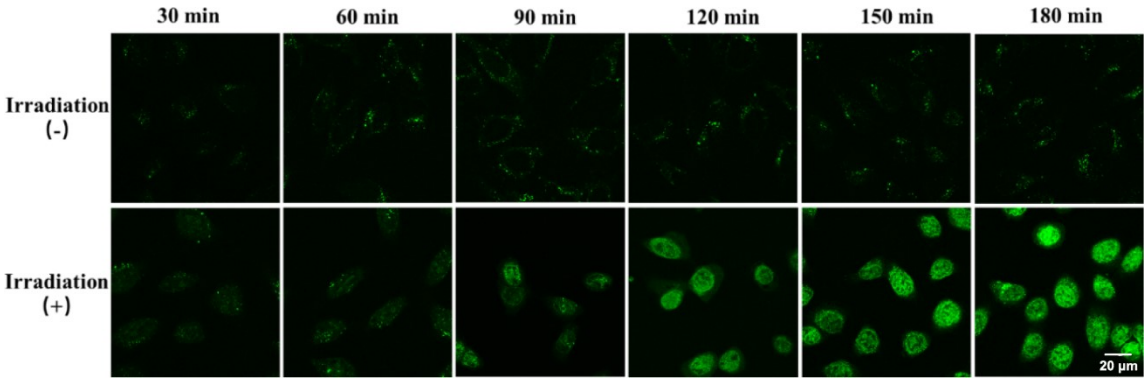


Fig. S10 Confocal images of HeLa cells treated with HG1-9-DNP (500 nM) without and with irradiation, and then further incubation for different times.

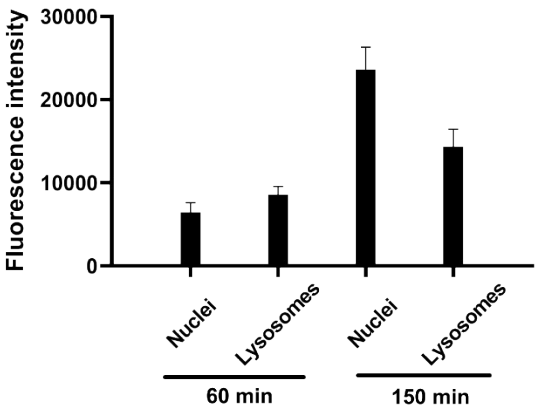


Fig. S11 Fluorescence intensity in lysosomes v.s. nuclei of HeLa cells with different further incubation time.

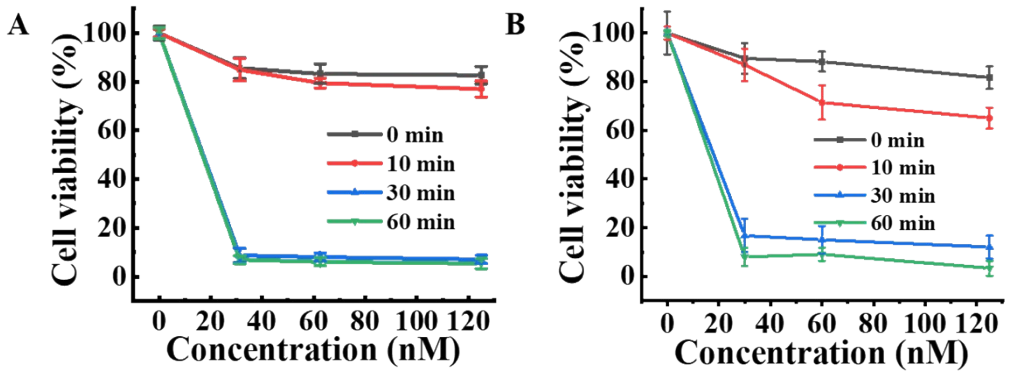


Fig. S12 Cytotoxicity of HG1-9-DNP (A) and ctr sq-DNP (B) to HeLa cells without (0 min) and with irradiation for different time.

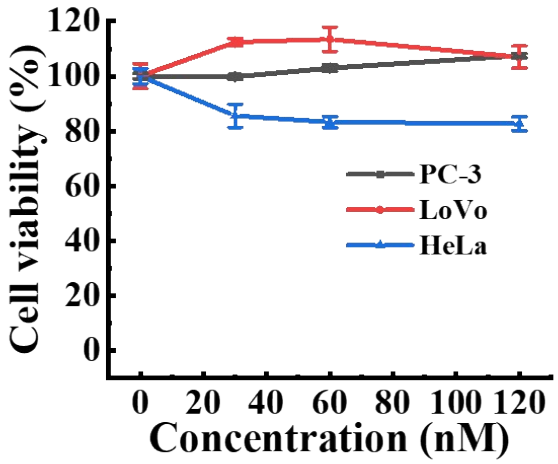


Fig. S13 Cytotoxicity of HG1-9-DNP to different cell lines without irradiation.

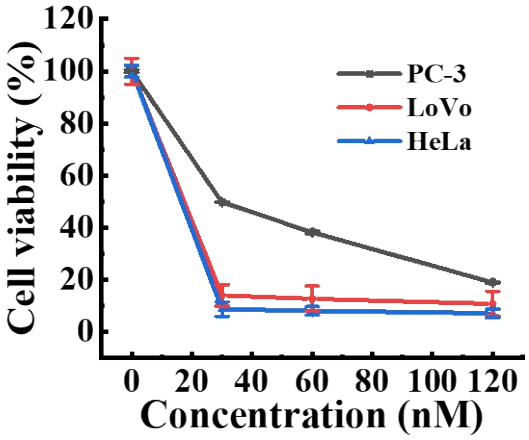


Fig. S14 Cytotoxicity of HG1-9-DNP to different cells after irradiation.

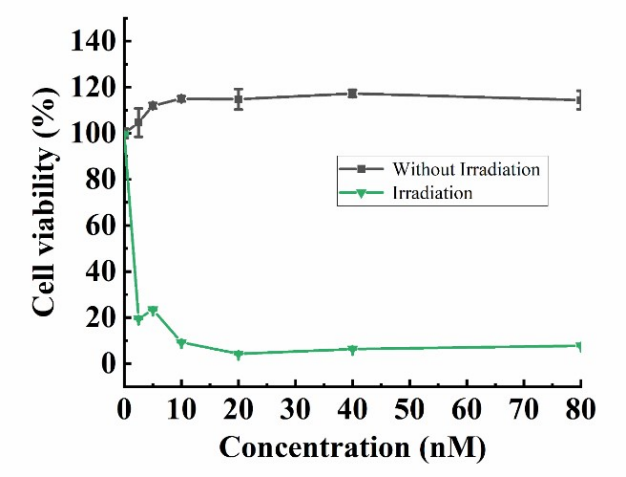


Fig. S15 Cytotoxicity of DNNH to HeLa cells without and with irradiation.

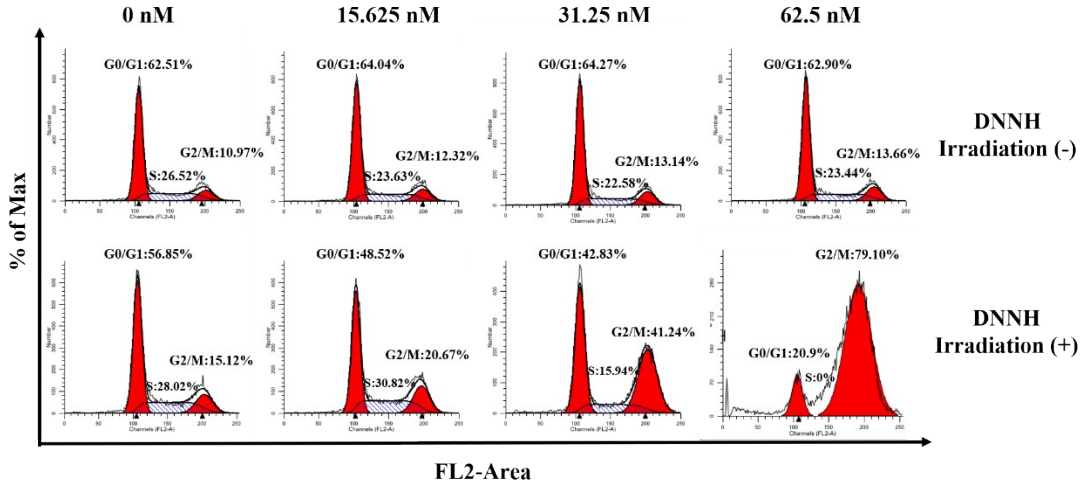


Fig. S16 Effect of different concentrations of DNNH on cell cycle of HeLa cells without or with irradiation.

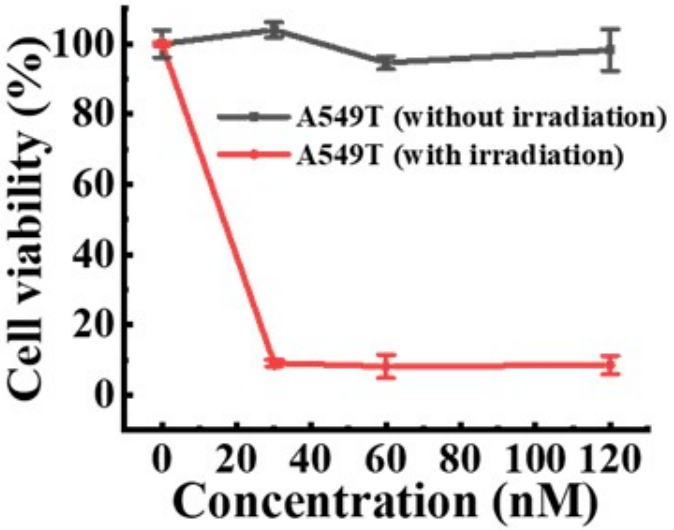


Fig. S17 Cytotoxicity of HG1-9-DNP to A549T cells without and with irradiation.

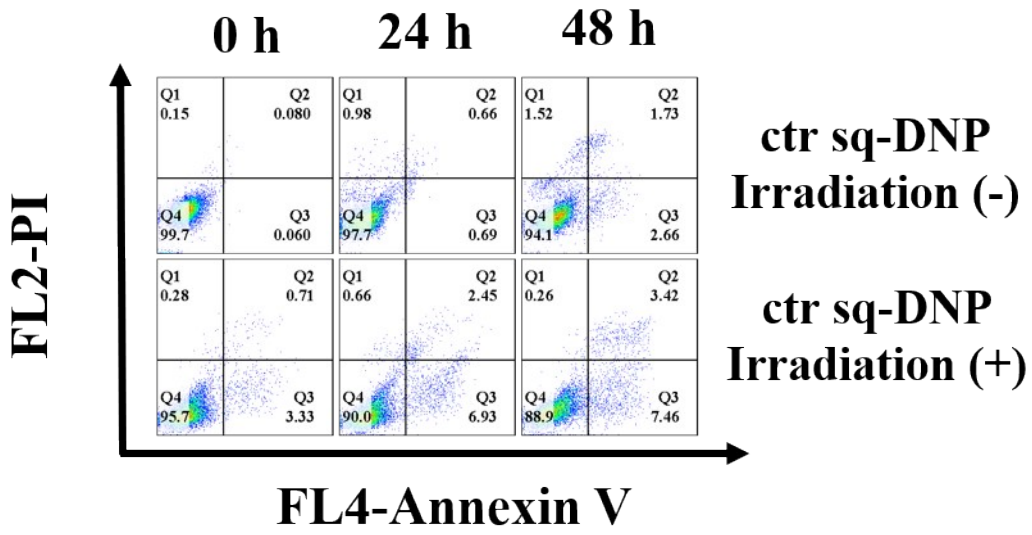


Fig. S18 Effect of ctr sq-DNP (50.0 nM) on apoptosis of HeLa cells without or with irradiation after washing away the free ctr sq-DNP in culture medium, and further incubated for different time.

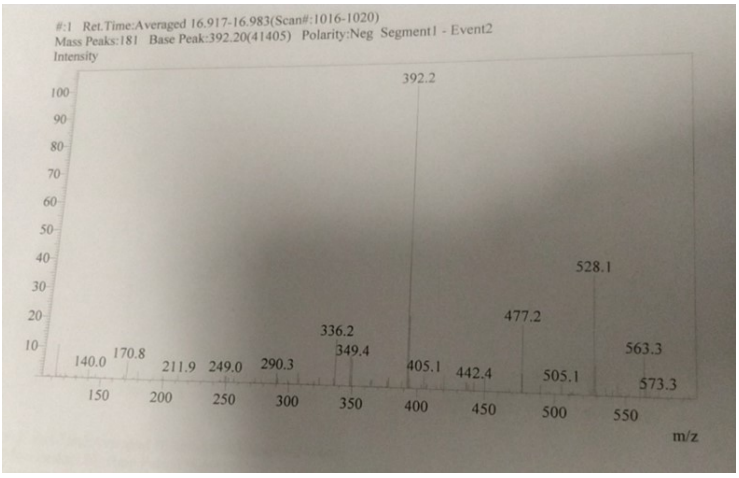


Fig. S19 MS (ESI) of DNP.