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Supplementary Information

Therapeutic effect of quantum dots for cancer treatment

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1. Chemicals and materials

All chemicals, unless indicated, were obtained from Sigma-Aldrich and were used without further purification. Fetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI)-1640 were from Invitrogen Corporation. ^{1}H NMR spectra were recorded on Bruker AV-400 spectrometers in D_2O. ESI-MS spectra were performed on a Thremo LCQ-DECA-XP spectrometer. Elemental contents were analyzed by a Perkin-Elemer 240 elemental analyzer. UV/Vis spectra were monitored with a Varian Cary 300 UV/Vis spectrophotometer equipped with a temperature controller (± 0.1 K). The fluorescence spectra were recorded by using a Cary Eclipse Fluorescence Spectrophotometer (American, Agilent, Co.).

2. Synthesis and characterization of polyamine-L-Arginine (PA)

A mixture of isoindoline-1,3-dione sylvinite and 1,4-dibromobutane (in a 1:3 molar ratio) in acetone was heated at refluxing temperature, and the reaction process was monitored by TLC. After completion, the reaction mixture was cooled to room temperature and the precipitate was filtered then the removal of the solvent resulted in a solid which was further purified to obtain the white solid 2-(4-bromobutyl)isoindoline-1,3-dione.

The BOC-L-Arginine (BOC-L-Arg) were synthesized. Briefly, L-Arg and di-tert-butyl dicarbonate ester ($(BOC)_2O$) (in a 1:3 molar ratio) in water was reacted with constant stirring at 0 °C for 1 h. Then the solution was heated to 25 °C with constant stirring for 12 h. After completion, the compound was extracted with chloroform and removed the solvent in the aqueous phase resulted in a slightly yellowish solid.

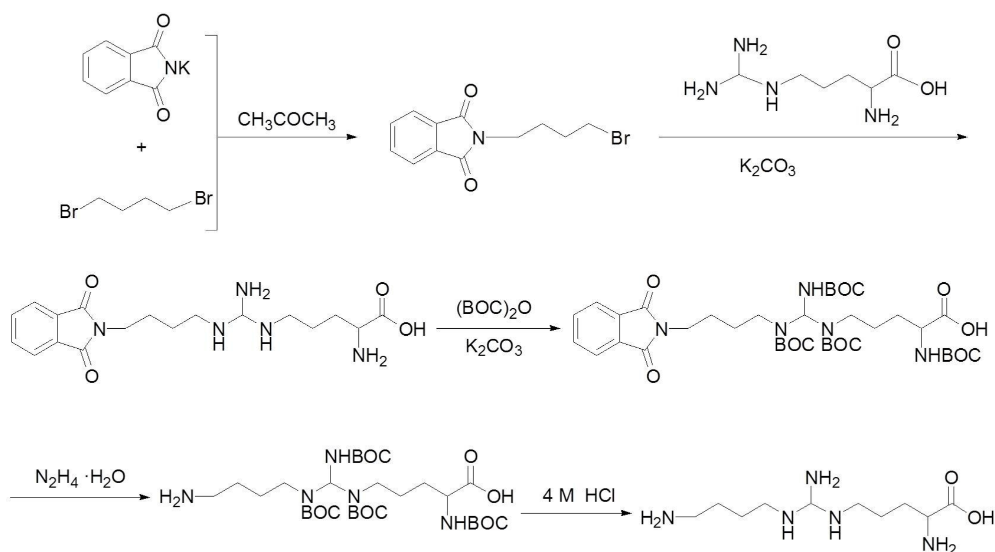
The respective N-Boc-L-Arg (2 mmol) and the anhydrous K_2CO_3 (2 mmol) were dissolved in H_2O (2 mL) and stirred at 25 °C for 15 min. Then the solution was heated to 45 °C and the 2-(4-bromobutyl)isoindoline-1,3-dione (2 mmol) was added slowly. The reaction mixture was stirred at 45 °C for 12 h. After completion of the reaction, the compound was extracted with chloroform, then the solvent was evaporated to dryness under reduced pressure and the residues were purified by silica gel column chromatography using a CH_2Cl_2 and CH_3OH mixture as the eluent to obtain to obtain a light yellow sticky solid.

A mixture of analogs that BOC-L-Arg and 2-(4-bromobutyl)isoindoline-1,3-dione and hydrazine hydrate (in a 1:10 molar ratio) in ethanol was stirred at 25 °C until the white solid appear. After completion, the reaction mixture was evaporated under reduced pressure, and the compound was extracted with chloroform. Removal of the solvent in the aqueous phase resulted in a yellow sticky solid.

The polyamine-BOC-L-Arg were dissolved in the solvents EtOH and stirred at 0 °C for 10 min. Then 4 M HCl was added dropwise at 0 °C. The reaction mixture was stirred at room temperature overnight. The solution typically gave a white solid as a precipitate. The solid was filtered, washed several times with absolute ethanol, and dried under vacuum to give the pure compounds polyamine-L-Arg. Scheme S1 showed the synthesis of polyamine-L-Arg.

2-Amino-5-(3-(4-aminobutyl)guanidino)pentanoic acid (polyamine-L-Arg): Yield 46.2%, yellowish solid. ^1H NMR (400 MHz, D_2O): δ 3.21-3.34 (m, 6H), 2.92-2.95 (t, 1H), 1.62-1.79 (m, 2H), 1.27-1.30 (m, 6H) (Figure S1). EMI-SI m/z：246.2

(M+H). Anal. Calcd for $C_{10}H_{23}N_5O_2$: C 42.69%, H 9.67%, N 24.89%; Found: C 42.98%, H 9.48%, N 24.52%.



Scheme S1 Synthesis of polyamine-L-Arg.

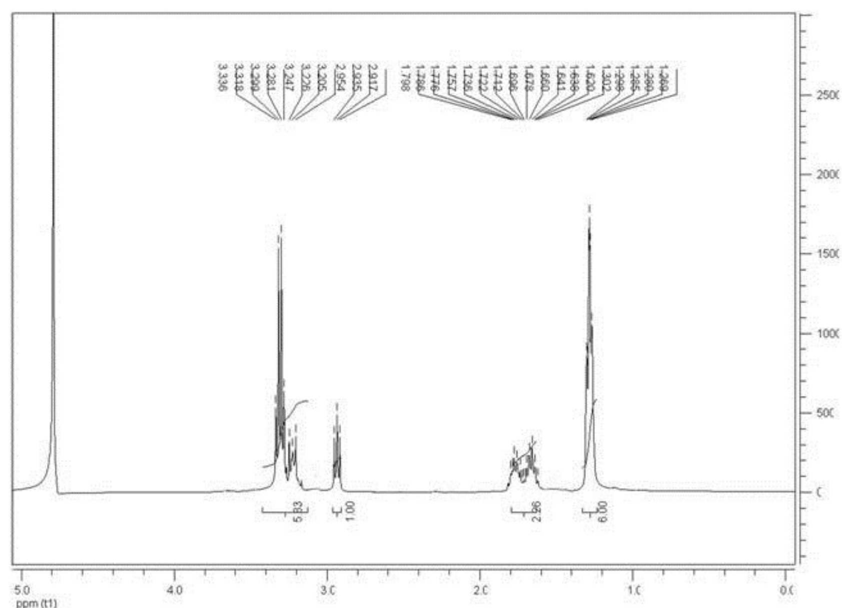


Figure S1. The ^{1}H NMR spectrum of complexes L-Arg and polyamine.

3. Synthesis of CdSe/ZnS Qdots

CdSe/ZnS Qdots were synthesized using organometallic procedures based on previously reported.[1] These Qdots showed high crystallinity and high quantum yields, narrow emission spectra, and narrow size distributions.

4. Synthesis and characterization of water-soluble Qdots@PA nanoparticles

The Qdots@PA nanoparticles were synthesized by an ultrasonic method. The purified nanocrystal complexes could be dissolved in water, phosphate buffered saline (PBS) or other various aqueous media. Then the solution was performed by UV-Vis absorption and fluorescence spectra. The absorption bands of Qdots were in the UV-Vis region at approximately 595 nm, which are typical absorption properties of CdSe/ZnS Qdots. And the Qdots displayed luminescence (613 nm) in PBS upon excitation at 480 nm. This suggests that the Qdots@PA showed highly optical properties in UV-vis spectra and fluorescence spectroscopy.

5. Cell viability assay

The cell viability of the samples was evaluated by a modified MTT assay. Briefly, cells were seeded at a density of 5×10^4 cells/well in 96-well plates with 100 μL of cells suspension per well. After 12 h, the Qdots were subsequently added at concentrations ranging from 0 to 50 μM and incubated for 48 h at 37 °C under 5% CO_2. MTT (20 μL of 2.5 mg mL^{-1}) was added to each well, and the plates were incubated for 4 h at 37 °C under 5 % CO_2. The medium was removed and each well was added with 100 μL DMSO. The OD570 of samples was measured at 690 nm using an Infinite M200 monochromator-based multifunction microplate reader.

6. The binding ability of Qdots with cells

HepG2 and QSG-7701 cells were seeded in 60 mm tissue culture dishes (Corning) at a density of 1×10^5 cells per mL in 1640 medium containing 10% FBS at 37 °C in a 5% CO_2. After incubation for 12 h, the culture medium was removed and replaced

with medium containing the samples. After incubation for 12 h, the cells were thoroughly washed with PBS buffer at pH 7.4. Then cells were trypsinised and washed twice with PBS buffer in order to eliminate Qdots that were not internalized. A flow cytometer (Becton Dickinson & Co., Franklin Lakes, NJ) was used to measure the fluorescence intensity with excitation at 488 nm. The monitor gate of 25 mm was set to accommodate the effective size of live cells, and the number of cells analysed for each sample was 10 000. The signal along the x axis indicates the fluorescence intensity, and the height along the y axis indicates the corresponding number (counts) of species showing the fluorescence.

For fluorescence microscopy observation of samples after treatment of cells for 12 h, the cells were washed with PBS and incubated with Hoechst 33342 (10 $\mu g\ mL^{-1}$) for 30 min at 37 °C, then viewed with a confocal microscope (Leica-SP8) by using a 63× oil immersion objective. The cells were scanned by dual excitation of 405 nm (blue) and 488 nm (red) laser lines.

7. ROS assay

Cellular accumulation of ROS was determined using the 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA). Specifically, after treatment with Qdots for 24 h, the cells were rinsed three times with PBS to remove unbound samples. The cells were then incubated with 10 μM of H_2DCF-DA for 30 min at 37 °C. Following this, the wells were rinsed twice with PBS to remove excess dye. Then, the fluorescence intensity of cells was determined immediately by a FACSCalibur flow cytometer and confocal microscopy equipped with a 63× oil-immersion

objective with excitation at 488 nm and emission at 530 nm.

8. Cellular apoptotic evaluation

Cell apoptosis was evaluated by Hoechst staining, annexin V-FITC apoptosis detection kit and caspase 3 activation kits by a confocal microscope (Leica-SP8) and FACSCalibur flow cytometer. Briefly, the HepG2 cells were seeded in 35 mm diameter culture dish containing 15 mm diameter glass cover-slips or 60 mm tissue culture dishes and exposed to Qdots for a specified time, then harvested and stained according to the manufacturer's protocol. Images were acquired using a confocal microscope. And the quantitative values subsequently analysed with a FACSCalibur flow cytometer.

Cellular ultrastructure examined by electron microscopy: HepG2 cells (5×10^5) were treated with Qdots at 37 °C for a specified time. Cells were washed twice with PBS and fixed with 2.5% glutaraldehyde at 4 °C for 1 h and then fixed with 2% osmium tetroxide solution. Cells were then dehydrated with washes in a graded series of ethanol and then embedded in epoxy resin. The ultrathin sections obtained were mounted in copper grids, counterstained with uranyl acetate (2%) and aqueous palladium (2%), and visualized in an electron microscope (JEM-1200EX, JEOL, Japan).

9. Analysis of Mitochondrial Membrane Potential (MMP)

HepG2 cells seeded in a 35 mm diameter dish containing 15 mm diameter glass coverslips were incubated for 15 min in complete medium containing 10 μg mL^{-1} JC-1. After washed twice with PBS, the stained cells were viewed using confocal

microscopy (Leica-SP8). We calculated the MMP by dividing the intensity of the 590 nm (red) images with their corresponding 530 nm (green) images.

10. Induced solid tumor in vivo

The animal experiment was carried out in accordance with the guidelines and approval of the Animal Welfare and Use Committees of Henan University to ensure animal welfare during experiments. Briefly, 20 male Kunming mice were randomly divided into two groups and were injected subcutaneously with H22 (mice hepotoma cells) cells (5×106 cells/mouse). Mice of group I (tumor control) and groups II were administered by caudal vein injection physiologic saline or Qdots (0.2 mg/kg), respectively, for 7 consecutive days. After 20 day of tumor cell implantation, all mice were sacrificed and their tumor were removed and weighed.

11. Survivability of Mice

For calculation of the survivability, mice were treated with Qdots (0.2 mg/kg) or physiologic saline by caudal vein injection was started 24 h after inoculation for 7 consecutive days. Then the mice from each group were kept in the laboratory for survival analysis.

12. References

1 J. J. Li, Y. A. Wang, W. Guo, J. C. Keay, T. D. Mishima, M. B. Johnson, X. Peng, *J. Am. Chem. Soc.* 2003, **125**, 12567.

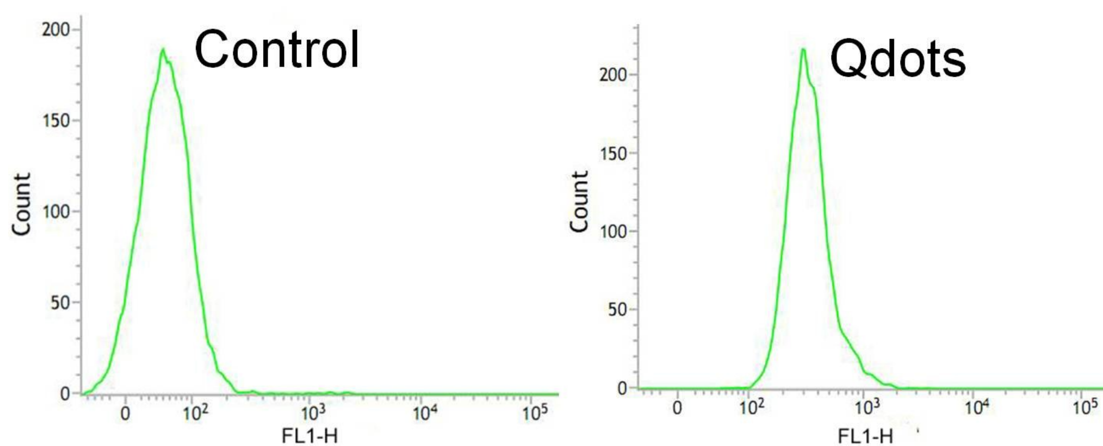


Figure S2. Flow cytometry results of ROS productions after HepG2 cells were treated with Qdots for 12 h.

Table S1. The cytotoxicity activity of PA-coated CdSe/ZnS Qdots.

Samples	IC_{50} (μM)[a]		
	HepG2	HeLa	QSG-7701
PA-coated CdSe/ZnS Qdots	2.51±0.12	2.67±0.14	26.65±0.11

[a] IC_{50} values are given in μM, and the data are presented as mean values standard deviations, and cell viability is assessed after 48 h of incubation.