Electronic Supplementary Material (ESI) for Journal of Materials Chemistry

Supplementary Information

One-step synthesis of surface passivated carbon nanodots by microwave assisted pyrolysis for enhanced multicolor photoluminescence and bioimaging

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Materials

4,7,10-trioxa-1,13-tridecanediamine (TTDDA, 97%) was purchased from Sigma–Aldrich. Quinine sulfate (98%, suitable for fluorescence) was supplied by Fluka. 3-(4, 5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT, 98%) was supplied by Alfa Aesar. All other reagents were of analytical grades and used without further purification.

Experimental Sections

Firstly, 5 ml glycerol and 2 ml 10 mM phosphate solution was mixed with different amount of TTDDA under vigorous stirring. Then the clear transparent solution was put into a domestic microwave oven (700 W) and heated for different time periods. When cooled down to room temperature, the color-changed solution was diluted and dialyzed against pure water through a dialysis membrane (MWCO of 1000) for 3 days. Finally, a clear, light yellow-brown aqueous solution containing surface passivated CDs was lyophilized to collect dry C-dots.

Instruments

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UV-Vis absorption was characterized by TU-1810 UV–Vis Spectrophotometer (Pgeneral, China). Photoluminescence (PL) emission measurements were performed using FLS920 fluorometer (Edinburgh Instruments, Britain). The morphology and microstructure of the CDs were examined by high-resolution transmission electron microscopy (HRTEM) on a Philips Tecnai G2 F20 microscope (Philips, Netherlands) with an accelerating voltage of 200 kV. The samples for HRTEM were made by dropping an aqueous solution onto a 300-mesh copper grid coated with a lacy carbon film. The FTIR spectra of the samples were measured on a Nicolet 380 spectrometer (Thermo, America). X-Ray diffraction (XRD) patterns of the prepared samples were recorded on a Rigaku-D/MAX 2500 diffractometer （Rigaku, Japan）equipped with graphite monochromatized CuKα (λ=0.15405 nm) radiation at a scanning speed of 4°/min in the range from 5° to 60°. The elementary construction of CD-TTDDA was further confirmed by elemental analysis with Vanio-EL (Elementar Analysensysteme GmbH, Germany). Thermogravimetry (TG) curves for the sample was obtained by a Perkin–Elmer DIAMOND TG/DTA Thermal Analyzer (Perkin–Elmer, America), in nitrogen atmosphere.

Measurement of fluorescence quantum yields

The quantum yield of the Carbon Dots (CDs) was determined by a comparative method. Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield: 54%) was selected as a standard sample to calculate the QY of test sample (i.e. CDs) which was dissolved in ultra pure water at different concentrations. All the absorbance values of the solutions at the excitation wavelength were measured with UV–Vis spectrophotometer. Photoluminescence (PL) emission spectra of all the sample solutions were recorded by FLS920 fluorometer at an excitation wavelength of 360 nm. The integrated fluorescence intensity is the area under the PL curve in the wavelength range from 380 to 700 nm. Then a graph was plotted using the integrated fluorescence intensity against the absorbance and a trend line was added for each curve with intercept at zero. Absolute values were calculated according to the following equation:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

Where the subscripts ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, Grad is the gradient from the plot of integrated fluorescence intensity *vs*

absorbance, and η is the refractive index of the solvent. In order to minimize re-absorption effects, absorbance in the 10 mm fluorescence cuvette should never exceed 0.1 at the excitation wavelength.

Cell culture, confocal microscopy and cytotoxicity assay

HepG-2 cells (human hepatocellular liver carcinoma line) were obtained from Peking Union Medical College (Beijing, China). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone) with high glucose, containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 ^{o}C in 5% CO_2 humidified atmosphere.

For confocal microscopy, HepG-2 cells were seeded on a coverslip in 6-well plate 12 h before use. Then the culture medium was replaced by 2.5 ml fresh medium containing 100 μg/ml C-dots and the cells were incubated for another 24 h. The cells were then washed with isotonic PBS (pH 7.4) three times, and fixed with 4% paraformaldehyde solution in PBS at 4 ^{o}C overnight. The samples were examined under a Leica confocal laser scanning microscope (Mannheim, Germany) equipped with a UV laser (351/364 nm), an Ar laser (457/488/514nm) and a HeNe laser (543/633 nm).

The cytotoxicity of TTDDA-passivated C-dots was assessed through MTT assay. HepG-2 cells were seeded in a 96-well plate, at a density of 2×10^4 cells/well and incubated overnight. The following morning, the culture medium in each well was replaced by 180 μL fresh DMEM/FBS. C-dots solutions with various concentrations were then added to each well. After incubation for 24 h, the medium containing C-dots was removed, and replaced with 200 μL fresh medium containing 20 μL MTT (5 mg/ml in PBS) and incubated for another 4 h. Finally all medium was removed and 150 μL/well DMSO was added, followed by shaking for 15 min. The absorbance of each well was measured at 490 nm using a Synergy HT Multi-Mode Microplate Reader (BioTek, USA) with pure DMSO as a blank. Non-treated cell (in DMEM) was used as a control and the relative cell viability (mean% ± SD, n = 3) was expressed as $Abs_{sample}/Abs_{control}$ ×100%.

Table S1 The results of elemental analysis of TTDDA-passivated C-dots

	C%	H%	N%	O (Calculated, %)
TTDDA-passivated C-dots	56.82	8.462	7.77	26.948
TTDDA （Calculated）	54.47	10.89	12.71	21.79
Glycerol （Calculated）	39.09	8.69	0	52.12

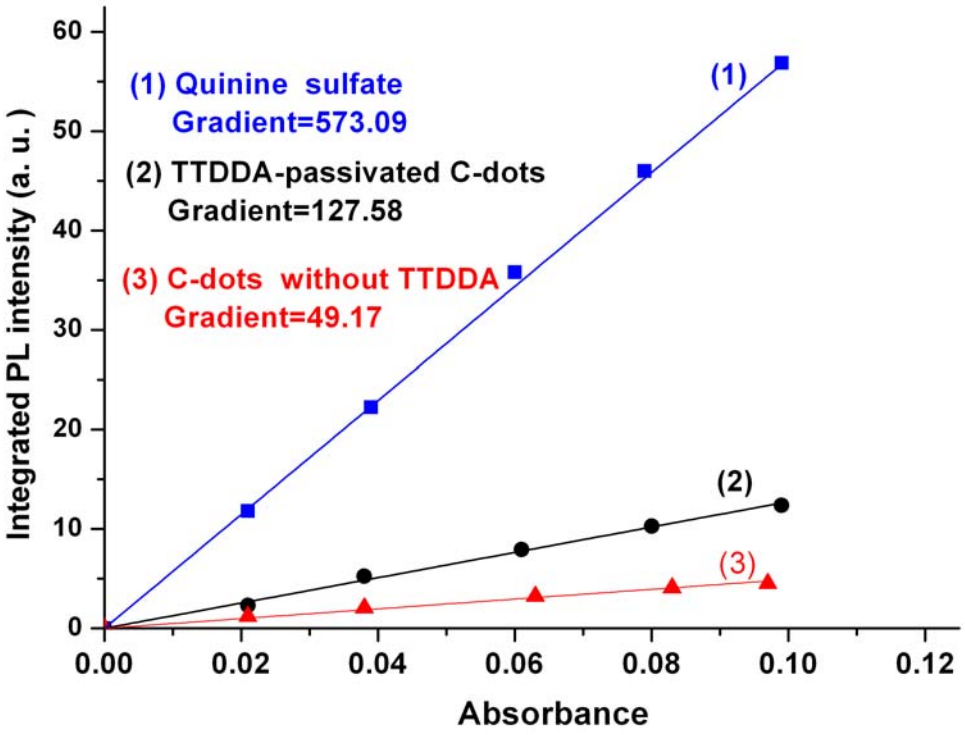

Figure S1 Photoluminescence and absorbance of C-dots（results: TTDDA-passivated C-dots 12.02%；C-dots without TTDDA 4.63%. Excited at 360 nm)

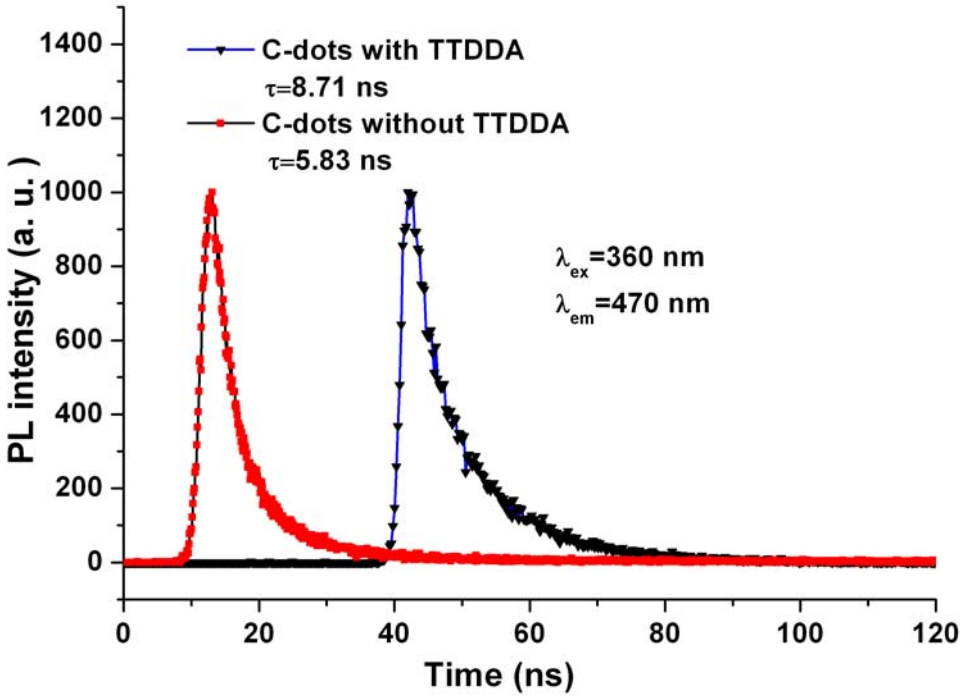

Figure S2 Photoluminescence decay curve for C-dots samples (results: TTDDA-passivated C-dots 8.71 ns；C-dots without TTDDA 5.83 ns.)

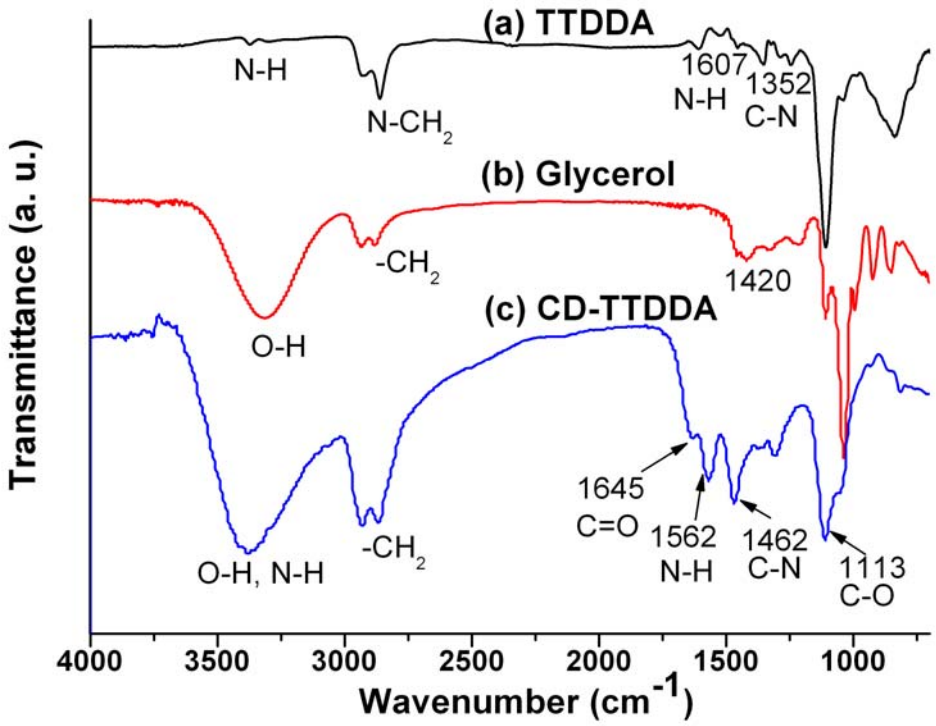

Figure S3 FT-IR spectra of raw TTDDA sample (a), raw glycerol sample (b) and TTDDA-passivated C-dots sample (c).

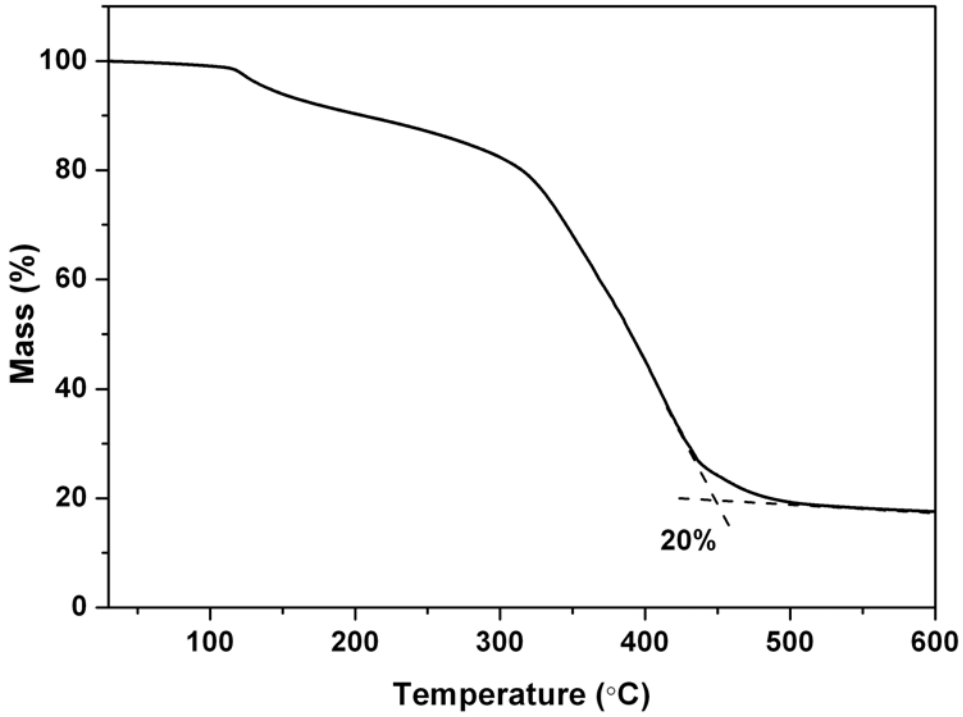

Figure S4 Thermogravimetric (TG) curve of the sample TTDDA-passivated C-dots. The analysis was performed under nitrogen atmosphere, at a flowing rate 20.0 ml/min and heating rate 10 ^{o}C/min.

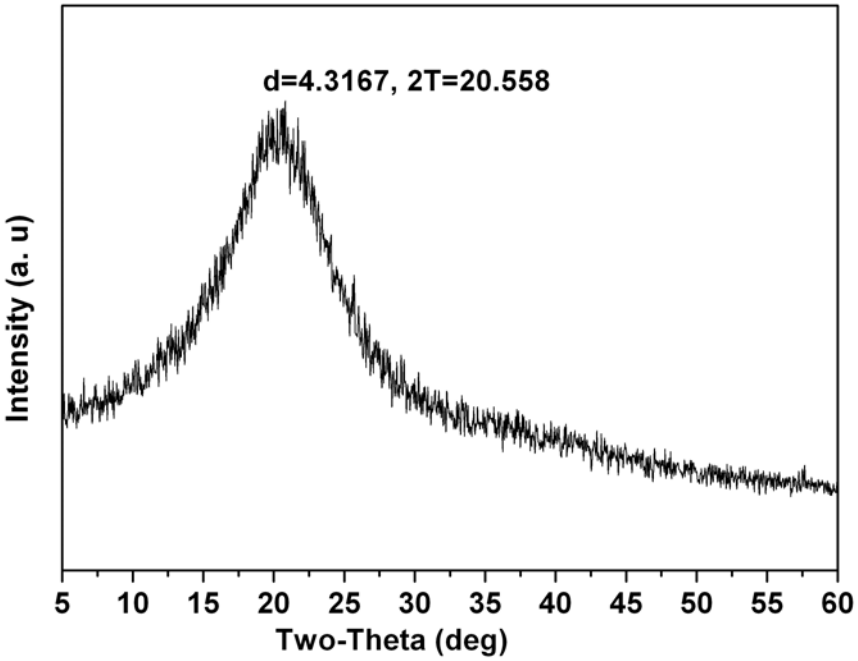

Figure S5 XRD pattern of TTDDA-passivated C-dots.

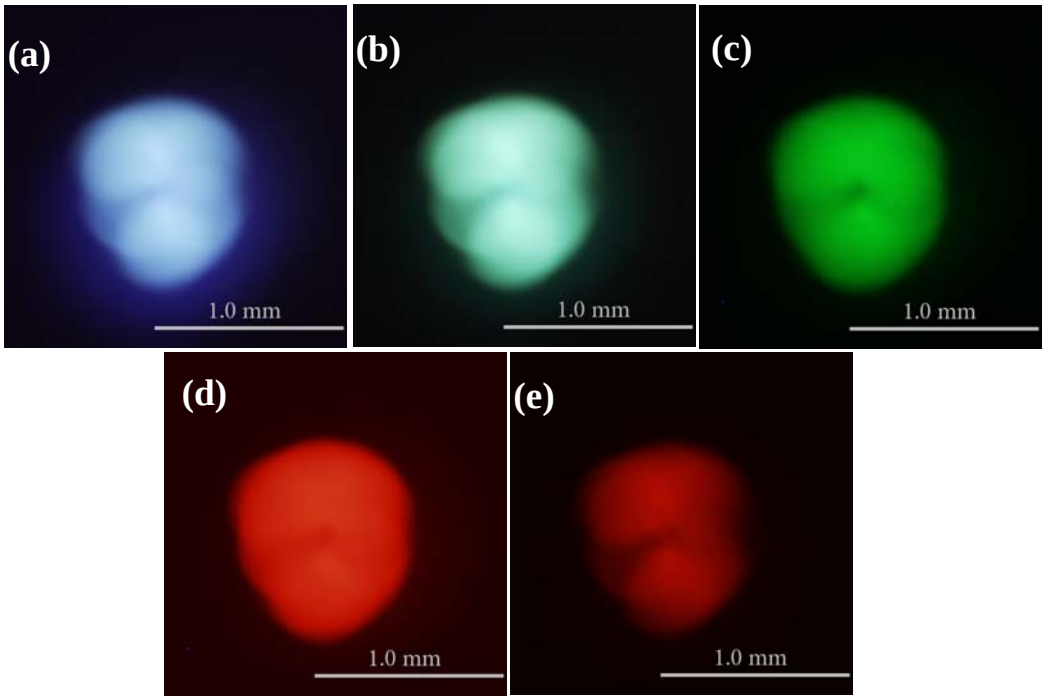

Figure S6 Fluorescent microscopy images (all scale bars: 1.0 mm) of diluted aqueous solution containing C-dots under (a) ultraviolet (330–385 nm), (b) violet (400–410 nm) , (c) blue (460–495 nm), (d) green (530-550 nm) and (e) yellow (545-580 nm) light excitation; the pictures were taken by an Olympus BX-51 optical system microscope (Tokyo, Japan).

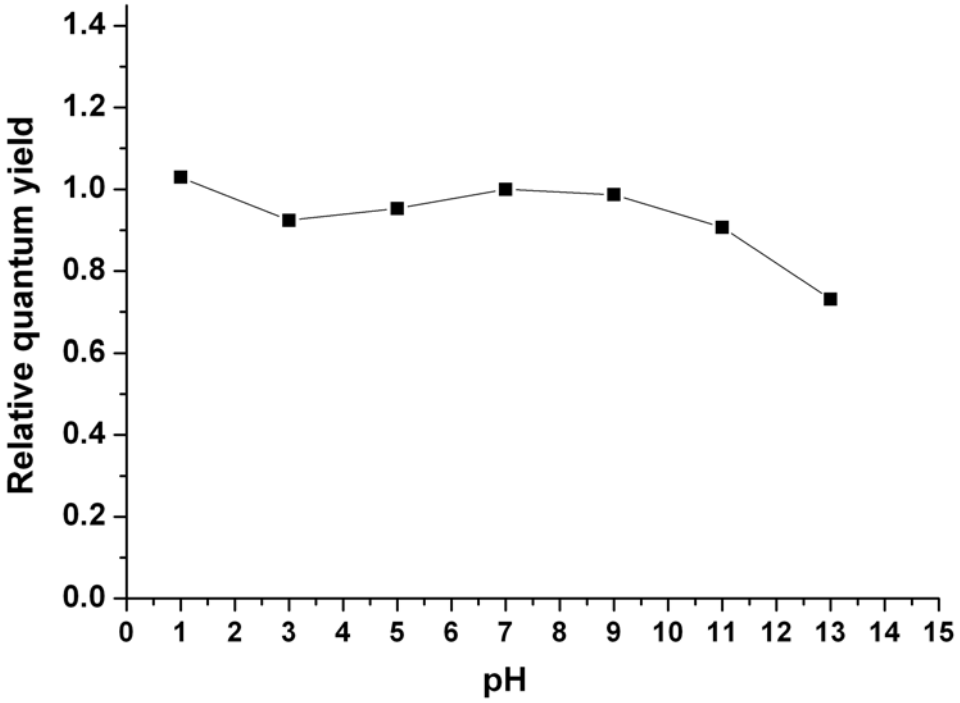

Figure S7 Relative quantum yields of C-dots in different pH solutions. The quantum yield of C-dots in pH 7 solution was defined as 1.0.

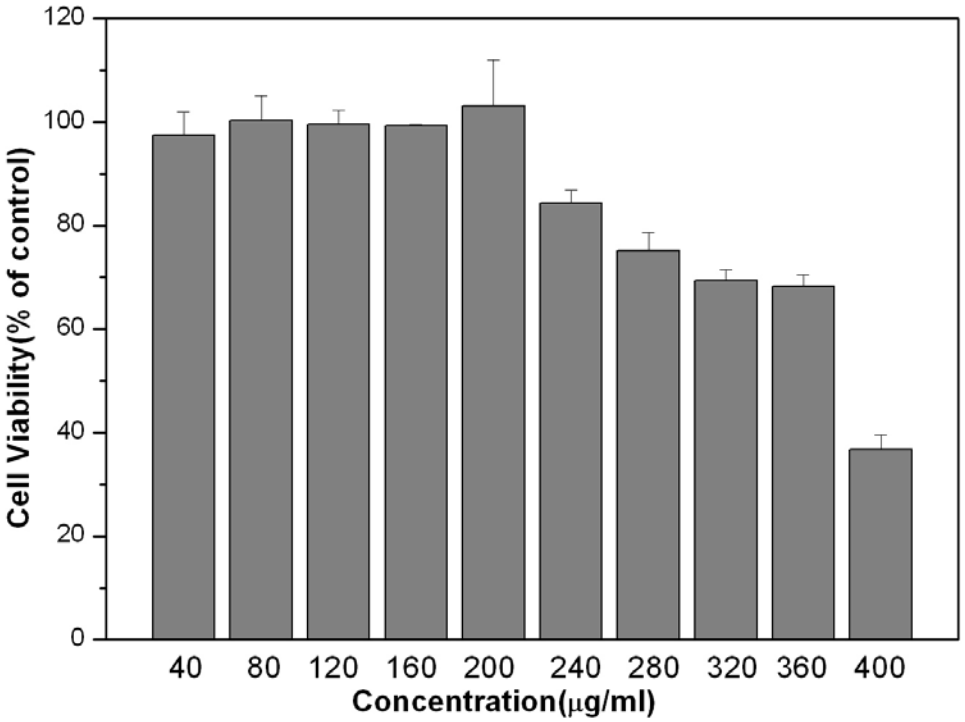

Figure S8 Cytotoxicity testing results via a MTT assay. The values represent percentage cell viability (means ± SD, n = 3)