Supporting Information

**Covalently Assembled Dopamine Nanoparticle as an Intrinsic Photosensitizer and pH-Responsive Nanocarrier for Potential Application of Anticancer Therapy**

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Experimental Section

Materials

Dopamine hydrochloride (DA), calcein acetoxyethyl ester (calcein-AM), propidium iodide (PI), 9,10-anthracenediylbis(methylene) dimalonic acid (ABDA), 2,2,6,6-tetramethyl-4-piperidone (TEMP) and 2’7’-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma Aldrich. Genipin was obtained from Wako Pure Chemicals, Japan. Methylene blue (MB) was purchased from Sinopharm Chemical Reagent Co., Ltd. Dimethyl sulfoxide (DMSO) and sodium hydroxide (NaOH) were obtained from Beijing Chemical Co., Ltd. Hoechst 33342, Alexa Fluor 488 and cell counting kit-8 (CCK-8) were purchased from Dojindo Laboratories, Kumamoto, Japan. The anticancer drug bortezomib (Btz) was supplied by Shanghai Aladdin Bio-Chem Technology Co., Ltd. Phosphate buffered saline (PBS) tablets were purchased from Solarbio Corporation. Dulbecco’s modified eagle medium (DMEM), fetal bovine serum (FBS) were obtained from Invitrogen. Ultrapure water used in all experiments was prepared in a Milli-Q apparatus (Millipore) and had a resistivity higher than 18.2 MΩ·cm.

Characterization

The morphology and size of the nanoparticles were characterized using scanning electron microscopy (SEM, Hitachi, S-4800), transmission electronic microscope (TEM, JEOL, JEM-7700), and dynamic light scattering (DLS, Malvin, Zetasizer Nano ZS). UV-Vis absorption spectra were recorded at room temperature using a SHIMADZU-2600 spectrophotometer. Fourier transform infrared spectroscopy (FTIR) was carried out on a Tensor-27 infrared spectrometer (Bruker) using the KBr pellet pressing method. Electron paramagnetic resonance (EPR) spectroscopy was carried out using a Bruker Model ESP 500E spectrometer. Confocal laser scanning microscopy (CLSM) images were taken with a confocal microscope (Olympus FV1000). A 635 nm laser from Changchun Leishi Photoelectric Co., Ltd was used as the light source. $^1$H NMR spectra of samples were obtained using NMR spectroscopy (Bruker Avance III 400 HD).
Preparation of nanospheres

In a typical experiment, 3 mM DA and 3 mM genipin were dissolved in ultrapure water, in which the molar ratio of DA and genipin was 1:1. Two solutions were mixed thoroughly under quick agitation. The pH of the mixture was adjusted to 7.5 by adding 0.1 M NaOH solution, and the mixture reacted at the room temperature with continuous magnetic stirring. After 24 h, the yellow solution changed into a dark blue suspension and the nanospheres were formed. The suspended nanospheres were collected by 3 cycles of centrifugation (10000 rpm, 15 min) and water washing.

Detection of $^{1}O_2$

9,10-Anthracenediy1-bis(methylene) dimalonic acid (ABDA) was used as the chemical probe for the detection of $^{1}O_2$ generation. ABDA stock solution (10 μM) was added to the obtained nanosphere dispersion and MB (as reference) aqueous solution. The mixed solution was laser irradiated at 635 nm, and the UV-Vis spectra were measured.

EPR spectra were also conducted to detect the generation of $^{1}O_2$. 40 μL of 1.0 M TEMP D$_2$O solution was added to 2 mL of 500 μg/mL nanosphere dispersion. The solutions were thoroughly mixed and then injected into the capillaries. Then, the mixture was irradiated with 635 nm laser at 1 W/cm$^2$ for 20 min and immediately subjected to EPR measurement at room temperature.

In vitro ROS detection was performed with HeLa cells. Cells were seeded into confocal dish for 24 h. DGNPs and DGNPs-Btz complex were added respectively and incubated for 6 h. Then cells were gently washed twice with PBS and incubated with DCFH-DA (1×10$^{-5}$ M) for 20 min. After irradiation with 635 nm laser for 20 min, the images were collected by the confocal laser scanning microscopy.

Preparation of Btz-loaded DGNPs

The anticancer drug Btz was dissolved in dimethyl sulfoxide (DMSO) and then mixed with the DGNPs dispersion with Btz/nanospheres mass ratio of 1:3. The mixture was stirred at 37
°C for 5 h. The obtained Btz-loaded DGNPs were collected by 3 cycles of centrifugation (10000 rpm, 15 min) and washing with DMSO–water solution (v/v=1:10). The amount of Btz loaded on the DGNPs was calculated by measuring the absorbance of the supernatant at 270 nm, and the entrapment efficiency was calculated with the following equation:

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\text{%Entrapment efficiency} = \frac{\text{Initial concentration of drug} - \text{Drug content in the supermatant}}{\text{Initial concentration of drug}} \times 100
\]

**In vitro cellular uptake**

HeLa cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin, and streptomycin under a humidified atmosphere of 5% CO₂ at 37 °C for 24 h. Then, the medium was replaced with 1.5 mL of DMEM medium containing assembled DGNPs-Btz complex and then incubated for 12 h at 37 °C. The cells were then washed three times with PBS, and 1 mL of DMEM medium was added. 10 μL of 1mg/mL Alexa 488 and 10 μL of 1mg/mL Hoechst 33342 were used to stain the cell membrane and nuclei, respectively. The intracellular localization of DGNPs was determined using an Olympus FV1000 microscope.

**In vitro drug release**

In the assessment of drug release behavior, the DGNPs-Btz complex were dispersed in 2 mL of different buffer solutions (pH 7.4 and pH 5.4, respectively) at 37 °C. The amount of Btz released from DGNPs-Btz complex was measured by the UV-vis absorbance of the supernatant at 270 nm. The cumulative amount of released drug was calculated, and the percentage of drug released from the nanoparticles was plotted against time.

**Cytotoxicity assay**

HeLa cells were seeded in 96-well plates at a density of 5×10³ cells per well and incubated at 37 °C with 5% CO₂ for 24 h. The culture media was removed and then replaced by 100 μL of DMEM supplemented with 10% fetal bovine serum containing equivalent concentrations of free Btz, DGNPs and DGNPs-Btz complex. After co-incubation for 6 h, the cells were washed
three times with PBS, and 1 mL of DMEM medium was added. The cells were irradiated with a 1 W/cm$^2$ 635 nm laser for 20 min. After another 20 h incubation, the viability of cells was detected by a CCK-8 assay.

Cytotoxicity was also measured with CLSM. In detail, HeLa cells were seeded into confocal dishes and treated with DGNPs and DGNPs-Btz complex for 6 h in incubator, respectively. Then, the cells were washed with PBS to remove the untaken drugs. 10 μL PI (1 mg/mL) and 10 μL calcein-AM (20 μg/mL) were added into the dishes. After that, the cells were irradiated with 1 W/cm$^2$ 635 nm laser for 20 min and continued to culture for another 12 h. Cells cultured without drugs were taken as the control group under the same irradiation.

Supplementary Figures

Figure S1 The photographs of DA and genipin mixture at different reaction time.
Figure S2 Dynamic light scattering measurement of DG nanoparticles (DGNPs) over time in the DMEM.
**Figure S3** (a) Time-dependent photodegradation of ABDA in the presence of methylene blue (MB). (b) Time-dependent decline percentage of ABDA in the presence of DG nanoparticles or MB under irradiation.

**Figure S4** Cellular ROS detection before and after irradiation with 635 nm laser for 20 min. The scale bars represent 100 μm.
Figure S5 $^1$H NMR spectra of Btz, DGNPs and DGNPs-Btz complex.

$^1$H NMR spectra of Btz, DGNPs and DGNPs-Btz complex were characterized to confirm the loading of Btz onto the DGNPs. As shown in Figure S5, free Btz was observed with sharp peaks such as the pyrazine protons (8.6-9.2 ppm) and the phenyl protons (7.1-7.3 ppm) (Figure S5a). After the complexation of Btz with catechol groups on the DGNPs, these characteristic peaks could still be distinguished (Figure S5c). In addition, the characteristic peaks at 5.5-6.0 ppm corresponding to the H atoms on the benzene of Btz-DA complex were occurred, further confirming the successful loading of Btz on the DGNPs.
Figure S6 Cell viability of HeLa cells incubated with different concentrations of DGNPs-Btz complex in darkness and under irradiation.

References

