Additional files

Supplementary figures and figure legends



The biological function of BBR-Glu in glioma U87 cells (A) Cell viability was analyzed in U87 cells treated by BBR-Glu (50µM), BBR-Water (50µM) and 5% glucose solution. (B) G2/M phase arrest was induced by BBR-Glu (50µM) for 48 h. (C) The number of cell colonies was observed in BBR-Glu (50µM), BBR-Water (50µM) and 5% glucose solution. (D) Cell apoptosis was detected in U87 cells treated with different concentrations of BBR-Glu (0µM, 25µM, 50µM, 100µM). (E) Mitochondrial apoptotic proteins were analyzed using western blotting in U87 cells treated with BBR-Glu (50µM), BBR (50µM) and 5% glucose solution for 48h. Each point represents Mean±SD of three experiments. *p<0.05, **p<0.01.

Supplementary Fig.S2



TEM appearances of BBR-Glu nanoparticles and its roles in the U87 mitochondrial functions. (A) Co-localization of BBR-Glu nanoparticles and mitochondria was observed in U87 glioma cells treated with BBR-Glu solution (50µM) for 48h. (B) TEM image exhibited the appearance of BBR-Glu nanoparticles, Glucose located the surface of BBR nanoparticles (Scale Bar=500nm). (C) TEM images exhibited the change of mitochondrial structure in U87 cells treated with BBR-Glu (50µM) and 5% glucose solution for 48h (Bar=500nm). (D) Mitochondrial ATPase activity was suppressed by 5% glucose, BBR-Water (50µM), BBR-Glu (50µM) solution at 48h. (E) High level of ROS generation was induced by 5% glucose, BBR-Water (50µM), BBR-Glu (50µM) solution treatment for 48h. Each point represents Mean±SD of three experiments.

Α Rotary heating at 68 $^\circ\!\mathrm{C}$ for different time in 5% GLu solution with BBR 1 h 15 min 30 min 3 h В Rotary heating at 68 $^\circ\!\!C$ for 2 h, then stand at room temperature for 1 h ddH₂O-BBR 0.1% GLu-BBR 1% GLu-BBR 5% GLu-BBR С Rotary heating at 68°C for 2 h, then stand at room temperature for 1 h ddH₂O-BBR 0.1% GLu-BBR 1% GLu-BBR 5% GLu-BBR 400 nm 400D Solutions standing at room temperature for 2 weeks ddH₂O-BBR 5% GLu-BBR

200 nm

The appearance of BBR nanoparticles in various concentrations of glucose solutions and at different times.

(A) Rotation with a small motor rotor and heating at 68°C at the different times in 5% glucose solution (15min, 30min, 1h, 3h), the appearance of BBR was changed, from tiny fibers to less polygonal and smaller size (400X magnification). (B) Rotation with a small motor rotor and heating at 68°C in various concentrations of glucose solution (ddH₂O, 0.1% Glu, 1% Glu, 5% Glu) for 2 h, the BBR nanoparticles became homogenized and smaller (400X magnification). (C) TEM data indicated the real size of BBR nanoparticles dissolved in different solutions for 2h. (D) The stability and appearance of BBR nanoparticles in ddH₂O and 5% Glu solutions for 2 weeks at room temperature, respectively. TEM observations were performed by a Hitachi 7700, AT 100 kV (Hitachi High Technologies America Inc., Dallas, TX, USA) for three-time replications.

Supplementary Fig.S4



The BBR and mitochondria distribution in different glioma cell lines. Mitochondria was detected by mito-tracker (deep-red dye, Excitation:633nm), BBR was detected using Excitation:488 nm (Green). most co-location was explored between BBR and mitochondria for 6h addition (630X magnification). All the data were repeated for three times.



The neuron specific Class III ß-Tubulin was detected in different times in BBR-water solution. The length of cytoskeleton was shortened but without obviously decreased immunofluorescence in 24h (400X magnification). All the data were repeated for three times.



50 µm of BBR-Glu in U251 cells

The neuron specific Class III ß-Tubulin was detected in different times in 5% Glu-BBR solution. After 6h, the length of cytoskeleton was shortened and with apparently decreased immunofluorescence (400X magnification). All the data were repeated for three times.

Supplementary Fig.S7



The cleavage caspase-3 was performed in different groups using immunofluorescence analysis (50µM BBR for 12h, 400X magnification). More cleavage caspase-3 was detected in BBR-Glu group. All the data were repeated for three times.