Materials and Methods

Materials

^{64}Cu was produced on a CS-15 biomedical cyclotron at Washington University School of Medicine (St. Louis, MO). 1,4,7,10-Tetraazadodecane-N,N',N'',N'''-tetraacetic acid (DOTA) was obtained from Macrocyclics (Dallas, TX). The BIACore sensor chip L1, HEPES buffered saline (HBS) running buffer (0.01 M HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], pH 7.4, 0.15 M NaCl), and regeneration buffer were purchased from BIACORE, Inc. (Piscataway, NJ). All the other chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO).

Synthesis and Characterization of Bis-DOTA-Hypericin

Synthesis of triacetylated emodin (2). One hundred milligrams (0.385 mmol) emodin (**1**) was added to a mixture of 2.5 mL of acetic anhydride and 2.5 mL pyridine. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated, and resulting yellow solid was recrystallized from dichloromethane(DCM)/ethanol, washed with water, and dried under reduced pressure. The triacetylated emodin (**2**) was obtained in 90% yield, high resolution-mass spectroscopy (HR-MS), m/z = 397.0886 for $[M+H]^+$ (calculated for $C_{21}H_{17}O_8$, $[M+H]^+$ 397.0923).

Synthesis of triacetylated emodin acid (3). Compound **2** (0.2 g, 0.5 mmol) was dissolved in a mixture of 2.5 mL CH_3COOH and 2.5 mL acetic anhydride at 55–60°C. A solution of 0.5 g CrO_3 in a mixture of 2.5 mL H_2O and 35 mL CH_3COOH was added dropwise over 30 min. The mixture was stirred at 70°C for 3 h during which the brown solution turned green. It was then added to 1.0 L of water, and the mixture was cooled overnight at 4°C to precipitate the

desired product (**3**) as a yellow powder in 90% yield. The powder was washed thoroughly with H_2O and dried over P_2O_5. HR-MS of the triacetylated emodin acid (**3**), m/z = 875.1221 for $[2M+Na]^+$ (calculated for $C_{21}H_{15}O_{10}$, $[M+H]^+$ 427.0665).

Synthesis of bis-protohypericin carboxylic acid (4). Compound **1** (0.1 g, 0.37 mmol), compound **3** (0.32 g, 0.75 mmol), and hydroquinone (0.162 g, 1.5 mmol) were dissolved in 5 mL of 0.8 M KOH, protected from light in a capped vial placed in a heavy steel reaction vial holder. The vial was placed in an oven at 155°C and allowed to react for 5 days during which it was agitated every 24 h to allow mixing. After cooling, the reaction mixture was acidified to pH 4-4.5 with 1 M HCl; and the dark-colored precipitate was separated by centrifugation, after which the filtrate was dissolved in methanol and purified by flash column chromatography with gradient elution starting from 100% CH_2Cl_2 to CH_2Cl_2/MeOH (80:20 V/V) to afford deep purple solid **4**. HR-MS, m/z = 565.0437 for $[M-H]^-$ (calculated for $C_{30}H_{13}O_{12}$, $[M-H]^-$ 565.0407).

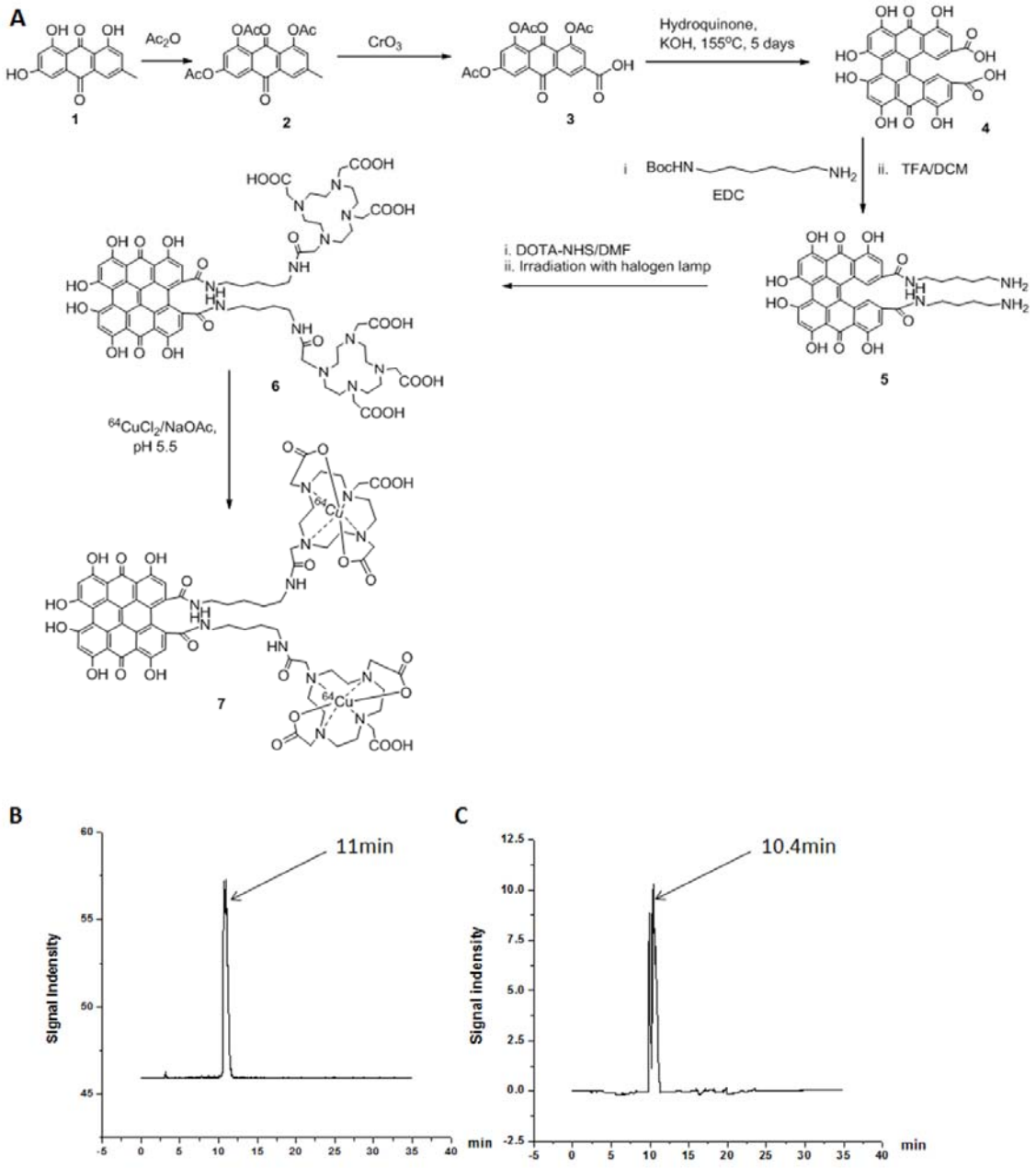
Synthesis of N,N'-bis(6-aminohexyl)-proto-hypericin dicarboxamide (5). Compound **4** (150 mg, 0.55 mmol) was dissolved in a mixture of 1 mL dimethylformamide (DMF) and 1 mL of pyridine, and 105 mg of ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.55 mmol) and 202 mg of N-Boc-1,5,-diaminopentane were added sequentially. The mixture was stirred at room temperature overnight. After evaporation of the solvent, the residual was treated with 90% trifluoroacetic acid (TFA) in dichloromethane (DCM), and the final product was purified by high-performance liquid chromatography (HPLC) to afford the titled compound **5**. HR-MS, m/z = 733.2541 for $[M-H]^+$ (calculated for $C_{40}H_{37}N_4O_{10}$, $[M-H]^-$ 733.2510).

Synthesis of bis-DOTA-hypericin dicarboxamide (6). The 100 mg (0.1 mmol) of **5** was conjugated with equivalent amount of DOTA-N-hydroxysuccinimide (DOTA-NHS) in

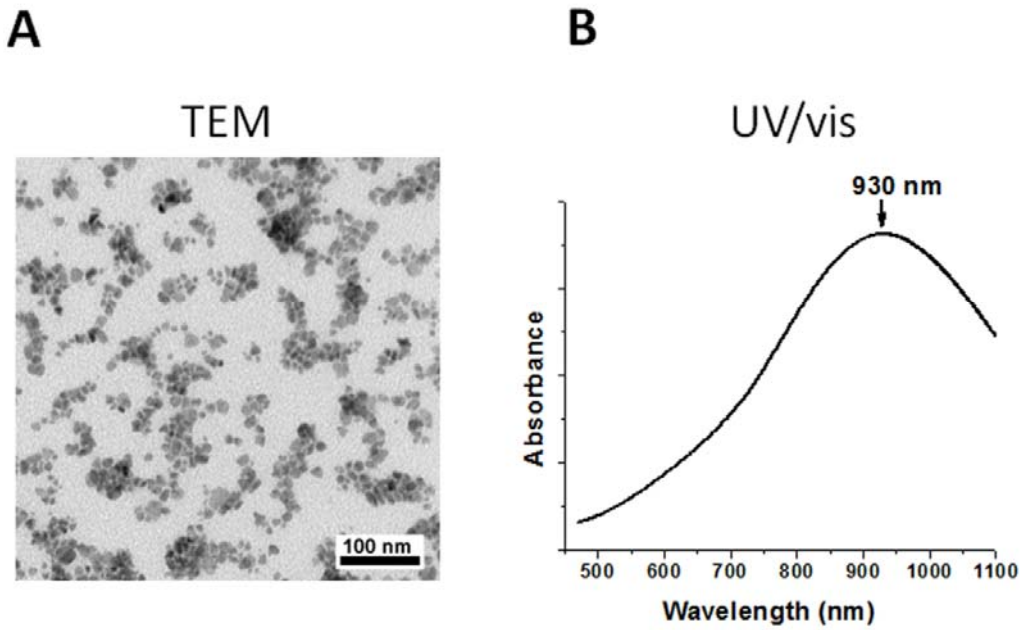
DMF/pyridine(1:1 v/v) at room temperature overnight. After the evaporation of the solvent, the residue was dissolved in dry methanol and the solution was irradiated with a 400-W halogen lamp for 30 min to give crude compound **6**. This compound was further purified by reverse-phase high-performance liquid chromatography (RP-HPLC) on an Agilent 1100 system (C-18, Vydac, 4.6 × 250 mm, 10 μm) eluted with a linear gradient of 10%–90% acetonitrile in a 0.1% aqueous TFA solution over 35 min at a flow rate of 1.0 mL/min. HR-MS, m/z = 1503.5952 for $[M-H]^-$ (calculated $C_{72}H_{87}N_{12}O_{24}$, $[M-H]^-$ 1503.5956).

Cell Uptake-Blocking Experiment

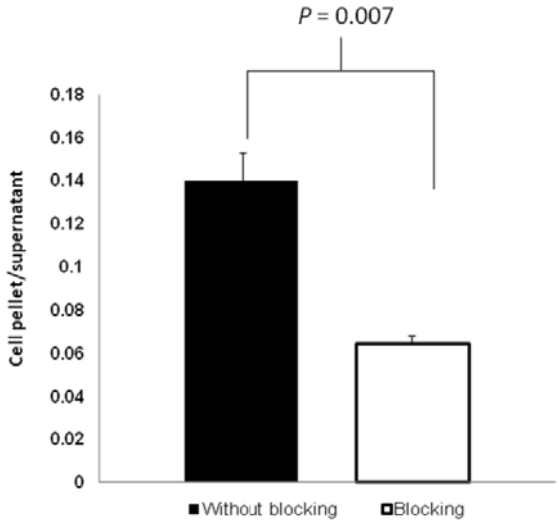
BT474 cells in a 12-well plate were incubated with ~3×1011 CuS NP/mL in 0.2 ml of DMEM medium at 37°C for 2 h. Cells were washed once with phosphate-buffered saline, and then DMEM medium supplemented with 10% FBS without phenol red was added. Cells were treated with the NIR at a dose of 12 W/cm^2 for 3 min. After irradiation, the cells were cultured with fresh complete DMEM medium supplemented with 10% FBS for 24 h. The cells were then scraped and transferred into 2-mL tubes and co-incubated with 0.37 MBq/μL (10 μCi/μL) ^{64}Cu-bis-DOTA-hypericin and cold hypericin (1mM) in 600 μL methanol at 37°C for 1 h. Next, 500 μL of a 75:25 mixture of silicon oil (density 1.05; Sigma-Aldrich) and mineral oil (density 0.872; Acros, Geel, Belgium) were added into the cell suspension. The mixture was centrifuged at 14,000 rpm for 10 min. The tubes were frozen in liquid nitrogen, the bottom tips containing the cell pellet were cut off, and the cell pellets and the supernatants were collected and counted for radioactivity. The data were expressed as activity ratios of the cell pellet to the medium [(cpm/ug protein in pellet/cpm/ug medium) × 100%].



Supplemental Figure 1. (A): Synthesis scheme for bis-DOTA-hypericin and ^{64}Cu-bis-DOTA-hypericin. (B) and (C): High-performance liquid chromatography analysis of ^{64}Cu-bis-DOTA-hypericin showing high labeling efficiency. The radiotracer was detected using a radiodetector (B) and a UV/Vis detector at 254 nm (C).



Supplemental Figure 2. Characterization of CuS-NPs. (A): TEM image of CuS NPs. (B): Extinction spectrum of CuS NPs.



Supplemental Figure 3. Blocking study of binding of ^{64}Cu-bis-DOTA-hypericin to apoptotic BT474 cells. BT474 cells were treated with CuS NPs and near-infrared laser to induce cell death. A significant decrease in cell-associated radioactivity was observed in cells co-incubated with cold ^{64}Cu-bis-DOTA-hypericin and hypericin (blocking) as compared to ^{64}Cu-bis-DOTA-hypericin alone.