Supporting information

Monitoring Isoniazid Metabolism In Vivo Using a Near-Infrared Fluorescent Probe

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1. Experimental section

1.1. Materials and instruments

Reagents/Instruments	Purity/Model	Manufacturer
Acetyl chloride	98%	Aladdin Chemical (Shanghai, China)
MTT	/	KeyGen BioTech (Nanjing, China)
Trypsin-EDTA solution	0.25%	Basifi BioTech (Nanjing, China)
Fetal bovine serums	/	Basifi BioTech (Nanjing, China)
DMEM	/	KeyGen BioTech (Nannjing, China)
DMSO	/	KeyGen BioTech (Nannjing, China)
Electronic balance	BS124S	Sartorius Instrument (Beijing, China)
Magnetic stirrer	85-2B	Sile Instrument (Shanghai, China)
UV-vis spectrophotometer	Lambda 25	Perkin Elmer, USA
NMR instruments	AV500	Bruker, USA
Mass spectrometer	/	Thermo fisher, USA
Fluorescence spectrophotometer	LS-55	Perkin Elmer, USA
Constant temperature incubator	/	Thermo Scientific，USA
Confocal laser microscopy	FV 1000	Olympus，Japan

1.2 In vitro spectral Measurement

Mother liquor with 1 mM probe Cy-HZ was prepared with Dimethyl sulfoxide and mother liquor with 20 mM hydrazine hydrate was prepared with pure water.

The time-dependent response curve. Extract 30 μL the mother liquor of the probe Cy-HZ and diluted to 10 μM. In the presence of the concentration of hydrazine was 0 μM, 100 μM, 200 μM, 400 μM, the fluorescence intensity of the probe at 737 nm was measured, respectively in 0 min, 1 min, 3 min, 5 min, 7 min, 9 min, 11 min, 13 min, 15 min, 17 min, 20 min.

The concentration-dependent response curve. Hydrazine solutions with different concentrations of 0 μM, 30 μM, 50 μM, 70 μM, 100 μM, 120 μM, 150 μM, 180 μM, 200 μM, 250 μM, 300 μM, 350 μM, 400 μM were set up respectively. Then, the probe (10 μM) incubated with above concentration of hydrazine for 10 min. The corresponding fluorescence intensity at 737 nm was measured.

The pH-dependent response curve. Under the condition of probe concentration 10 μM and hydrazine hydrate concentration 400 μM, different pH test groups were arranged (pH = 4, pH = 5, pH = 6, pH = 7, pH = 8, pH = 9, pH = 10). The fluorescence intensity of the probe at 737 nm was measured.

The selective experiment of the probe. Configuration 400 μM hydrazine, while configuration 1 mM aniline, isoniazid, L-phenylalanine, L-cysteine, glutamic acid, L-aspartic acid, tyrosine, serine, phthalamide, thioacetamide, benzoylhydrazine, SO_4^{2-}, Cu^{2+}, Ca^{2+}, Cl^-, NO_2^-, Al^{3+}, Zn^{2+}, carbidopa. Then, the probe (10 μM) incubated with above solutions for 10 min. The corresponding fluorescence intensity at 737 nm was measured.

1.3 Cytotoxicity test

The logarithmic phase cells (L02, HepG2 cells) were collected and mixed evenly in the medium. The mixed cells were then added to 96-well plates at the volume of 100 μL per hole, and the edges of 96-well plates were filled with water and then the cells were incubated for 24 hours at 37 °C in 5% CO_2 cell incubator until the monolayer of cells was filled with the bottom of the pores, and then probe were added. The probe Cy-HZ was dissolved in DMSO and diluted to 0 μM, 6 μM, 12 μM, 18 μM, 24 μM, 30 μM, 36 μM, 42 μM, 48 μM, 54 μM gradient with cell medium. Then, add 200 μL per hole, set 6 holes to prevent experimental error and incubate for 24 hours at 37 °C in 5% CO_2 cell incubator. Then 20 μL MTT solution (5 mg/mL) was added to each hole and cultured for 4 hours. 4 hours later, carefully sucked out the culture solution, and then added 150 μL DMSO into each hole. Placed the 96-hole plate on a shaker and shake for 30 minutes. The absorbance value was measured by enzyme-labeled instrument. The viability of cell growth was calculated by using the following formula: viability (%) = (average absorbance of test pore - average absorbance of medium control pore) / (average absorbance of untreated pore - average absorbance of medium control pore) × 100%.

1.4 Cell co-localization experiment

HepG2 cells were collected in logarithmic phase, mixed evenly in the medium, then the cells (5×10^5) were added into the confocal microscope culture dish, and when the cell occupied about 80% of the confocal dish, the commercial mitochondrial-tracker (1 μM, λex = 488 nm) and lysosomal-tracker (1 μM, λex = 405 nm) and probe Cy-HZ (10

μM) were added and incubated for 20 mins. After incubation, the medium was sucked out, and washed three times with PBS, then fixed with 1mL 4% polyoxymethylene solution, and imaged with laser confocal microscope.

1.5 Imaging of hydrazine in cells

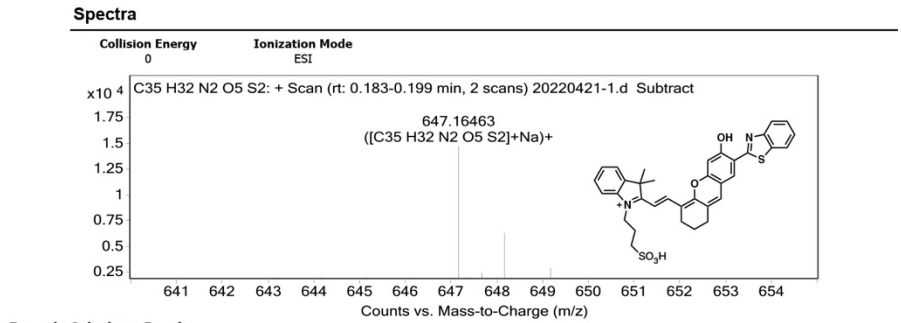
The logarithmic phase cells (L02, HepG2 cells) were collected, the cell suspension concentration was adjusted, the cells (5×10^5) were added into confocal culture dishes, the cells were cultured to 80% of the confocal dish, then the probe Cy-HZ (10 μM) was added. Different concentrations of hydrazine (0 μM, 100 μM, 200 μM and 400 μM) were added to different culture dishes, respectively. After co-incubation, the medium was sucked out, then washed three times with PBS, fixed with polyoxymethylene, and imaged with laser confocal imaging.

1.6 Imaging of hydrazine in vivo

To study the imaging of the probe to hydrazine in vivo, the control group was injected with 50 μM probe Cy-HZ only, and the experimental group was first injected with 50 μM probe Cy-HZ, then 200 μM hydrazine was injected intravenously into mice. And the in vivo imaging of hydrazine was observed at 5 min, 10 min, 20 min, 30 min, 60 min, 120 min, 180 min, 240 min and 360 min by using in vivo imaging system.

To investigate the distribution of drug metabolizing hydrazine in mice, the mice were first injected with 50 μM probe Cy-HZ intravenously and then given 200 μM isoniazid intragastrically. Using the probe Cy-HZ to monitor the distribution of drug metabolism hydrazine at 5 min, 10 min, 20 min, 30 min, 60 min, 120 min, 180 min, 240 min and 360 min by the in vivo imaging system.

1.7 HR-MS spectrum and NMR spectrum of Cy-H and Cy-HZ



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	CalculatedMz
C35 H32 N2 O5 S2	TRUE	624.17794	624.17526	-4.28	C35 H32 N2 Na O5 S2	647.16448

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Figure S1. HR-MS spectrum of Cy-H

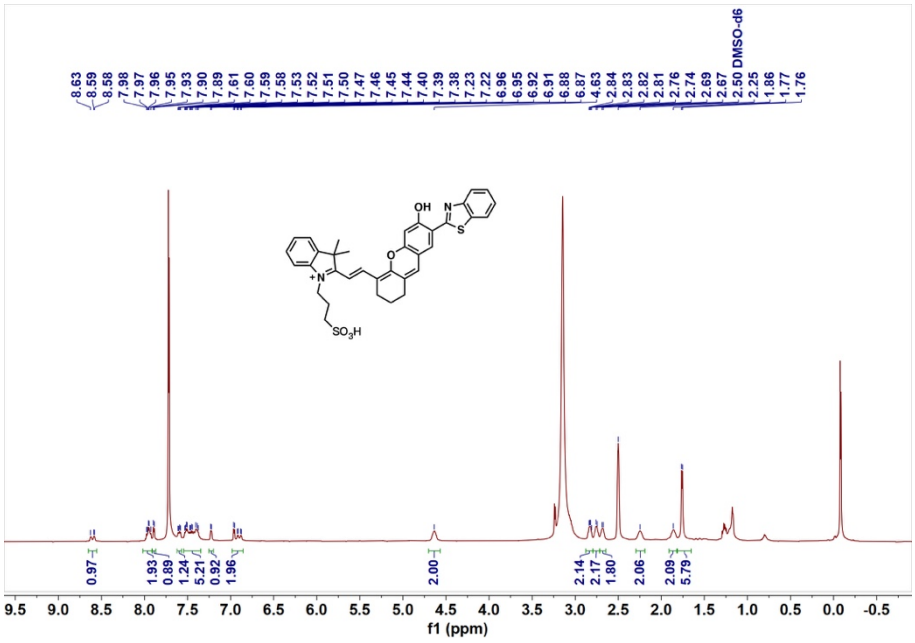
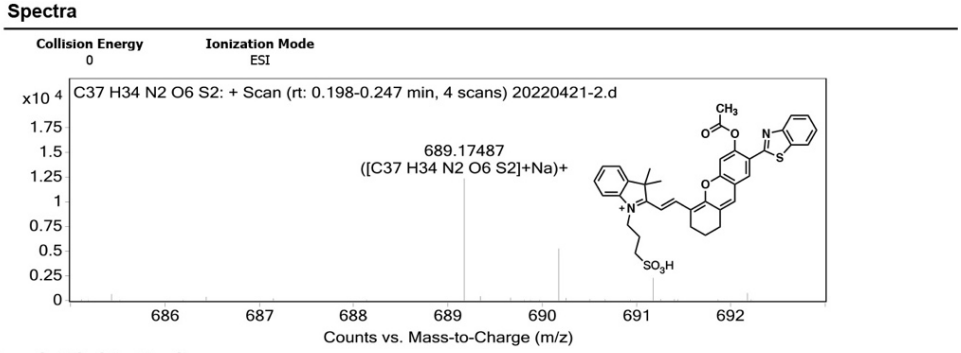


Figure S2. ^{1}H-NMR spectrum of Cy-H



Formula Calculator Results

Formula	Best	Mass	Tgt Mass	Diff (ppm)	Ion Species	CalculatedMz
C37 H34 N2 O6 S2	TRUE	666.18564	666.18583	0.29	C37 H34 N2 Na O6 S2	689.17505

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Figure S3. HR-MS spectrum of Cy-HZ

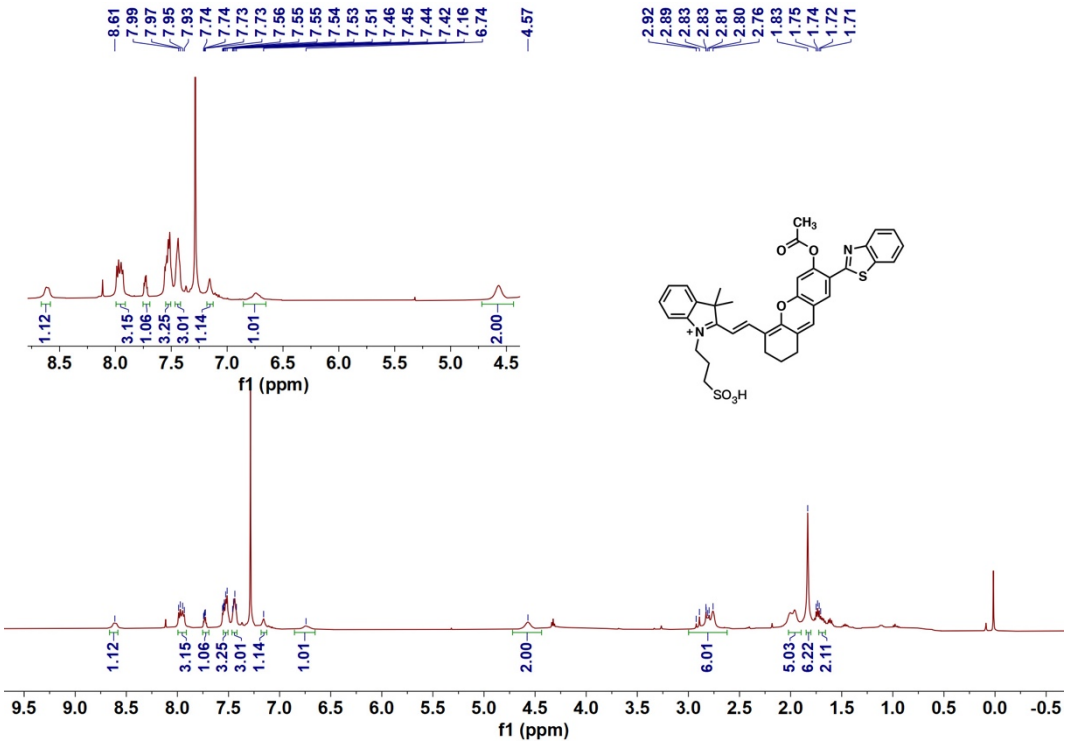

Figure S4. ^{1}H NMR spectrum of Cy-HZ

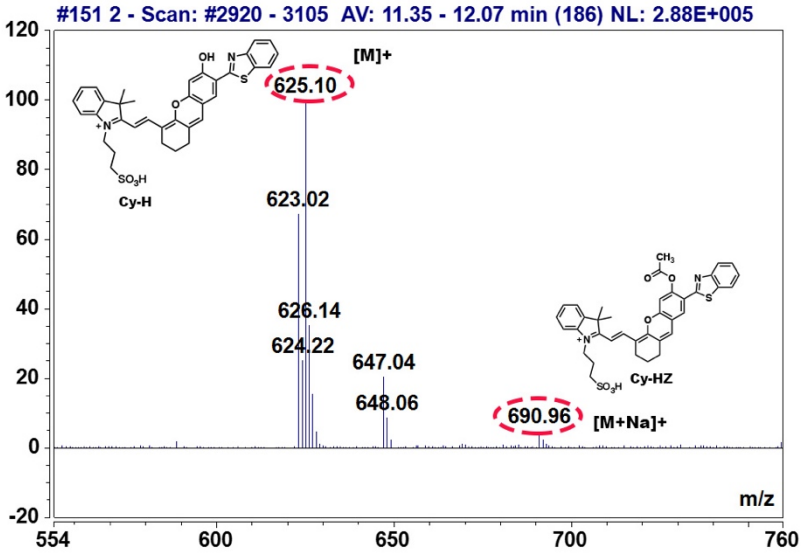

Figure S5. LC-MS spectrum of Cy-HZ reacted with hydrazine.

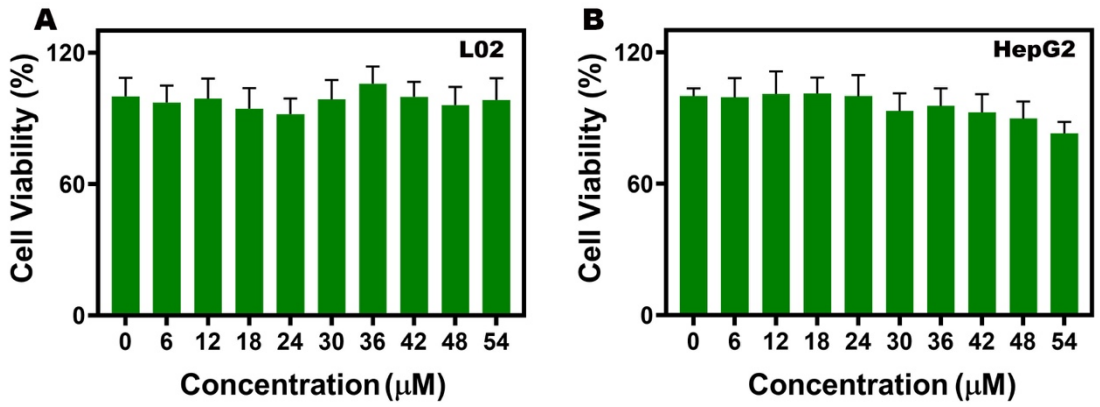

Figure S6. The cell viability of L02 (A) and HepG2 (B) cells with different concentration of Cy-H.