

## Supporting information

### **A NIR facile cell-compatible fluorescent sensor for glutathione based on Michael addition induced cascade spirolactam opening and its application in hepatocellular carcinoma**

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Figure S1.  $^1\text{H}$ NMR spectra of 4-Maleimidobenzoic acid in  $\text{CD}_3\text{OD}$ .

Figure S2.  $^1\text{H}$  NMR spectra of Compound  $\text{M}_2$  in  $\text{CDCl}_3$ .

Figure S3.  $^{13}\text{C}$  NMR spectra of Compound  $\text{M}_2$  in  $\text{CDCl}_3$

Figure S4.  $^1\text{H}$  NMR spectra of Compound  $\text{M}_3$  in  $\text{DMSO-d}_6$

Figure S5. HRMS spectrum of Compound  $\text{M}_3$ .

Figure S6.  $^1\text{H}$  NMR spectra of RhNM in  $\text{CDCl}_3$

Figure S7.  $^{13}\text{C}$  NMR spectra of RhNM in  $\text{CDCl}_3$

Figure S8. Mass spectra of RhNM in  $\text{CH}_3\text{OH}$ .

Figure S9. HRMS spectrum of the RhNM-GSH complex

Figure S10. The UV-vis spectra of RhNM ( $10\ \mu\text{M}$ ) in EtOH/PBS buffer solution (V/V, 3:2, pH = 7.4) after the concentration of GSH ( $0\text{-}5\ \mu\text{M}$ ) is added.

Figure.S11 (a) The fluorescence intensity of probe ( $10\ \mu\text{M}$ ) upon the addition of other amino acids ( $10\ \mu\text{M}$ ) in the presence of GSH ( $10\ \mu\text{M}$ ) in EtOH/PBS buffer solution (V/V, 3:2, pH = 7.4) (b) The linear curve of probe toward GSH

Figure.S12 Cell viability values was estimated by MTT assay. HepG2 cells were cultured in the presence of  $10\ \mu\text{M}$  probe for different time. Data are expressed as the mean $\pm$ SD

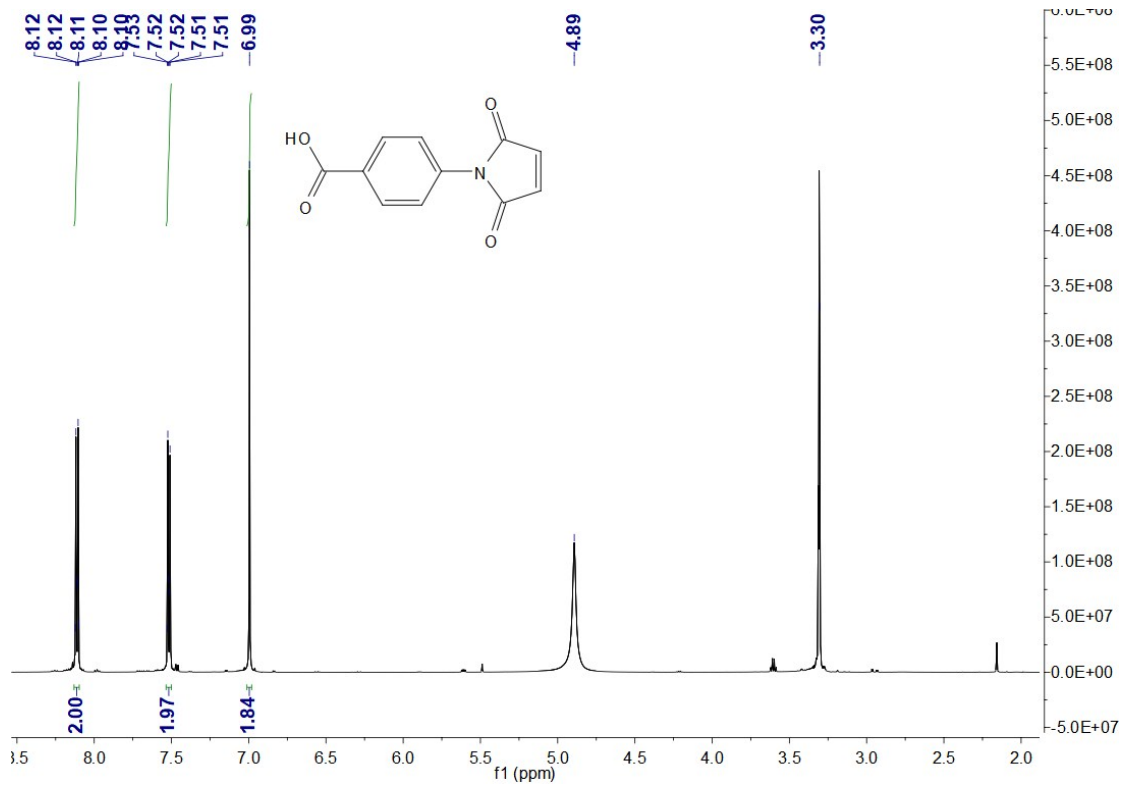


Figure S1. <sup>1</sup>H NMR spectra of 4-Maleimidobenzoic acid in CD<sub>3</sub>OD.

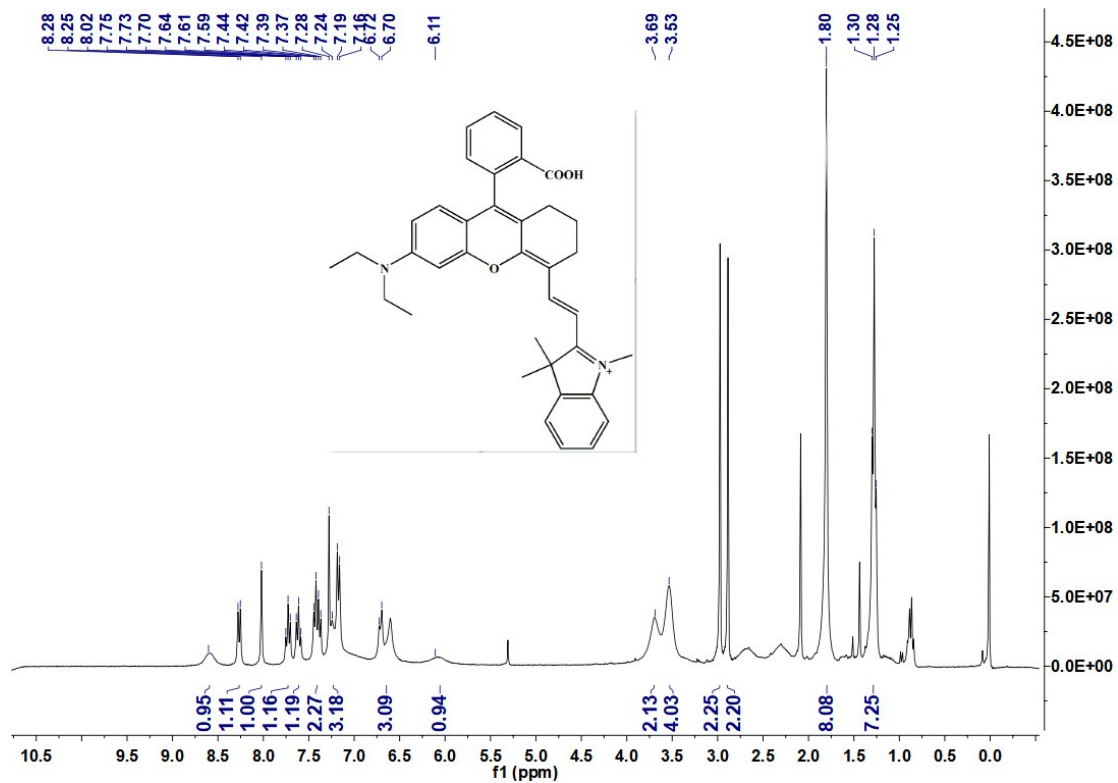


Figure S2. <sup>1</sup>H NMR spectra of compound M<sub>2</sub> in CDCl<sub>3</sub>.

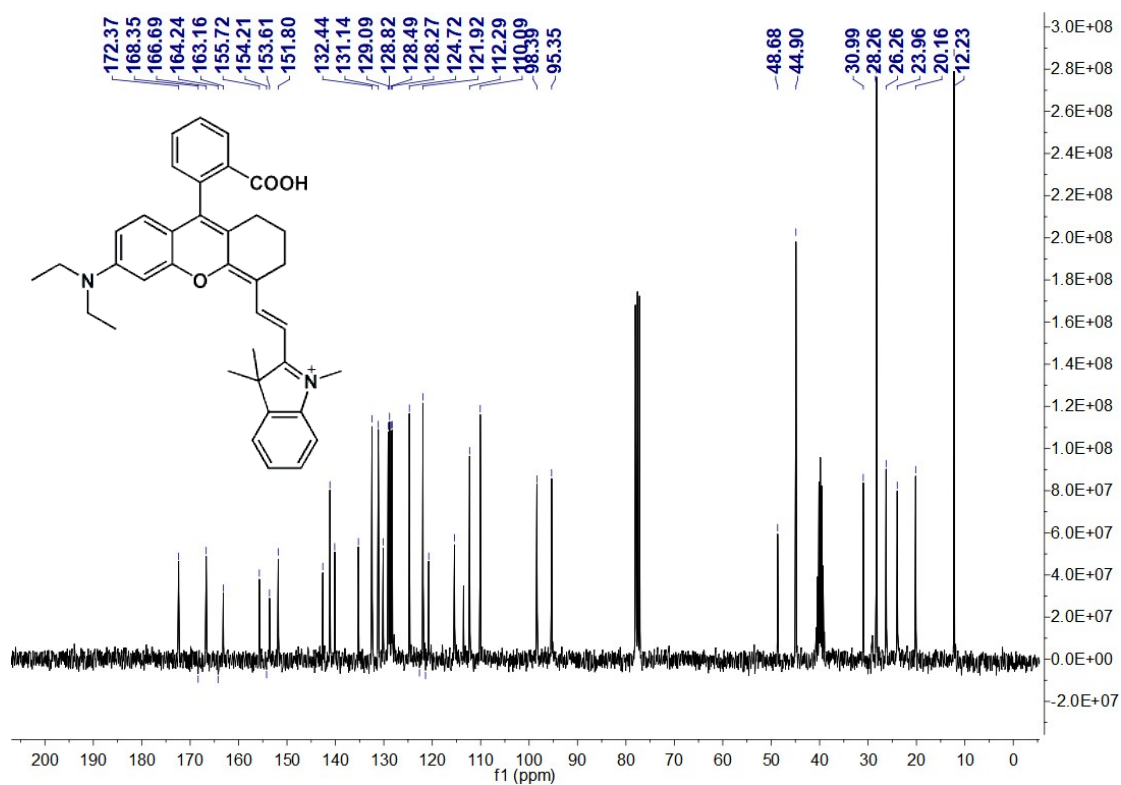


Figure S3. <sup>13</sup>C NMR spectra of compound M<sub>2</sub> in CDCl<sub>3</sub>.

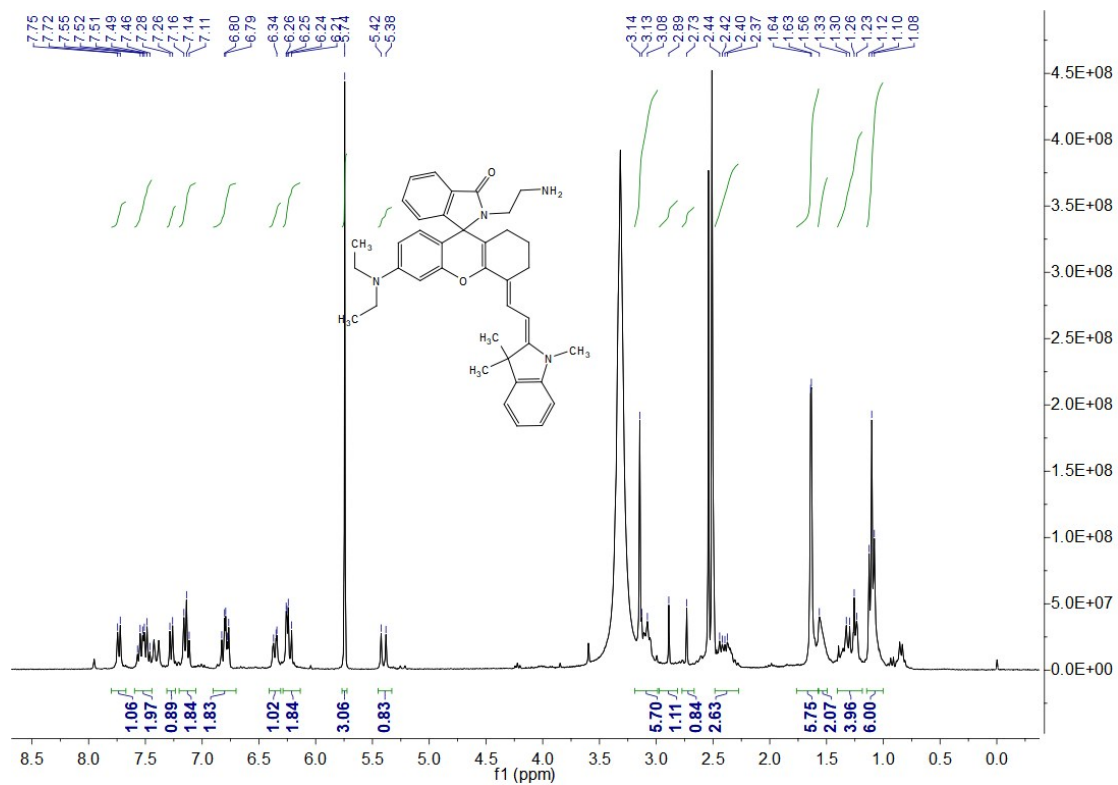


Figure S4. <sup>1</sup>H NMR spectra of compound M<sub>3</sub> in DMSO-d<sub>6</sub>

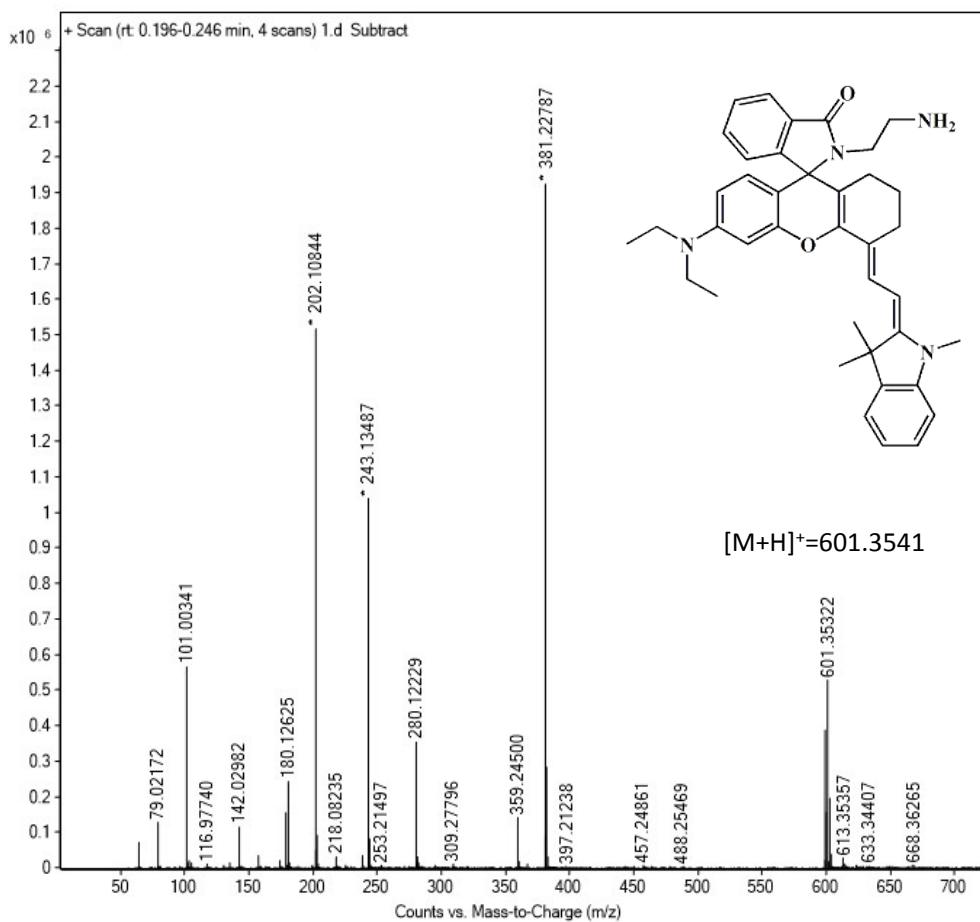


Figure S5. Mass spectra of Compound M<sub>3</sub>.

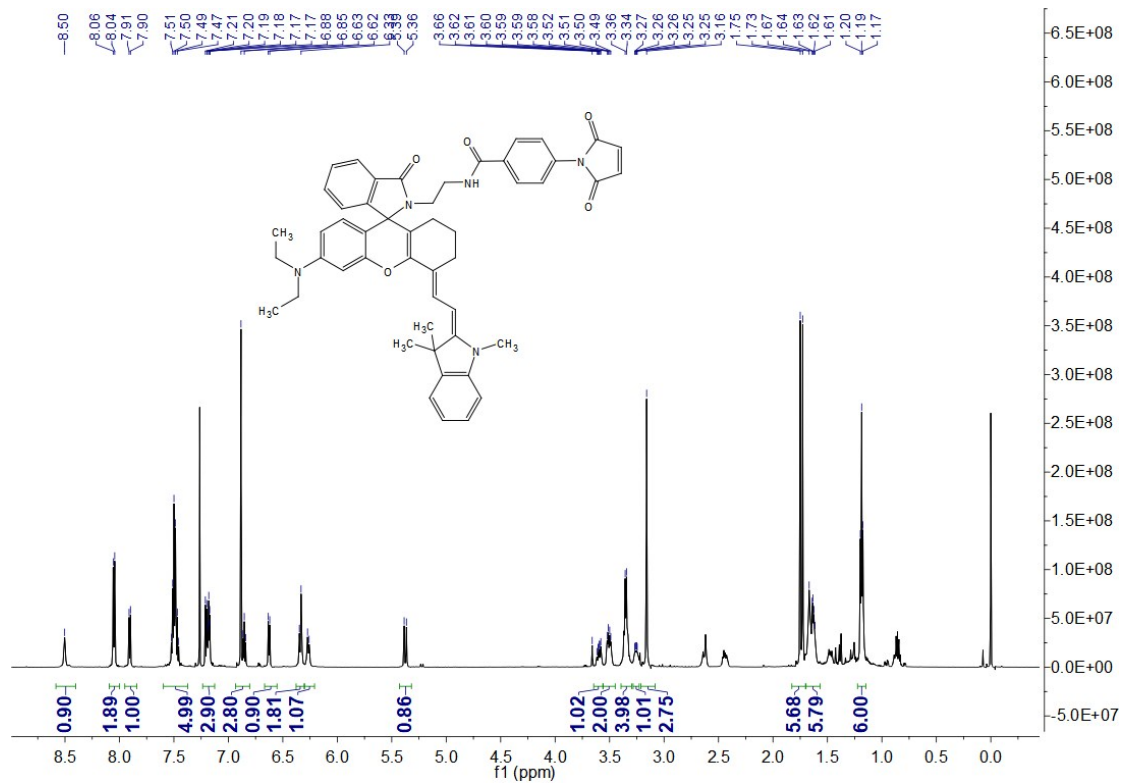


Figure S6. <sup>1</sup>H NMR spectra of RhNM in CDCl<sub>3</sub>

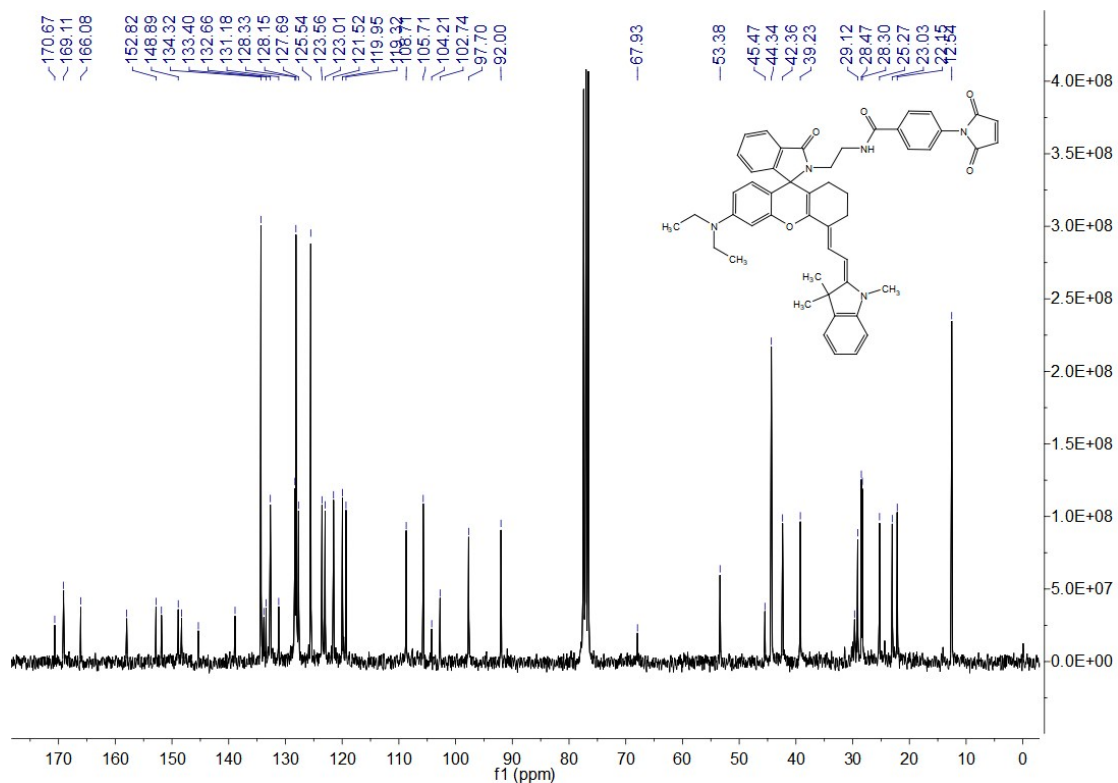


Figure S7. <sup>13</sup>C NMR spectra of RhNM in CDCl<sub>3</sub>

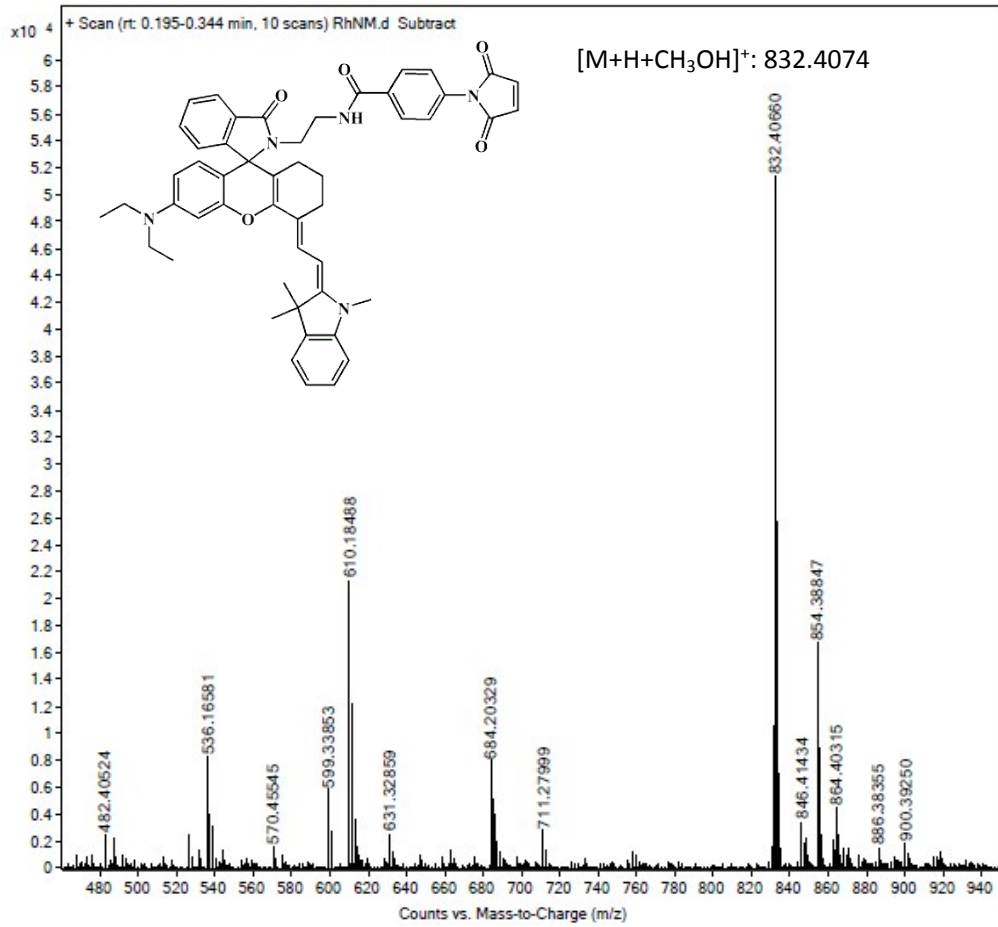
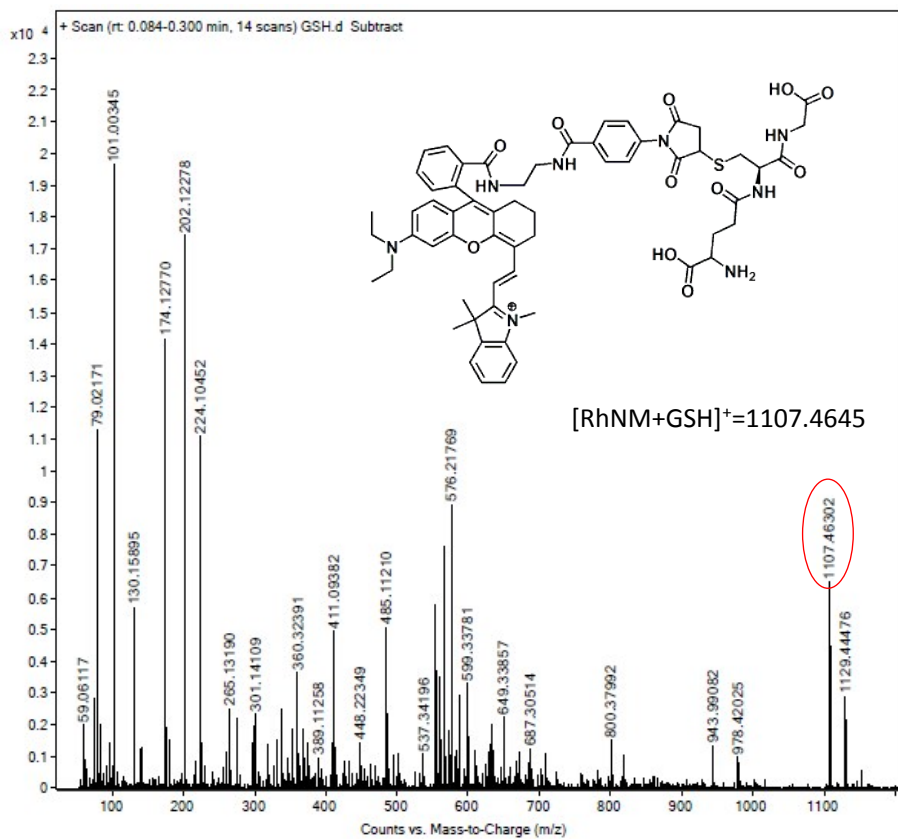
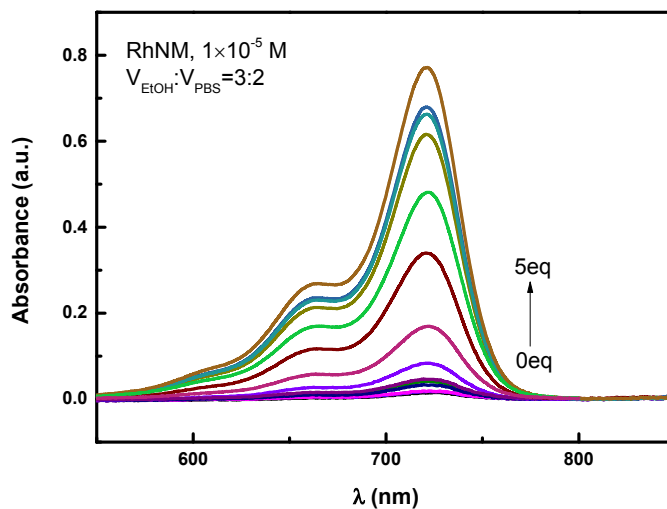


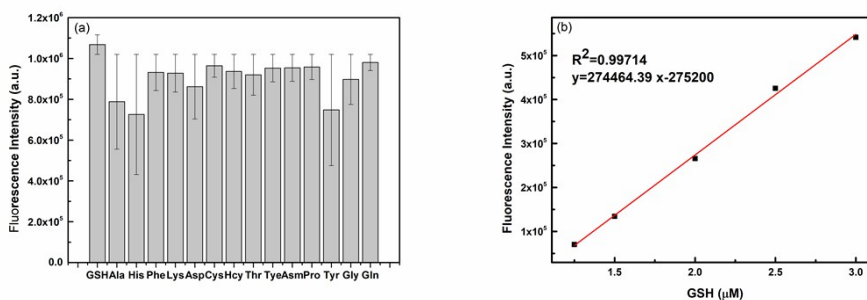
Figure S8. HRMS spectrum of RhNM in CH<sub>3</sub>OH.



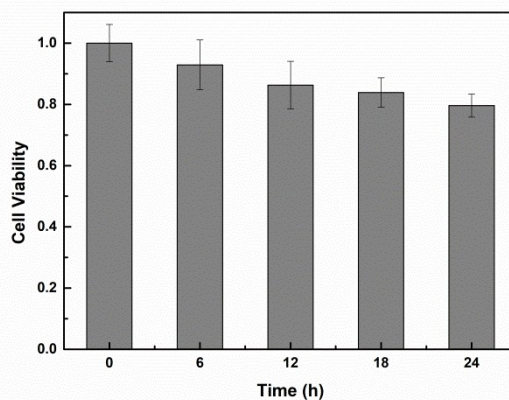
**Figure S9.** HRMS spectrum of the probe-GSH complex



**Figure. S10** The UV-vis spectra of RhNM (10  $\mu\text{M}$ ) in EtOH/PBS buffer solution (V/V, 3:2, pH = 7.4) after the concentration of GSH (0-5 $\mu\text{M}$ ) is added.



**Figure.S11** (a) The fluorescence intensity of probe (10  $\mu\text{M}$ ) upon the addition of other amino acids (10  $\mu\text{M}$ ) in the presence of GSH (10  $\mu\text{M}$ ) in EtOH/PBS buffer solution (V/V, 3:2, pH = 7.4) (b) The linear curve of probe toward GSH



**Figure.S12** Cell viability values was estimated by MTT assay. HepG2 cells were cultured in the presence of 10  $\mu\text{M}$  probe for different time. Data are expressed as the mean $\pm$ SD