

Supporting information for

Development of fast-responsive turn-on phosphorescence probe for
biothiols based on ruthenium(II) complex

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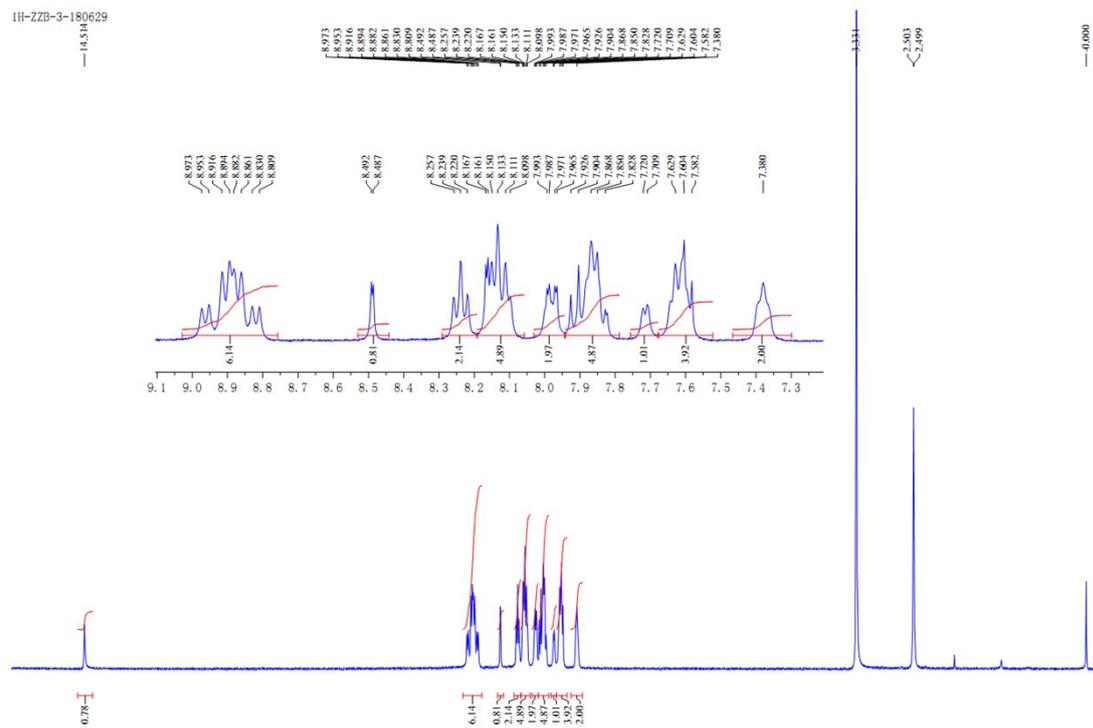


Fig. S1. ^1H NMR spectrum of Ru-BIPP-DNBS.

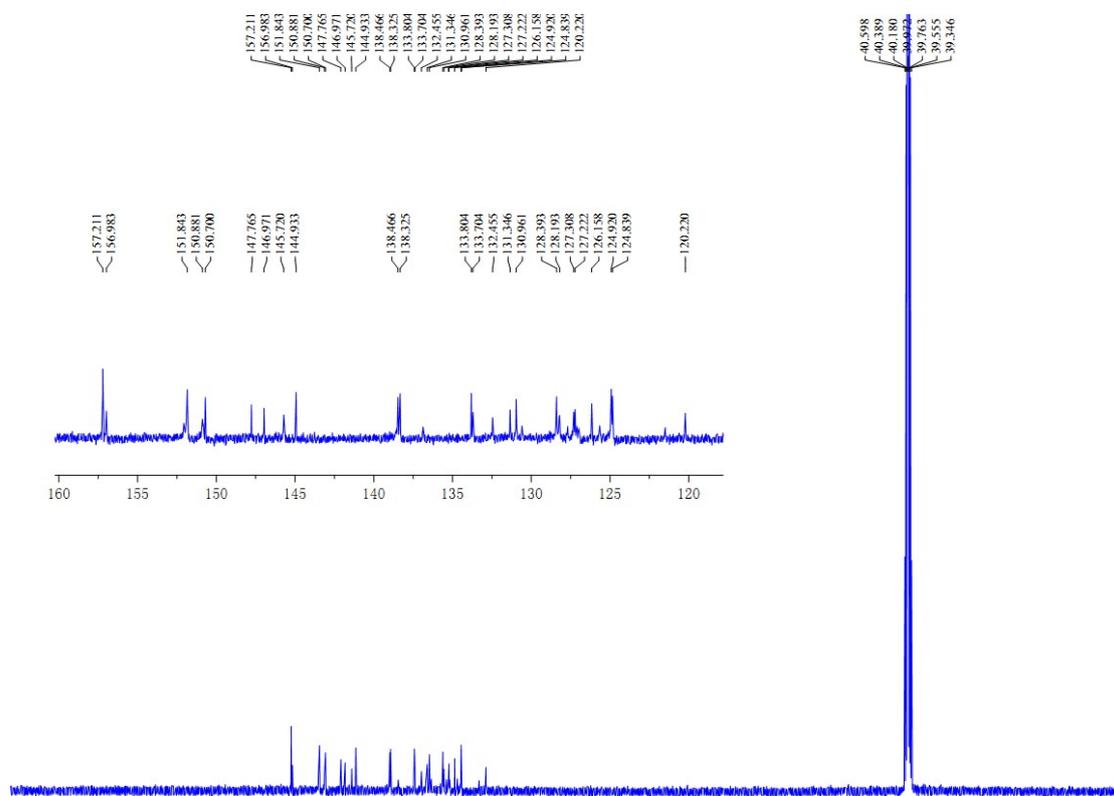


Fig. S2. ^{13}C NMR spectrum of Ru-BIPP-DNBS.

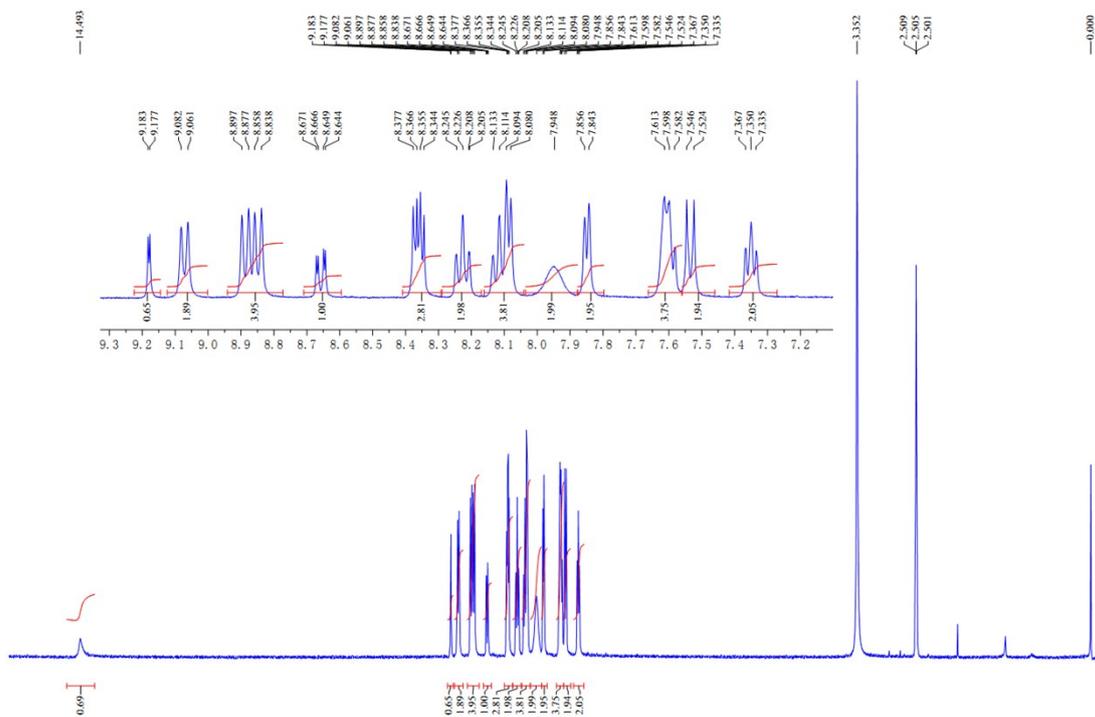


Fig. S3. ^1H NMR spectrum of Ru- *p*-hPIP-DNBS.

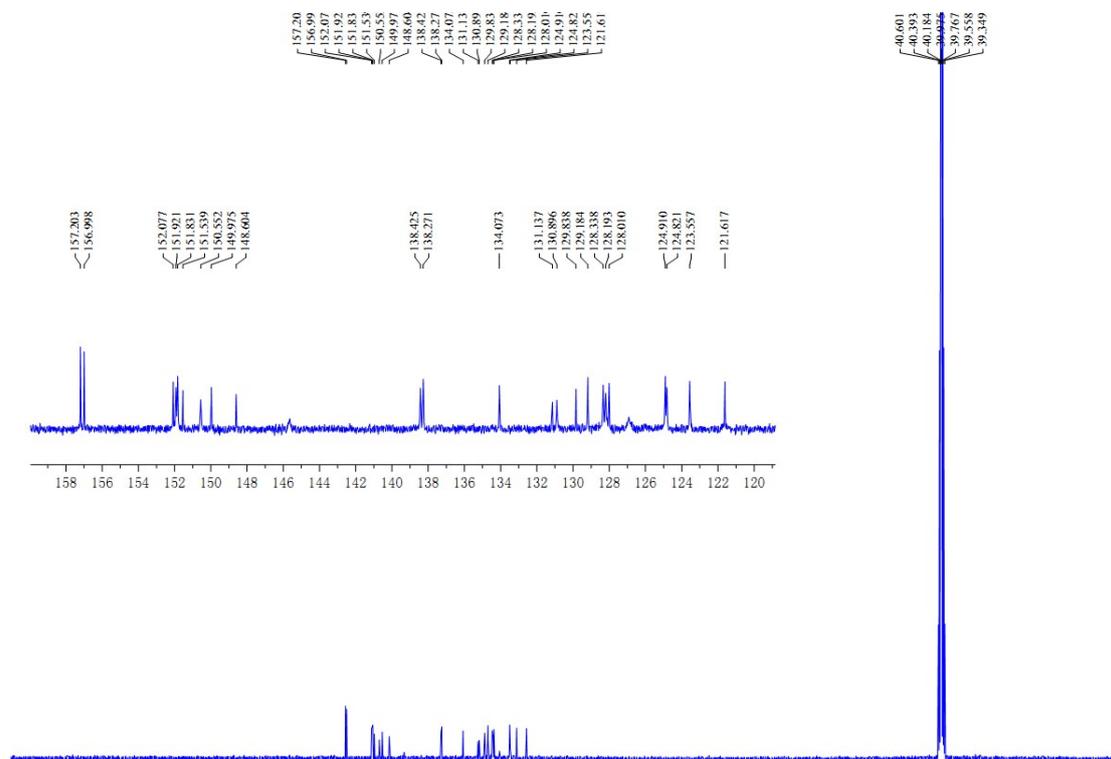


Fig. S4. ^{13}C NMR spectrum of Ru- *p*-hPIP-DNBS.

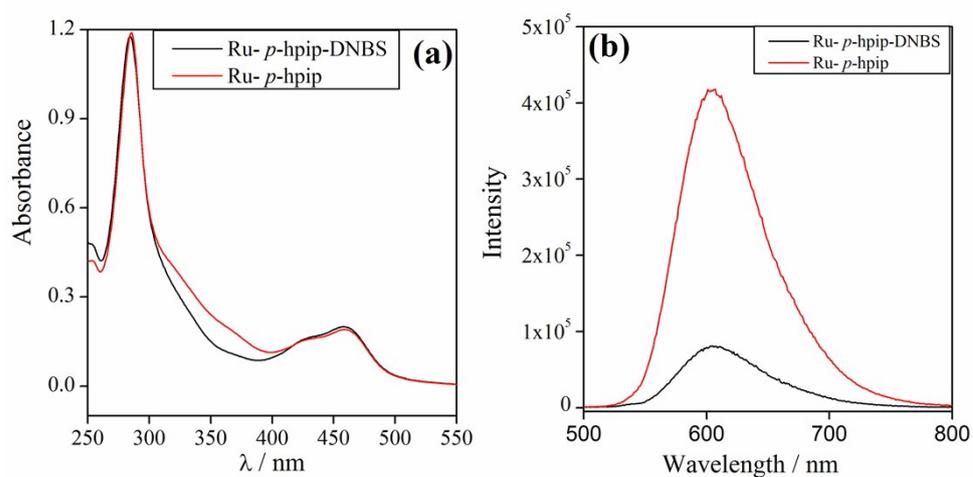


Fig. S5. UV-vis absorption spectra (a) and fluorescence spectra (b) of Ru-*p*-hPIP-DNBS and Ru-*p*-hPIP (10 μ M) in 20 mM HEPES-CH₃CN (100:1, pH 7.2).

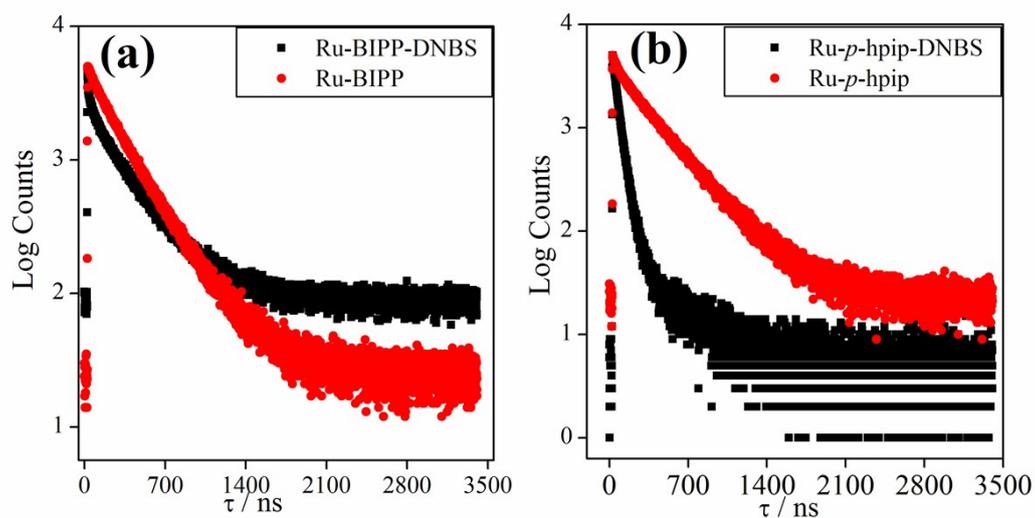


Fig. S6. Luminescence decay curves of 10 μ M Ru-BIPP-DNBS and Ru-*p*-hPIP-DNBS (a), 10 μ M Ru-*p*-hPIP-DNBS and Ru-*p*-hPIP (b) in HEPES (20 mM, pH = 7.2)/CH₃CN (100/1, v/v) solutions.

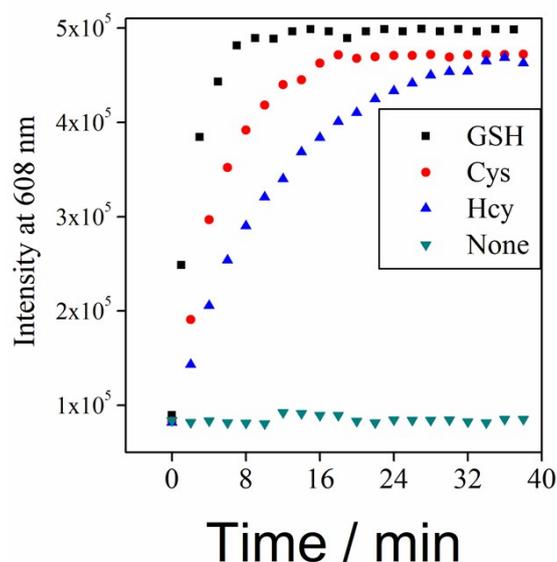


Fig. S7. Time-dependent phosphorescence intensity at 608 nm of **Ru- *p*-hPIP-DNBS** (10 μ M) in the absence and presence of 2.0 equiv. GSH, Cys, and Hcy in 20 mM HEPES-CH₃CN (100:1, pH 7.2). (λ_{ex} = 458 nm, slit 2.0 nm/2.0 nm)

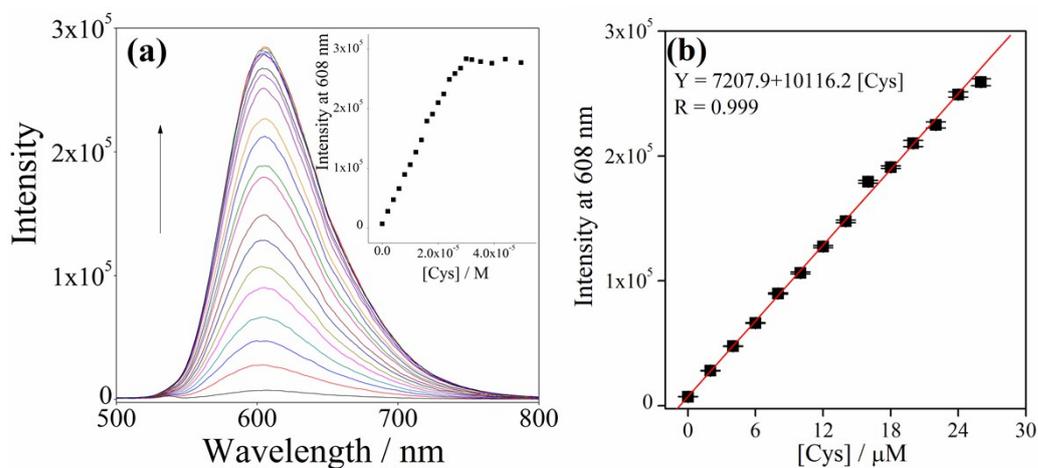


Fig. S8. (a) Emission spectra of **Ru-BIPP-DNBS** (10 μ M) in the presence of different concentrations of Cys in 20 mM HEPES-CH₃CN buffer (100:1, pH 7.2). The insets show changes in emission intensity at 608 nm (λ_{ex} = 458 nm, slit 2.0 nm/2.0 nm) vs. Cys concentrations. (b) The linear relationship between the **Ru-BIPP-DNBS** (10 μ M) phosphorescence intensity at 608 nm and Cys concentration (0.0-13.0 μ M). The error bars represent the standard deviation of three independent measurements.

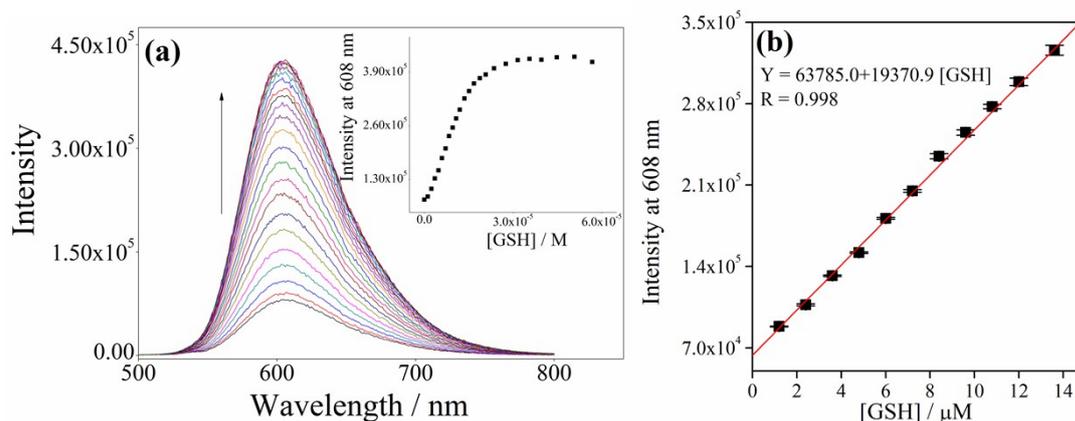


Fig. S9. Emission spectra of **Ru- *p*-hPIP-DNBS** (10 μM) in the presence of different concentrations of GSH in 20 mM HEPES- CH_3CN buffer (100:1, pH 7.2). The insets show changes in emission intensity at 608 nm ($\lambda_{\text{ex}} = 458$ nm, slit 2.0 nm/2.0 nm) vs. GSH concentrations. (b) The linear relationship between the **Ru- *p*-hPIP-DNBS** (10 μM) phosphorescence intensity at 608 nm and GSH concentration (0.0-13.0 μM). The error bars represent the standard deviation of three independent measurements.

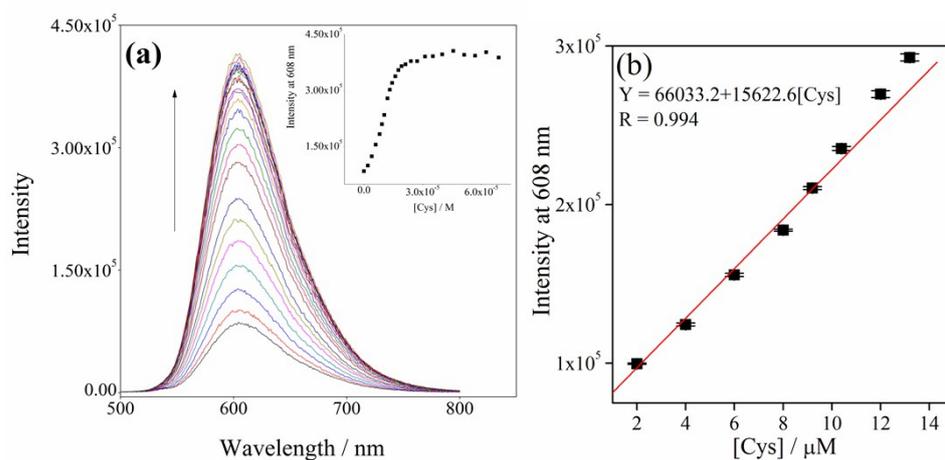


Fig. S10. (a) Emission spectra of **Ru- *p*-hPIP-DNBS** (10 μM) in the presence of different concentrations of Cys in 20 mM HEPES- CH_3CN buffer (100:1, pH 7.2). The insets show changes in emission intensity at 608 nm ($\lambda_{\text{ex}} = 458$ nm, slit 2.0 nm/2.0 nm) vs. Cys concentrations. (b) The linear relationship between the **Ru- *p*-hPIP-DNBS** (10 μM) phosphorescence intensity at 608 nm and Cys concentration (0.0-6.0 μM). The error bars represent the standard deviation of three independent measurements.

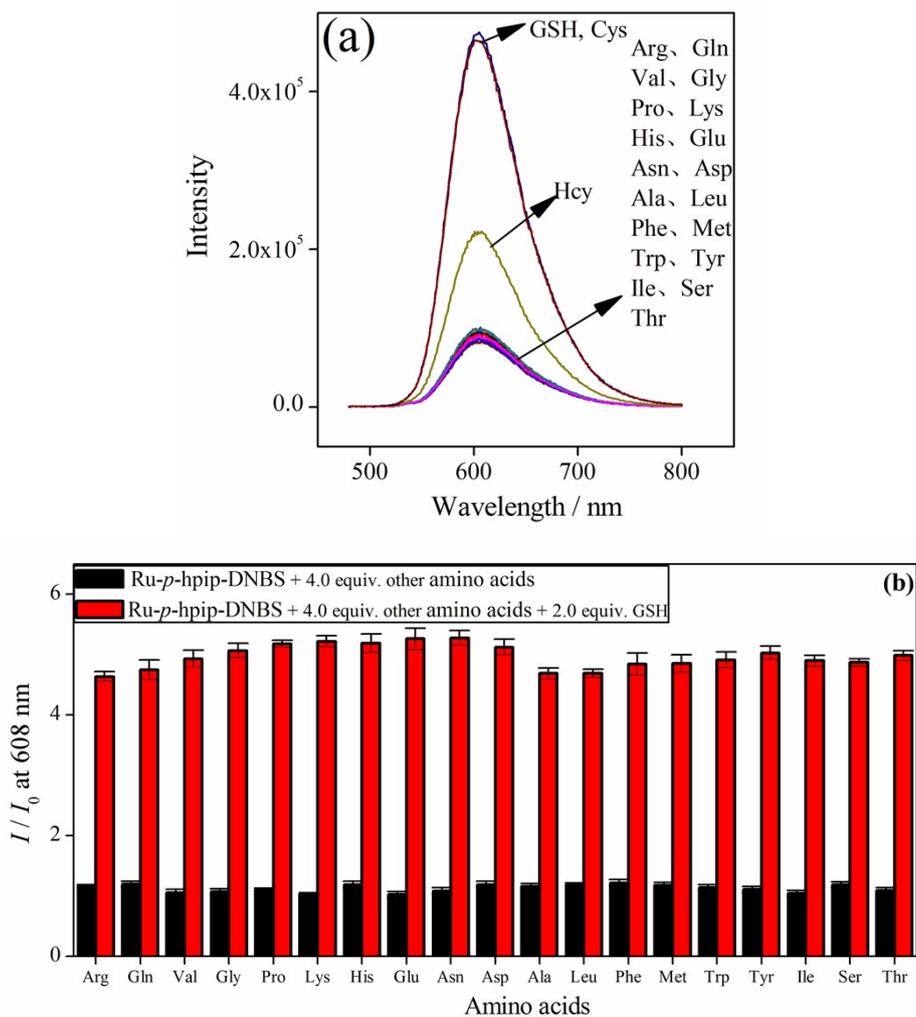


Fig. S11. (a) Emission spectra of Ru-*p*-h_{pip}-DNBS (10 μM) upon addition of 4.0 equiv. different amino acids including Arg, Gln, Val, Gly, Pro, Lys, His, Glu, Asn, Asp, Ala, Leu, Phe, Met, Trp, Tyr, Ile, Ser, Thr. All spectra were recorded in 20 mM HEPES-CH₃CN buffer (100:1, pH 7.2), $\lambda_{\text{ex}} = 458 \text{ nm}$, slit 2.0 nm / 2.0 nm. (b) Phosphorescence intensity ratios of Ru-*p*-h_{pip}-DNBS (10 μM) with GSH (2.0 equiv.) in the presence/absence of 4.0 equiv. various relevant amino acids in 20 mM HEPES-CH₃CN buffer (100:1, pH 7.2).

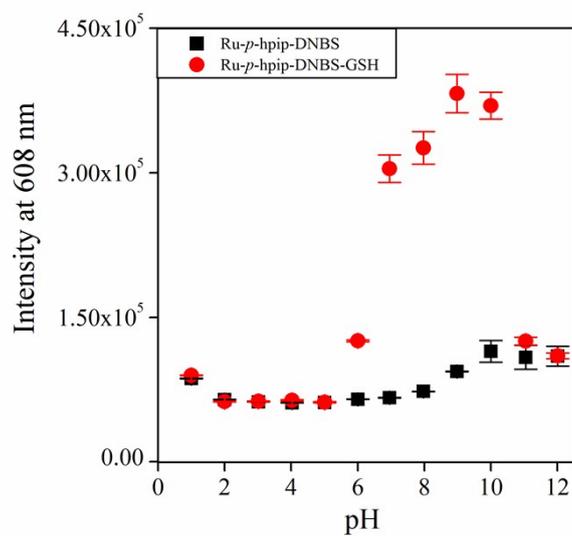


Fig. S12. Effects of pH on the phosphorescence intensities of **Ru- *p*-hip-DNBS** (10 μ M) in the absence / presence of GSH (2.0 equiv.) The error bars represent the standard deviation of three independent measurements.

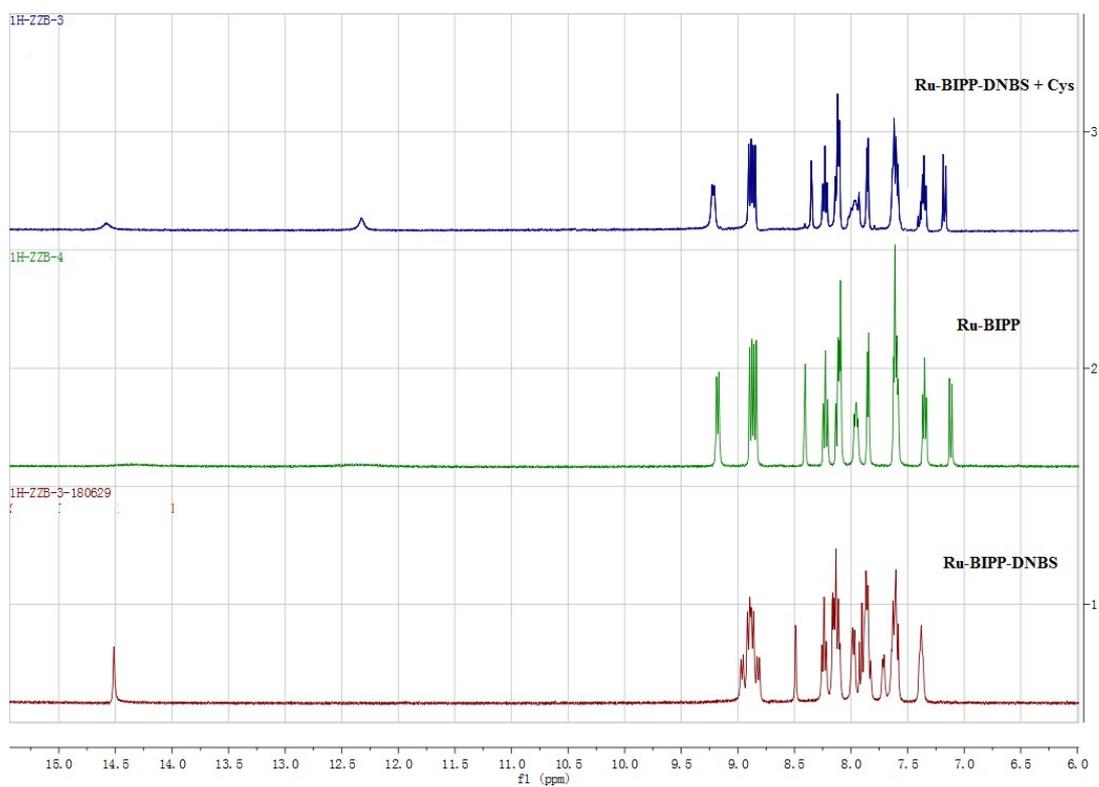


Fig. S13. Partial ^1H NMR spectra of **Ru-BIPP-DNBS**, **Ru-BIPP** and the reaction product of **Ru-BIPP-DNBS** with cys in d_6 -DMSO

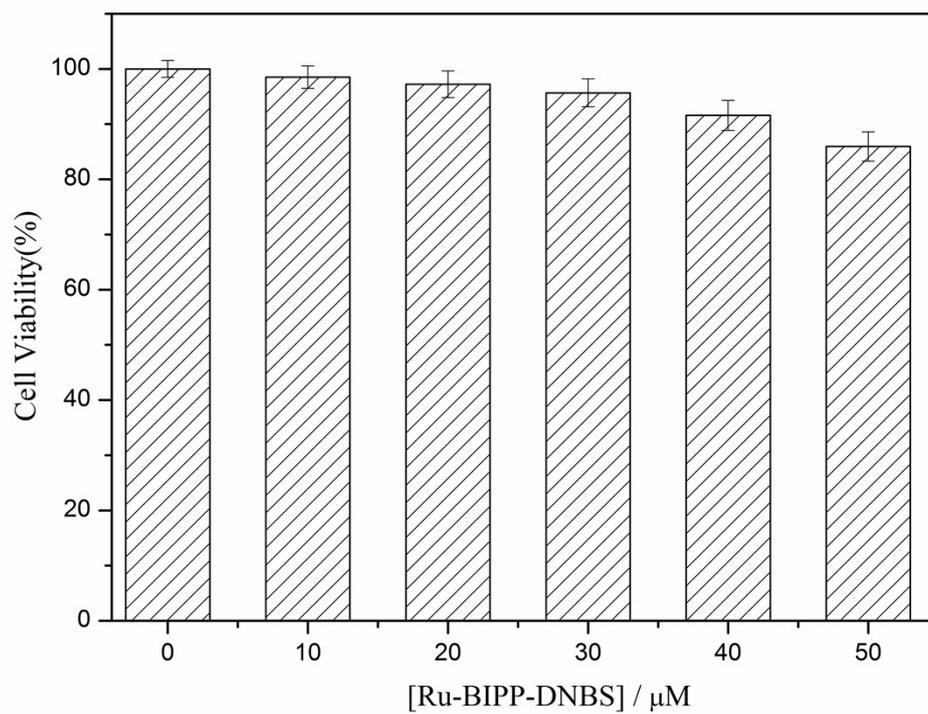


Fig. S14. Viabilities of Glioma cells incubated with different concentrations (0, 10, 20, 30, 40, 50 μM) of **Ru-BIPP-DNBS** for 24 h.