Supplementary Information

Reaction-based probes for Co^{2+} and Cu^{+} with dual output modes: fluorescence live cell imaging

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Contents

1. General method for measurements of photophysical properties

UV-vis spectra were recorded on a Perkin Elmer Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer LS 55 spectrophotometer. 1 cm cells were used for emission titration. For titrations stock solution of ligands **ResCo** and **ResCu** were prepared (c = 2000 μM) in DMSO. The solutions of guest cations were prepared in DMSO in the order of 10^{-3} M. Working solutions of **ResCo** and **ResCu** and metal ions were prepared from the stock solutions. Excitation was carried out at 540 nm for **ResCo** and **ResCu** with 10 nm excitation and 10 nm emission slit widths.

2. Additional photophysical measurements

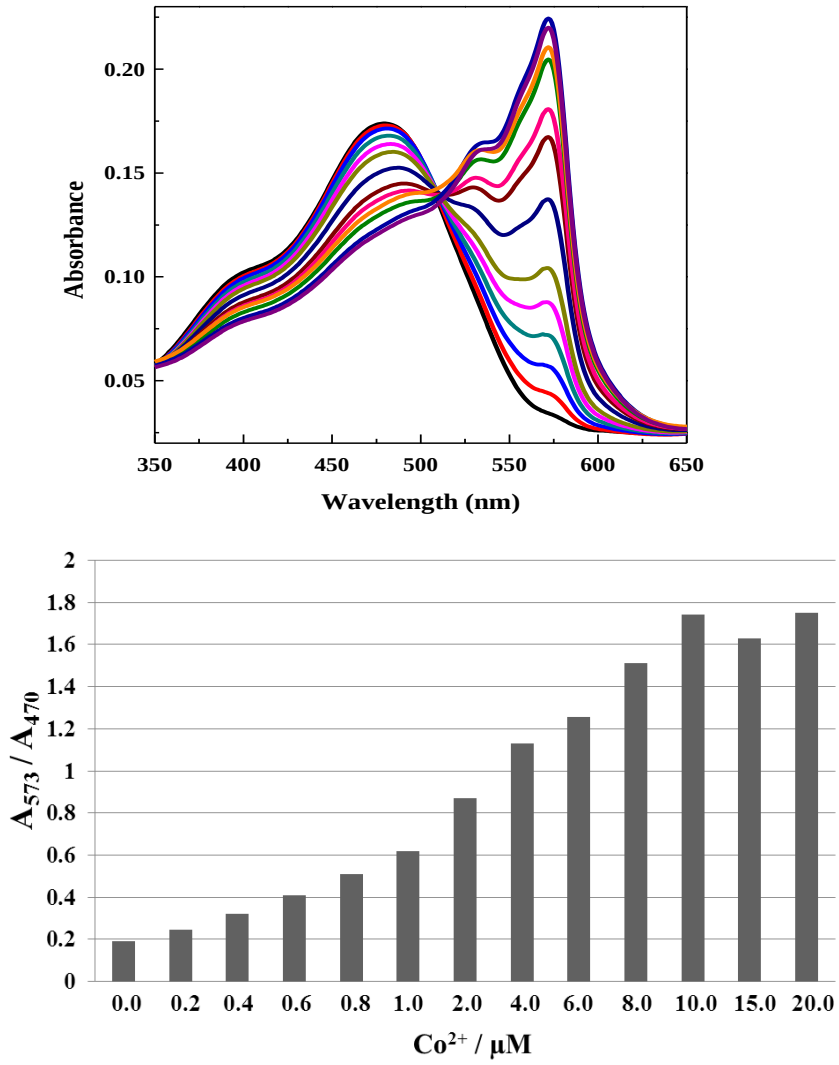


Fig. S1 Absorption spectra (top) and ratiometric responses (bottom) of **ResCo** (10.0 μM) upon addition of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 15.0, 20.0 μM of Co^{2+} after 2 h in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH).

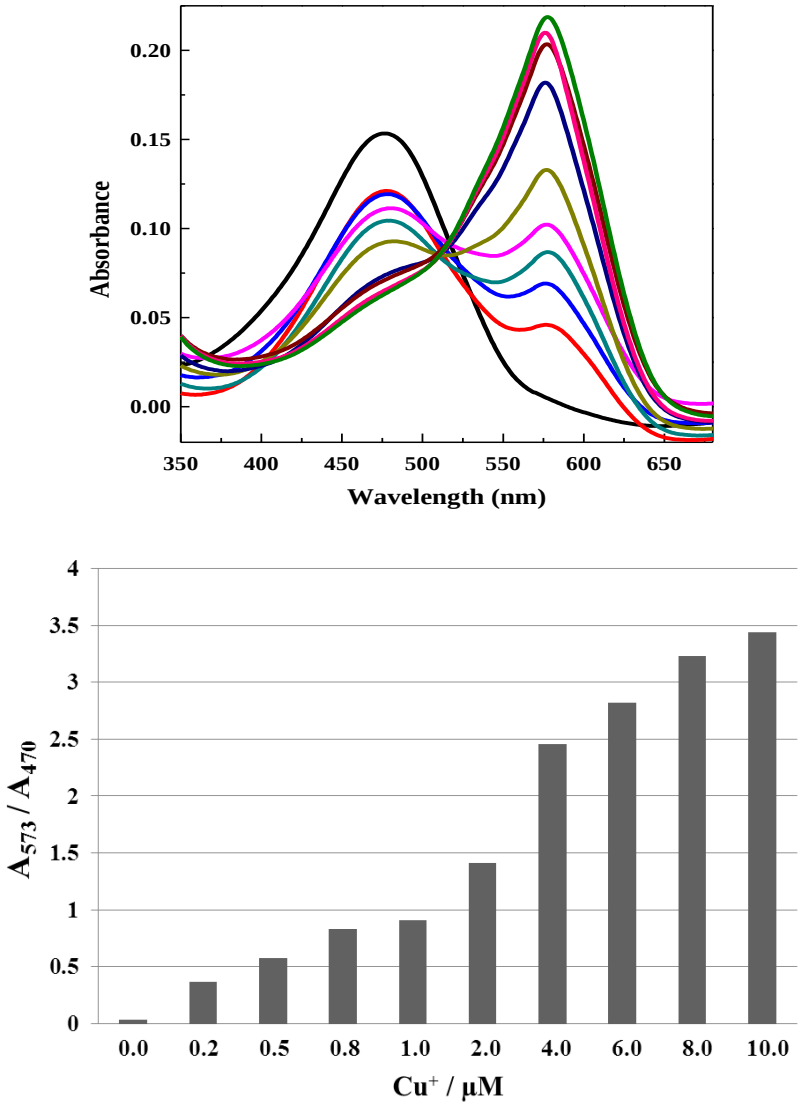


Fig. S2 Absorption spectra (top) and ratiometric responses (bottom) of **ResCu** (10.0 μM) upon addition of 0.0, 0.2, 0.5, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 μM of Cu^{+} after 2 h in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH).

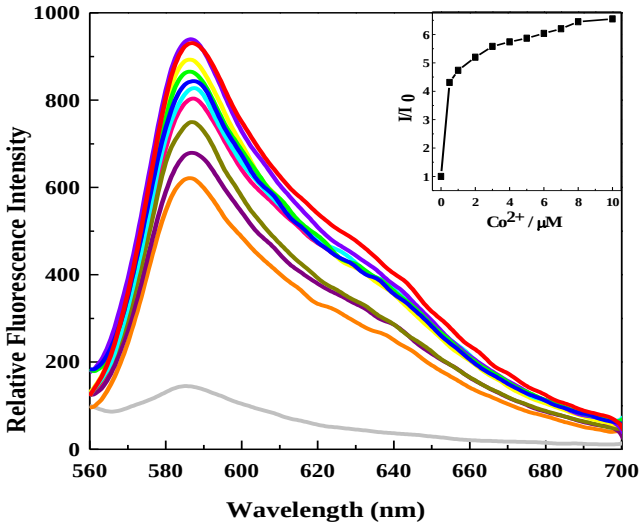


Fig. S3 Fluorescence responses of **ResCo** (1.0 μM) upon addition of 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0 μM of Co^{2+} after 2 h in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH) (λ_{ex} = 540 nm).

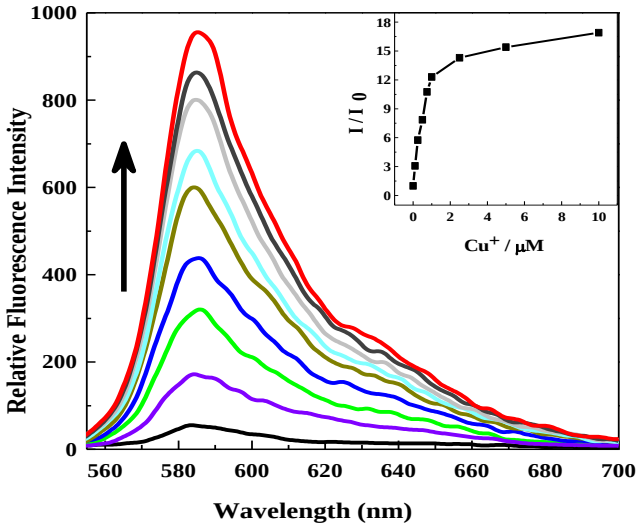


Fig. S4 Fluorescence responses of **ResCu** (1.0 μM) upon addition of 0.0, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, 10.0 μM of Cu^{+} after 2 h in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH) (λ_{ex} = 540 nm).

3. pH study

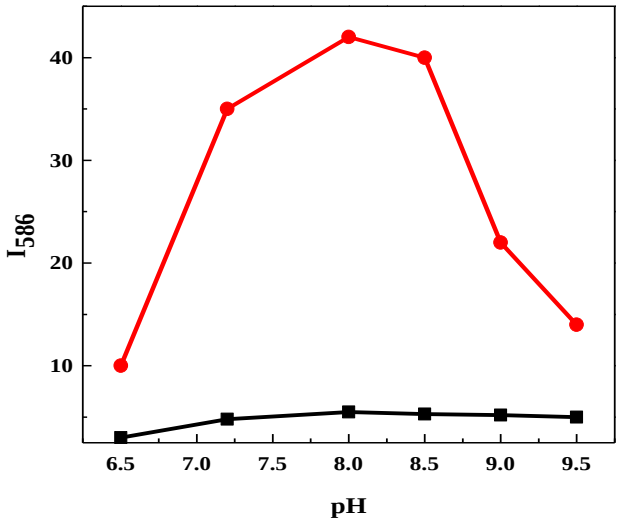


Fig. S5 Effect of pH on the 'switch-on' emission of **ResCo**. Red trace: **ResCo** (1.0 μM) with 10.0 μM of Co^{2+} after 2 h in aqueous solution. Black trace: **ResCo** (1.0 μM) without Co^{2+} after 2 h in aqueous solution.

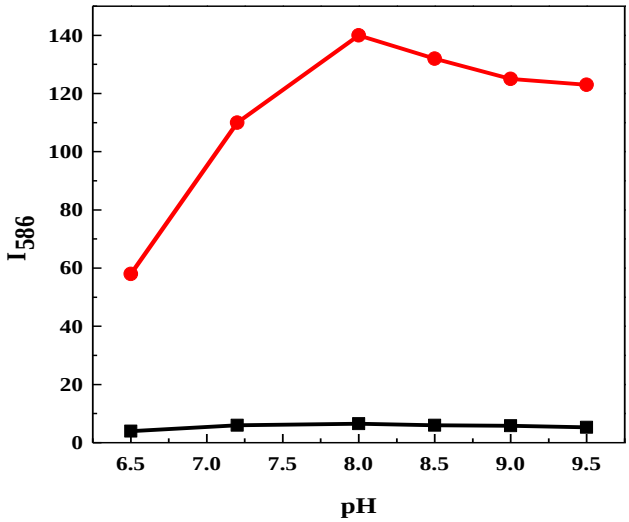
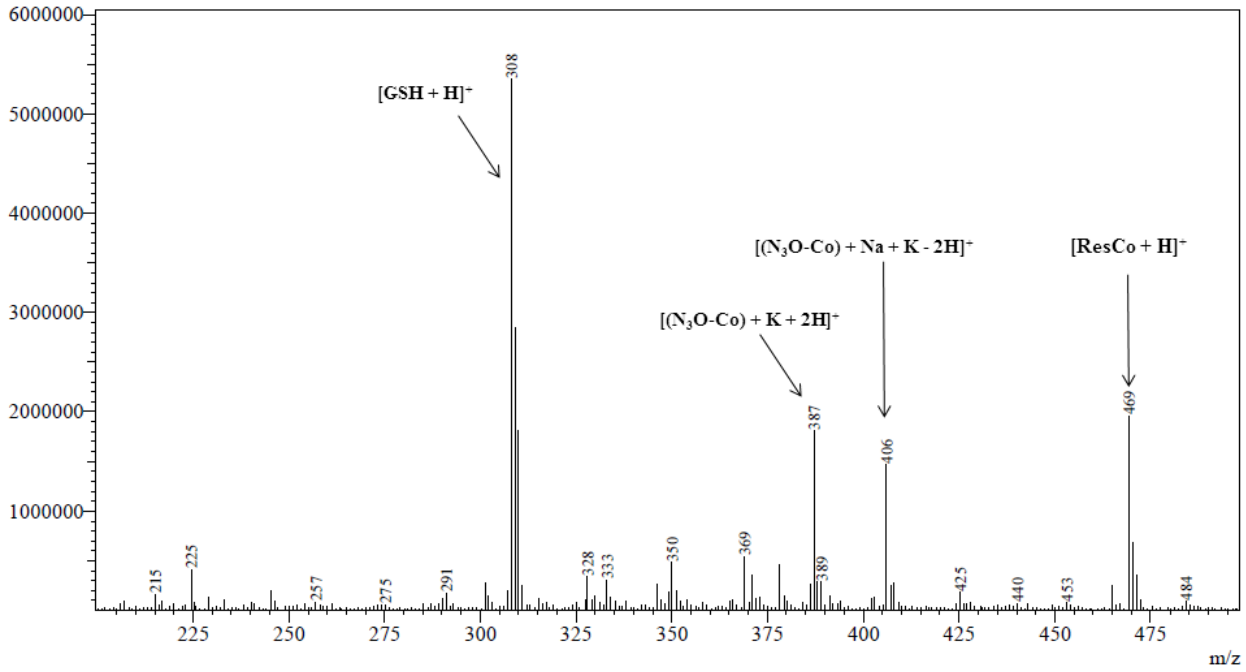
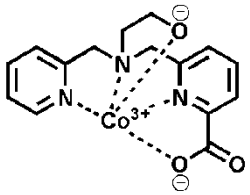


Fig. S6 Effect of pH on the 'switch-on' emission of **ResCu**. Red trace: **ResCu** (1.0 μM) with 10.0 μM of Cu^{+} after 2 h in aqueous solution. Black trace: **ResCu** (1.0 μM) without Cu^{+} after 2 h in aqueous solution.

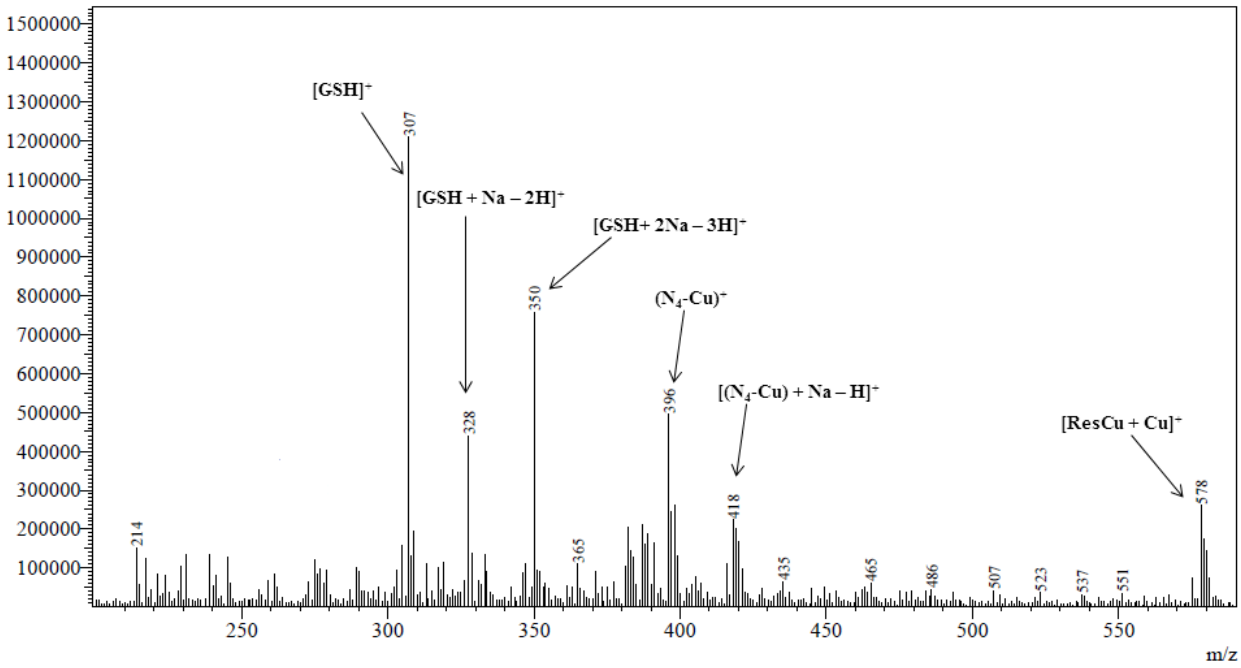
4. Mass study

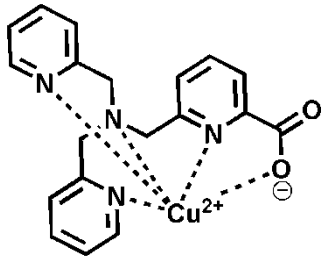




Calculated m/z = 387:04 for $C_{15}H_{17}CoN_3O_3 + K^+ + 2H^+$ (**N_3O**-Co complex)

Fig. S7 ESI mass spectra (positive ion mode) for the reaction of 10.0 μM **ResCo** with 100.0 μM Co^{2+} in water in presence of 100.0 μM GSH. Mass peaks observed at 387 ($[M + K + 2H]^+$) and 406 ($[M + Na + K – 2H]^+$) are corresponds to **N_3O**-Co complex $C_{15}H_{17}CoN_3O_3$. Mass peak observed at 469 ($[M + H]^+$) correspond to **ResCo** (calculated 468.18 for $C_{27}H_{24}N_4O_4$). Mass peak observed at 308 ($[M + H]^+$) correspond to GSH.





Calculated m/z = 396.06 for $C_{19}H_{17}CuN_4O_2$ (**N_4**-Cu complex)

Fig. S8 ESI mass spectra (positive ion mode) for the reaction of 10.0 μM **ResCu** with 100.0 μM Cu^+ in water in presence of 100.0 μM GSH. Mass peaks observed at 396 ($[M + H]^+$) and 418 ($[M + Na – H]^+$) are correspond to **N_4**-Cu complex $C_{19}H_{17}CuN_4O_2$. Mass peak observed at 578 ($[M + Cu]^+$) correspond to copper bound **ResCu** (calculated 578.12 for $C_{31}H_{25}CuN_5O_3$). Mass peaks observed at 307 ($[M]^+$), 328 ($[M + Na – 2H]^+$), 350($[M + 2Na – 3H]^+$) is corresponds to GSH.

5. GSH dependent study

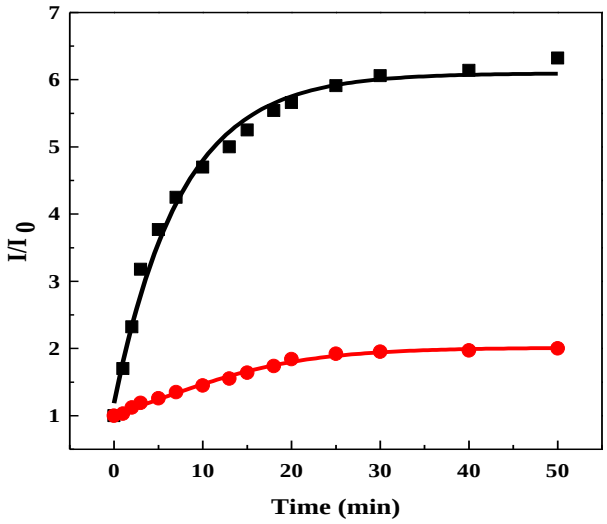


Fig. S9 Time dependent fluorescence study of 1.0 μM **ResCo** incubated with 10 μM of Co^{2+} in aqueous solution (50 mM HEPES, pH 7.2) with (**black trace**) and without (**red trace**) 2 mM GSH.

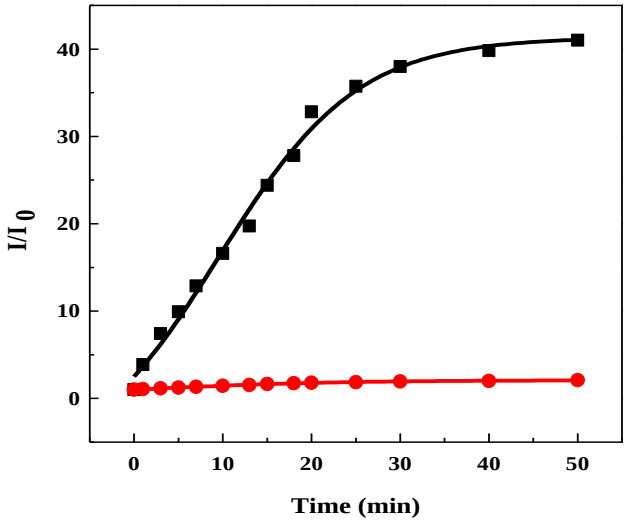


Fig. S10 Time dependent fluorescence study of 1.0 μM **ResCu** incubated with 10 μM of Cu^{+} in aqueous solution (50 mM HEPES, pH 7.2) with (**black trace**) and without (**red trace**) 2 mM GSH.

6. Colourimetric changes

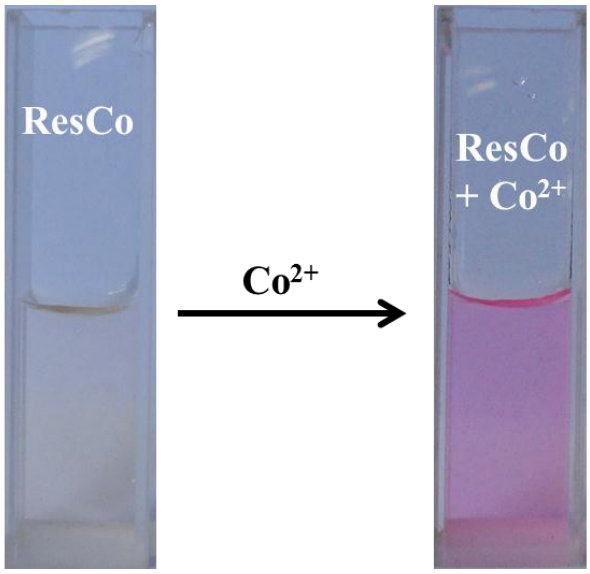


Fig. S11 Colourimetric changes of **ResCo** (10.0 μM) after addition of 20.0 μM of Co^{2+} in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH)

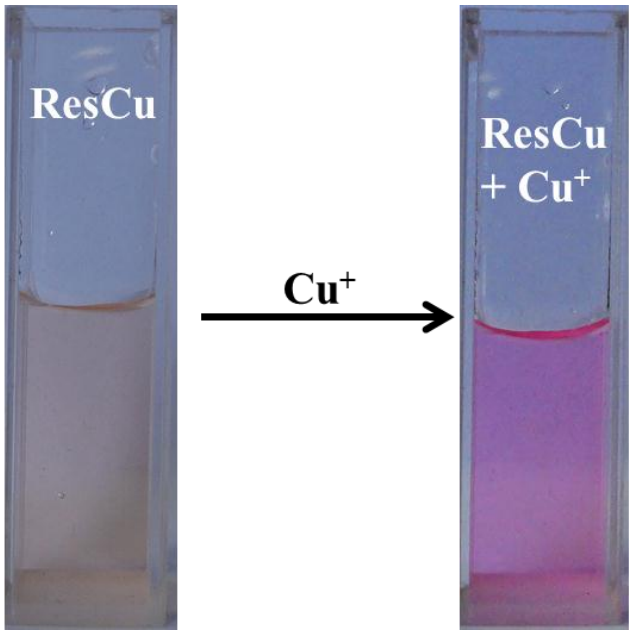
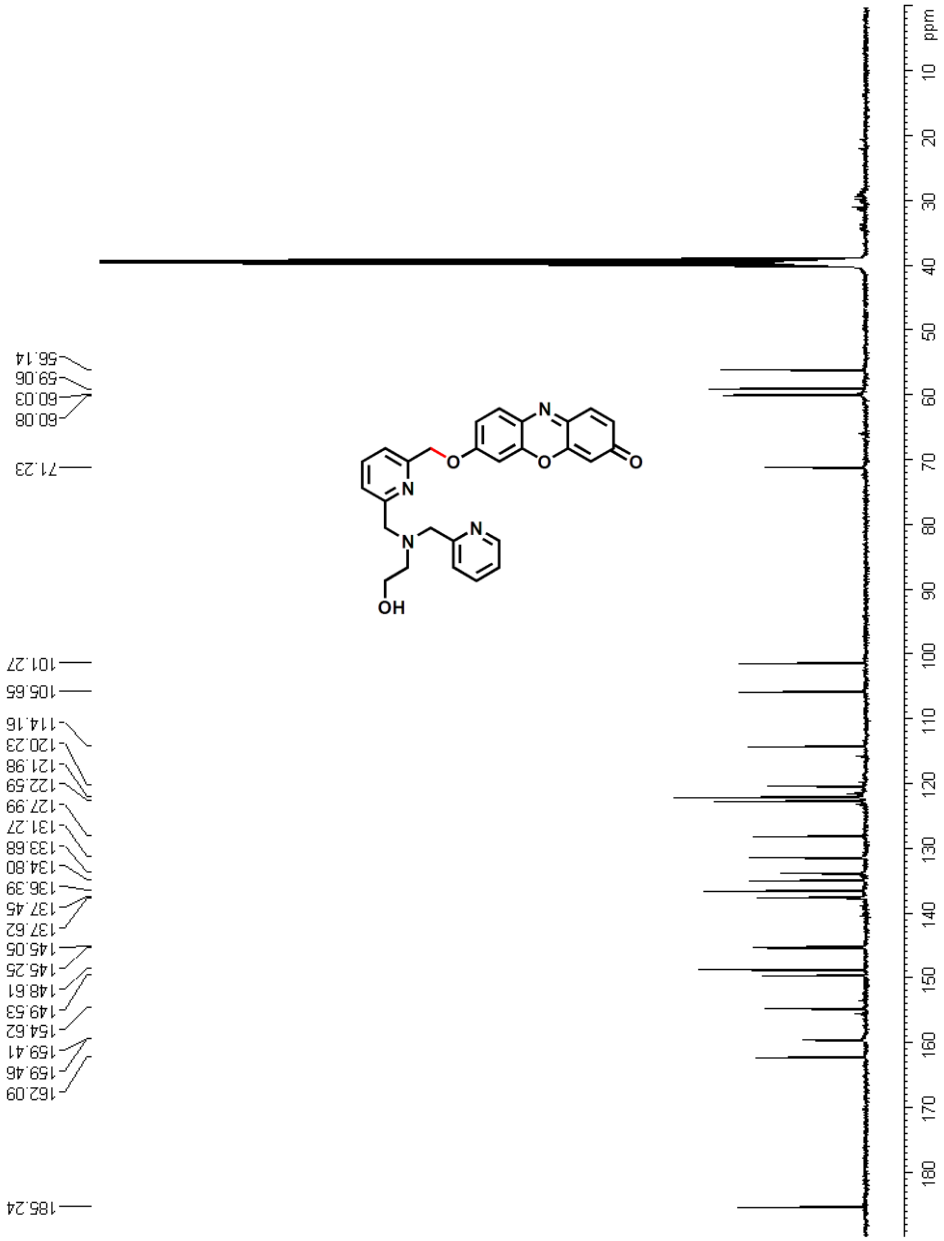


Fig. S12 Colourimetric changes of **ResCu** (10.0 μM) after addition of 20.0 μM of Cu^{+} in aqueous solution (50 mM HEPES, pH 7.2, 2 mM GSH)

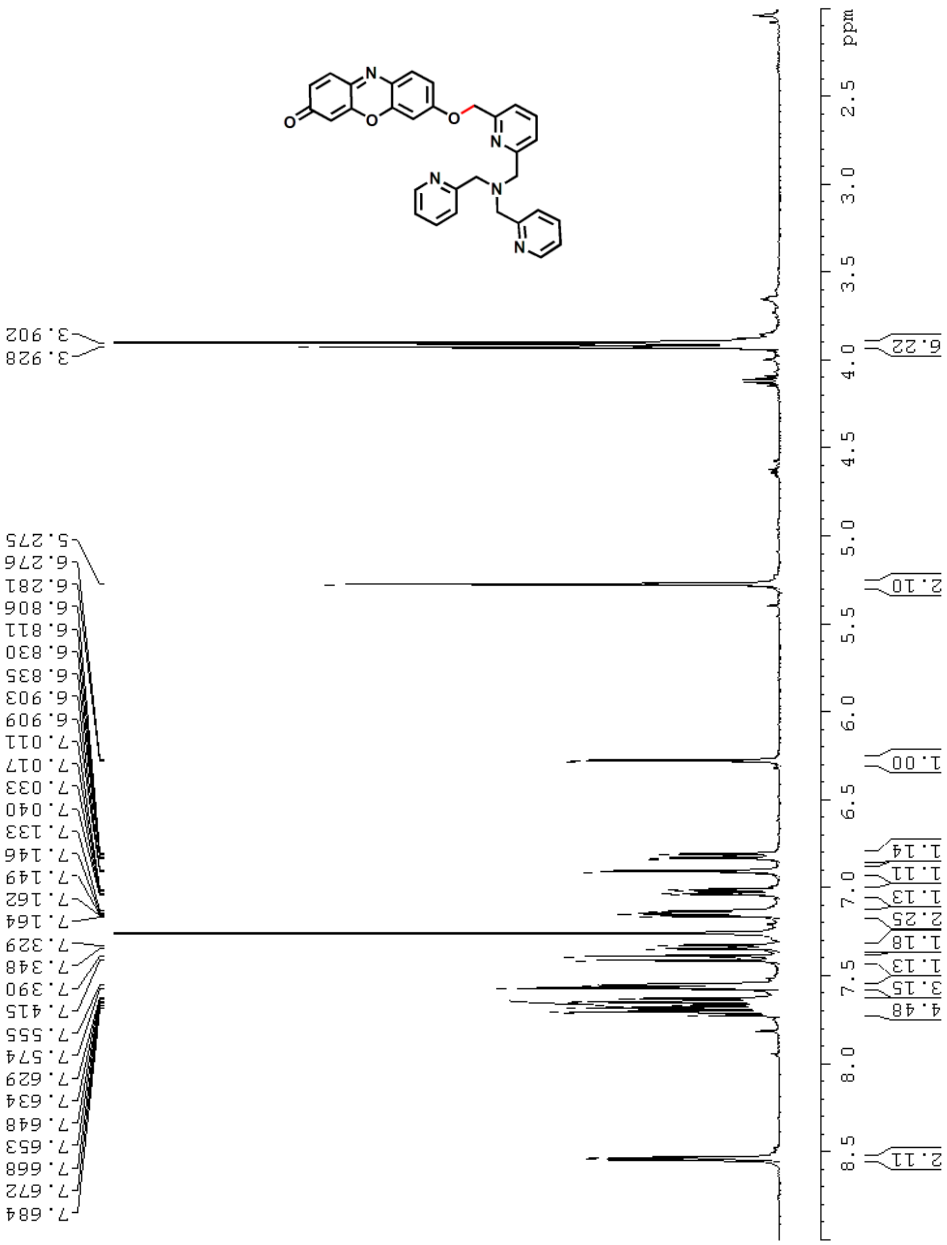
7. NMR spectra

^{1}H NMR spectrum of ResCo

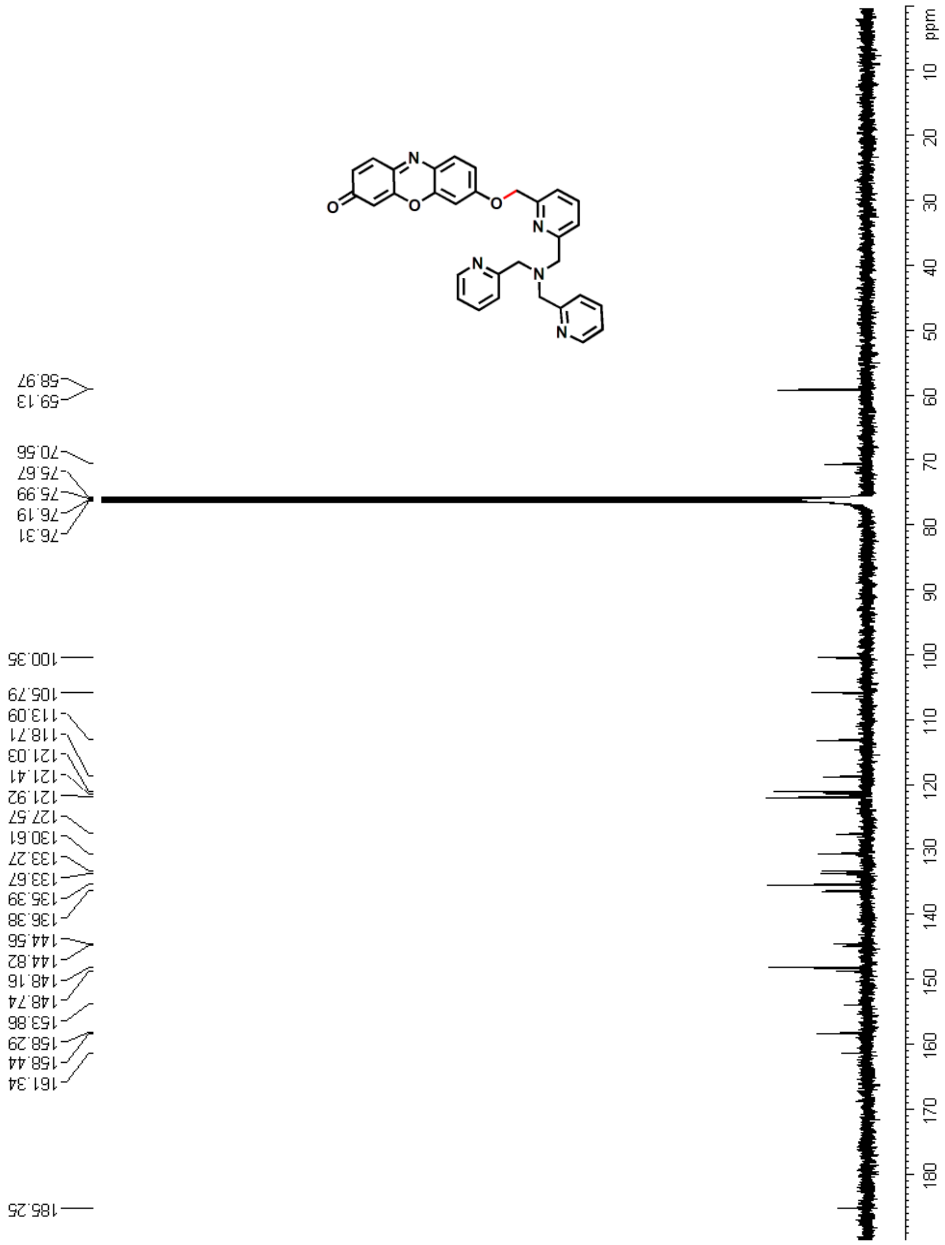
^{13}C NMR spectrum of ResCo



^{1}H NMR spectrum of ResCu



^{13}C NMR spectrum of ResCu



8. HRMS spectra

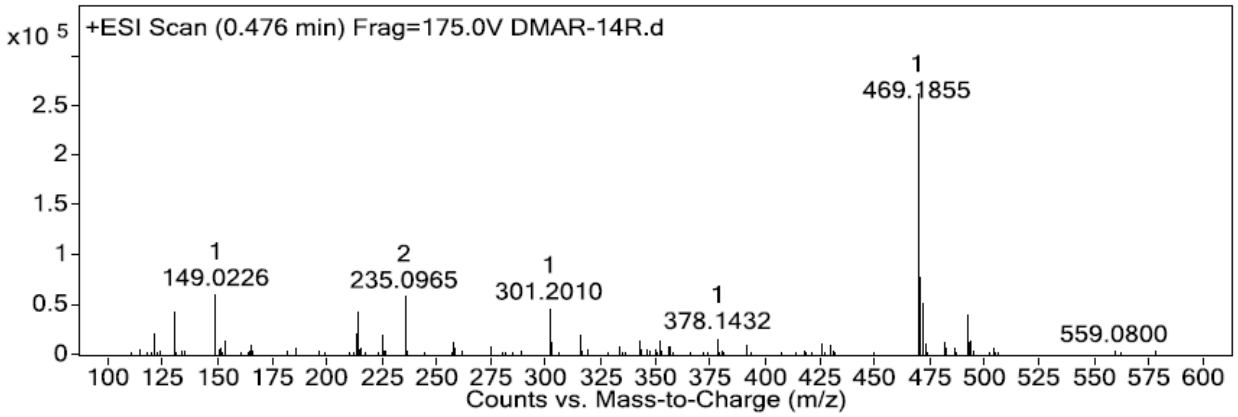


Fig. S13 HRMS spectra of **ResCo.** Observed m/z = 469.1855 $[M + H]^+$ and calculated m/z = 469.1876 for $C_{27}H_{25}N_4O_4$.

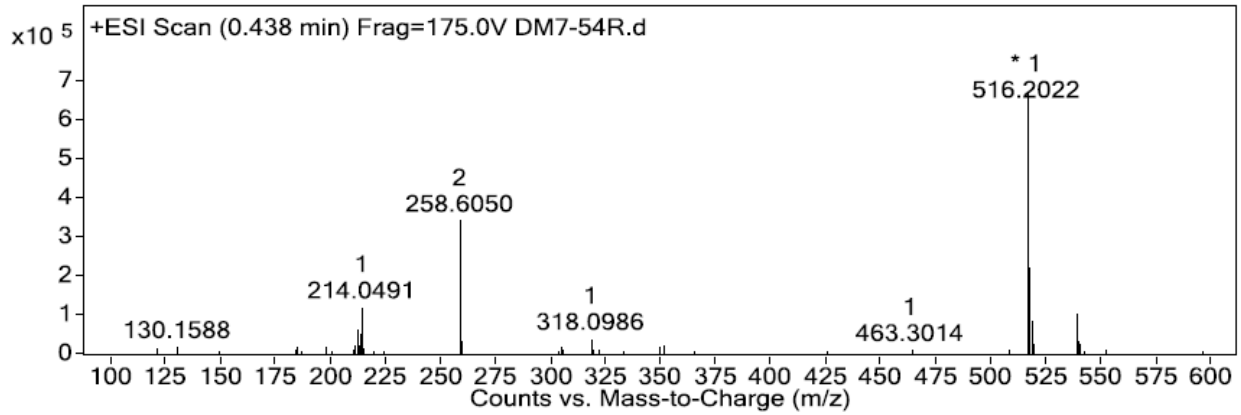


Fig. S14 HRMS spectra of **ResCu.** HRMS: observed m/z = 516.2022 $[M + H]^+$ and calculated m/z = 516.2036 for $C_{31}H_{26}N_5O_3$.

9. References

1 H. Y. Au-Yeung, E. J. New and C. J. Chang, *Chem. Commun.*, 2012, **48**, 5268-5270.

2 B. Lucchese, K. J. Humphreys, D. Lee, C. D. Incarvito, R. D. Sommer, A. L. Rheingold and K. D. Karlin, *Inorg. Chem.*, 2004, **43**, 5987-5998.