Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary information

Glutathione selective "off-on" fluorescence response by probe displaced modified ligand for its detection in biological domain

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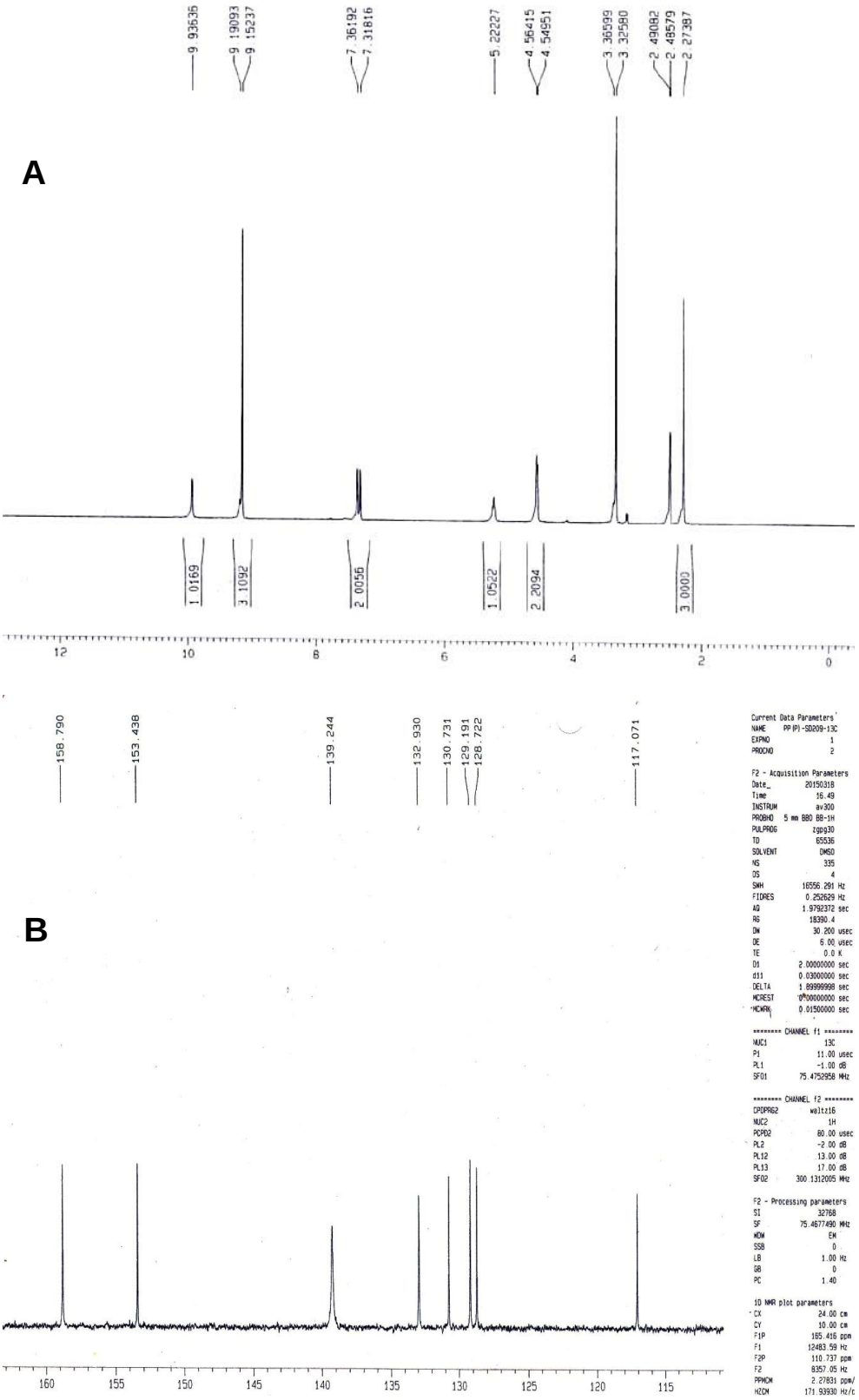

Fig. S1. (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of L_1 in DMSO-d_6.

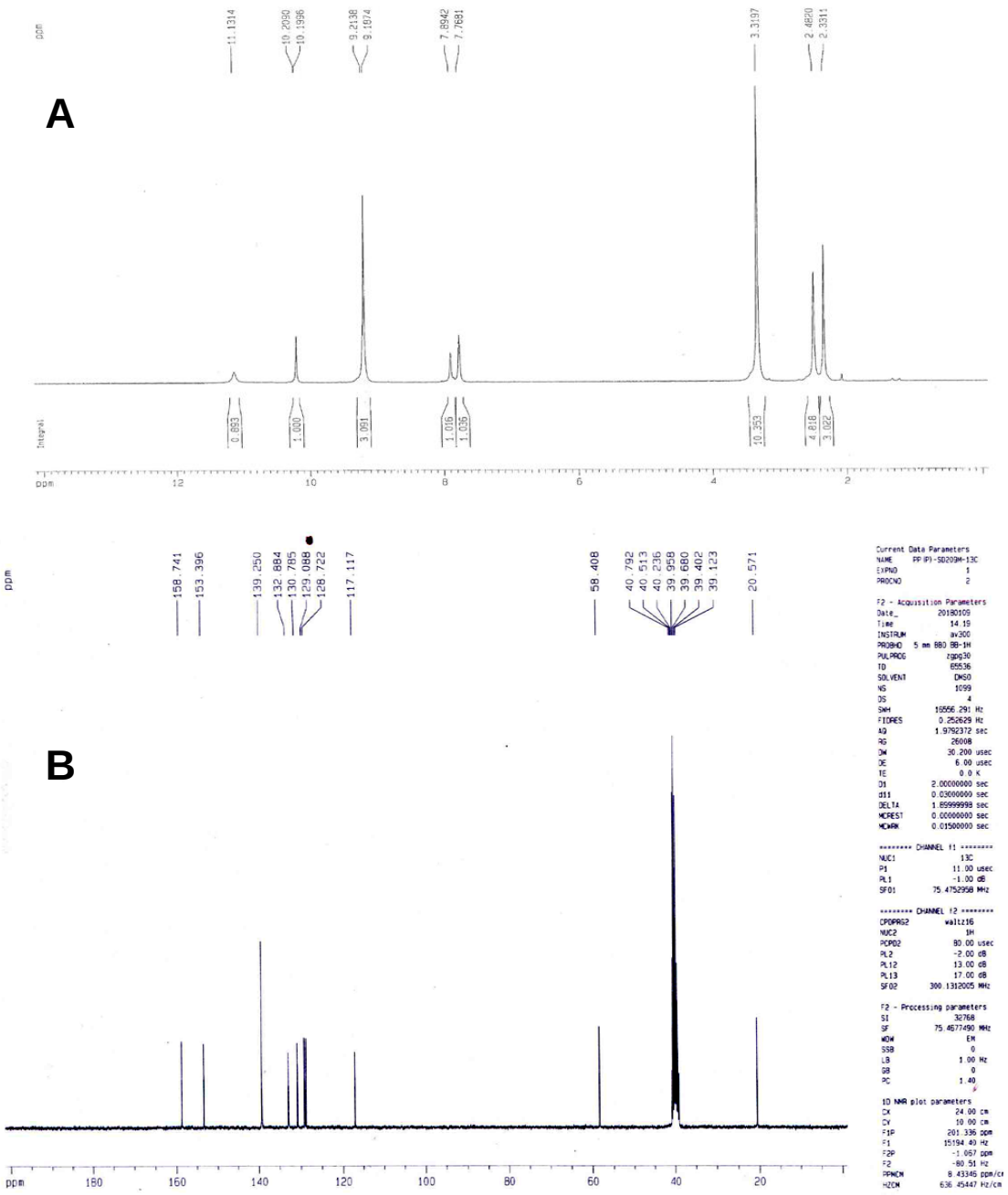

Fig. S2. (A) ^{1}H-NMR and (B) ^{13}C-NMR spectra of L_1/ in DMSO-d_6.

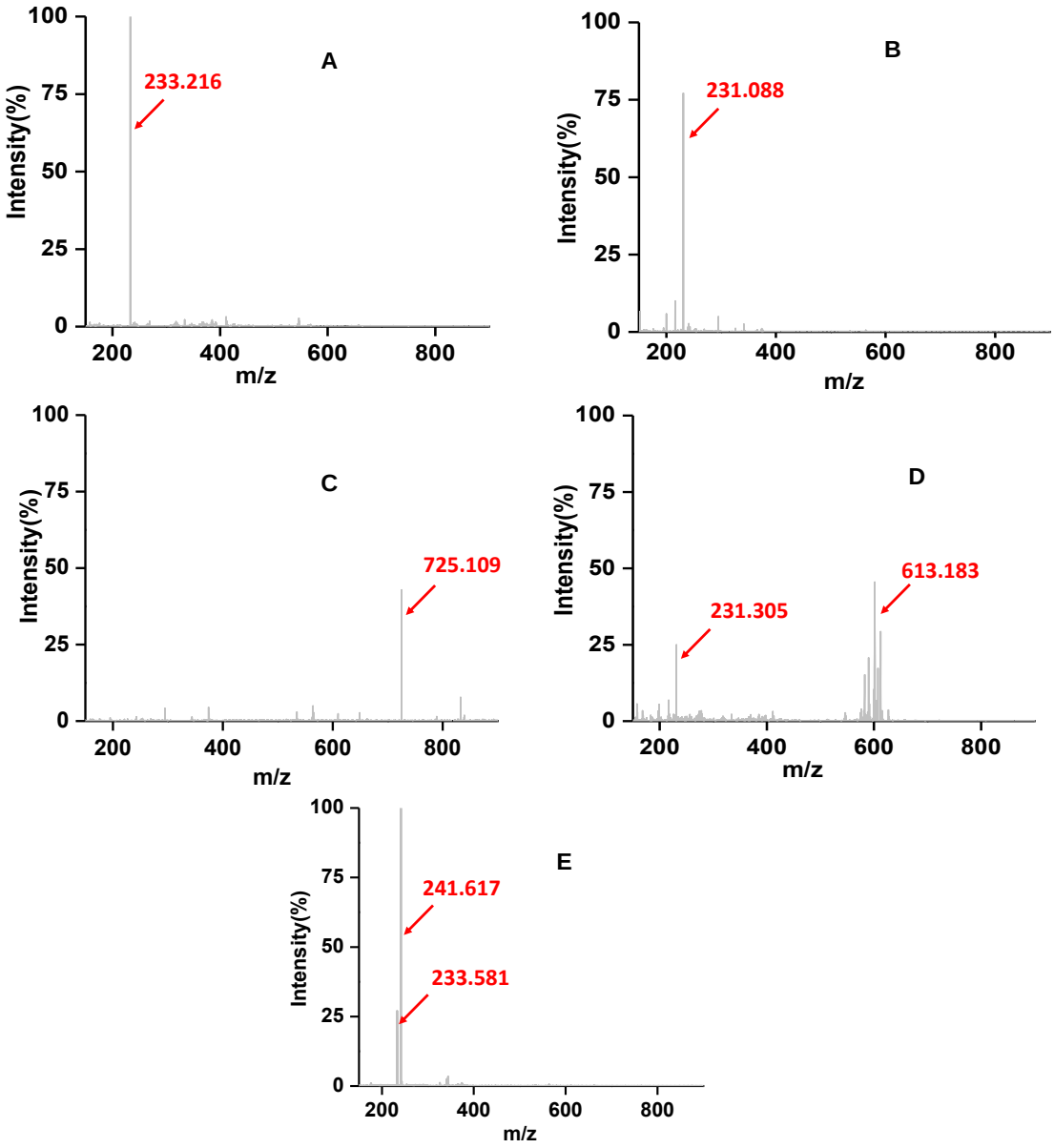

Fig. S3. ESI-MS$^+$ of (A) L_1 (*m/z*: found 233.216; calcd. 233.251) for $[L_1+H]^+$), (B) $L_1^/$ (*m/z* : found 231.088; calcd. 231.235, *m/z* for $[L_1^/+H]^+$, (C) **1** (*m/z*: found 725.109; calcd. 725.038 for $[C_{22}H_{22}N_8O_4Cu_2+ClO_4]^+$), (D) **1**+GSH (*m/z*: found 231.305 for $[L_1^/+H]^+$, and 613.183 for $[GSSG+H]^+$: calcd. 613.355), and (E) **1**+Cys (*m/z*: found 233.581 for $[L_1+H]^+$; and 241.617 for $[Cystine+H]^+$) calcd. 241.341) in water.

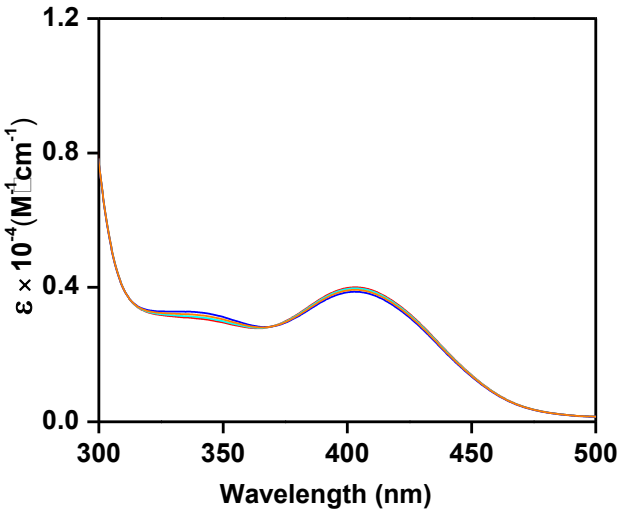

Fig. S4. UV-vis absorption studies of **1** (5 μM with respect to L_1) in buffer medium at different pH: cyan, pH 6.0; red, pH 7.0; blue, pH 8.0; orange, pH 9.0.

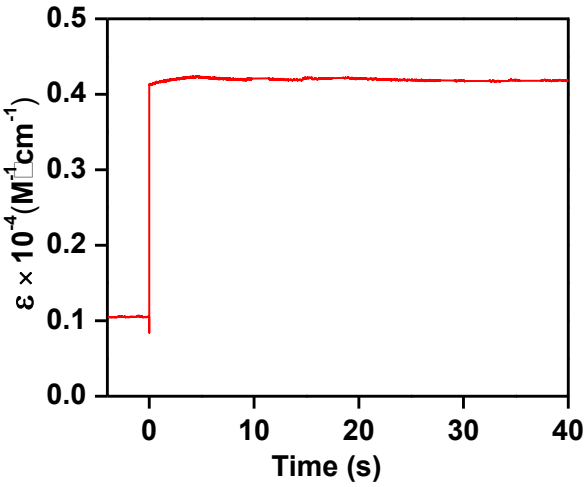

Fig. S5. Time response UV-vis absorption intensity at 405 nm by addition of $Cu(ClO_4)_2$ (40 µM) in 20 mM HEPES-NaOH buffer, pH 7.3 containing L_1 (5 µM) at 25°C.

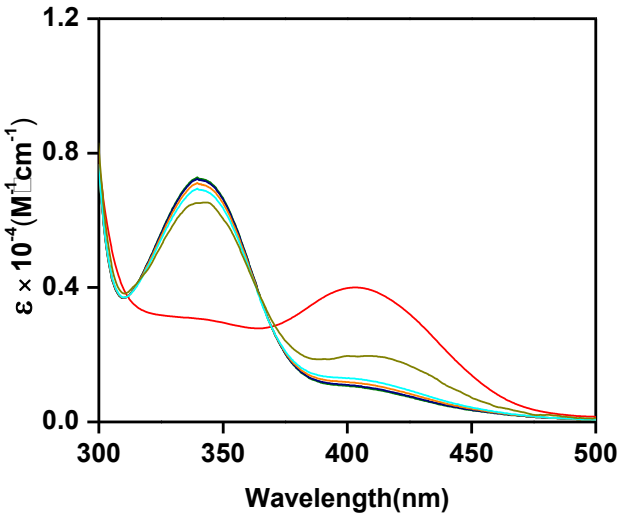

Fig. S6. UV-vis absorption spectra of L_1 (5 µM) in 20 mM HEPES-NaOH buffer, pH 7.3, containing different metal ions (0–40 µM) at 25°C: red, Cu^{2+}; dark yellow, Ni^{2+}; cyan, Co^{2+}; orange, Fe^{2+} and dark blue, Mn^{2+}; The spectrum in absence of metal ions is shown by green for comparison.

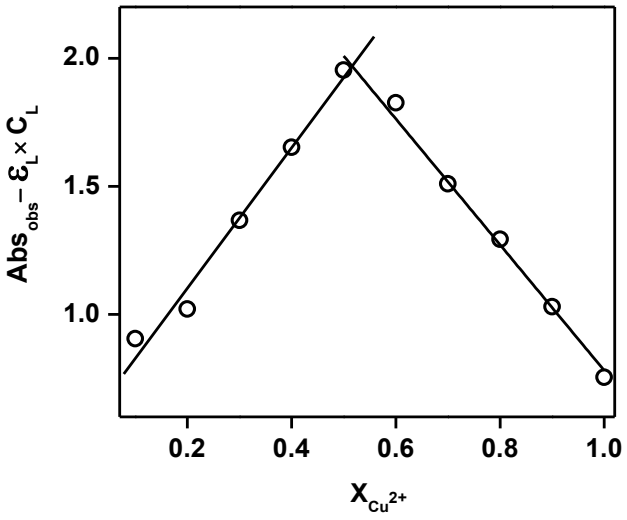

Fig. S7. Job's plot for determining the stoichiometry of the complex between L_1 and Cu^{2+}. The difference between the observed and L_1 absorbance at 405 nm were plotted with mole fraction of Cu^{2+} ($X_{Cu}{}^{2+}$) in the various mixture of L_1 and $Cu(ClO_4)_2$ (ε_L and C_L are the extinction coefficient and concentration of L_1, respectively).

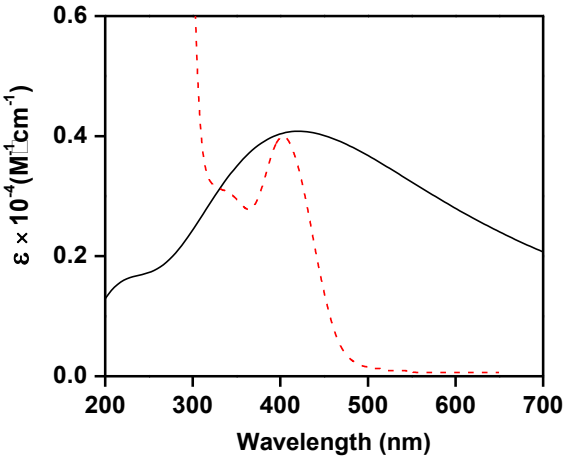

Fig. S8. Electronic excitation wavelength (λ) and extinction coefficient (ε) for **1** obtained by the TD-DFT/B3LYP/6-31+G(d,p) calculation on ground state geometries with CPCM solvation model in water. The experimentally obtained UV-vis absorption and TD-DFT calculated spectra depicted by red and black respectively.

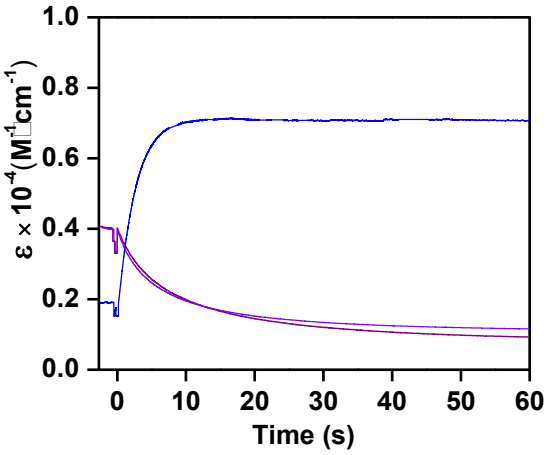

Fig. S9. Biothiols induced time response UV-vis absorption intensities at (blue) 440 nm for GSH (100 µM), (purple) 405 nm for Cys (250 µM) and (violet) 405 nm for Hcy (250 µM each) in 20 mM HEPES-NaOH buffer, pH 7.3 containing **1** (5 µM with respect to L_1) at 25°C.

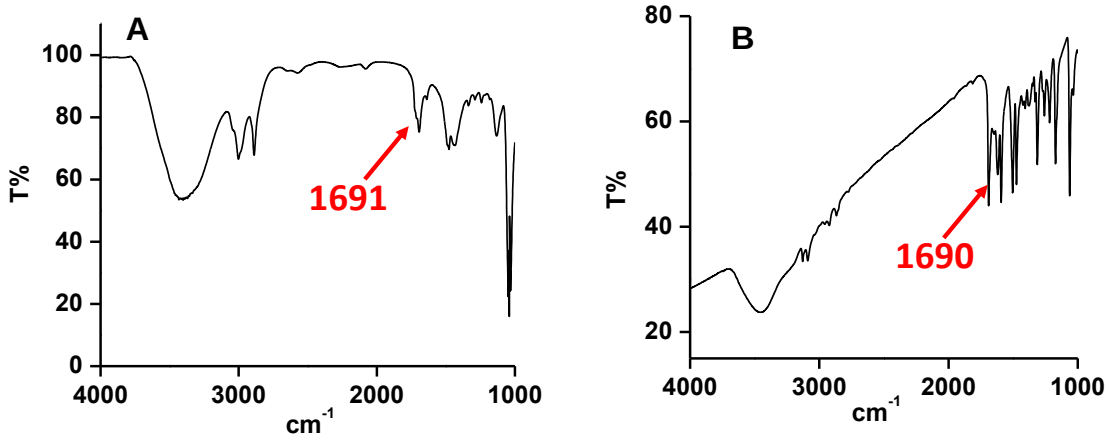

Fig. S10. FT-IR spectrum of (A) **1**+GSH and (B) synthesized L_1'.

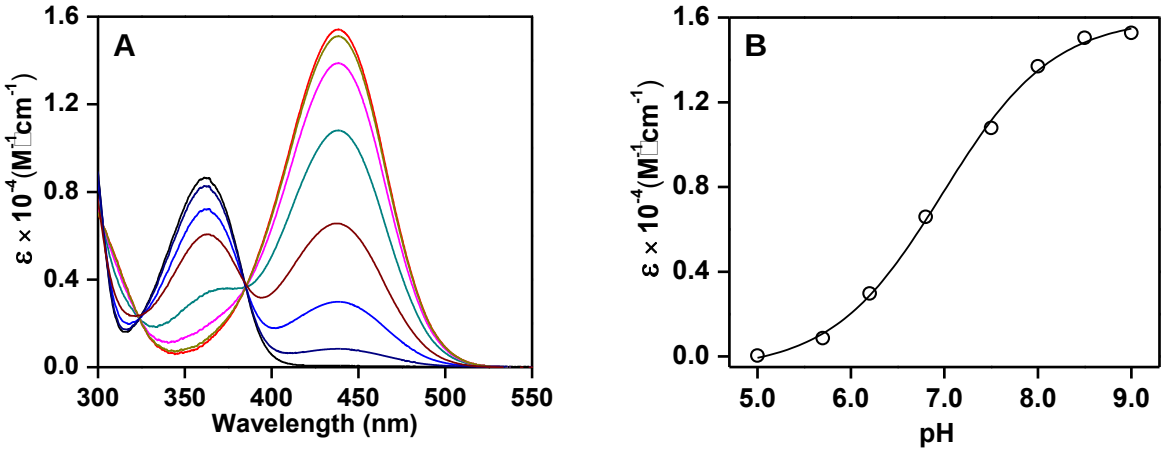

Fig. S11. (A) pH dependent UV-vis absorption spectra of synthesized L_1': black, pH 5.0; dark blue, pH 5.7; blue, pH 6.2; purple, pH 6.8; dark cyan, pH 7.5; magenta, pH 8.0; dark yellow, pH 8.5 and red, pH 9.0. (B) Extinction coefficients at 440 are plotted with pH, fitted the data points with sigmoidal-Boltzmann equation.

Note: Transition mid-point of the fitted curve represents the pK_a ~7.0.

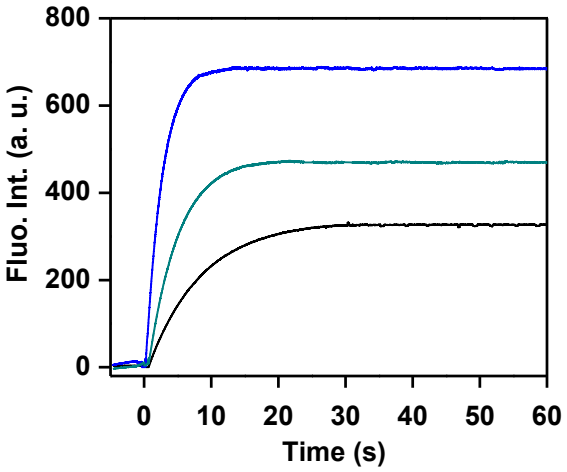

Fig. S12. Time response fluorescence intensities at 555 nm by addition of various concentration of GSH (black, 12 equiv.; dark cyan, 15 equiv. and blue, 20 equiv.) in 20 mM HEPES-NaOH buffer, pH 7.3 containing **1** (5 μM with respect to L_1) at 25°C. Excitation wavelength were 440 nm.

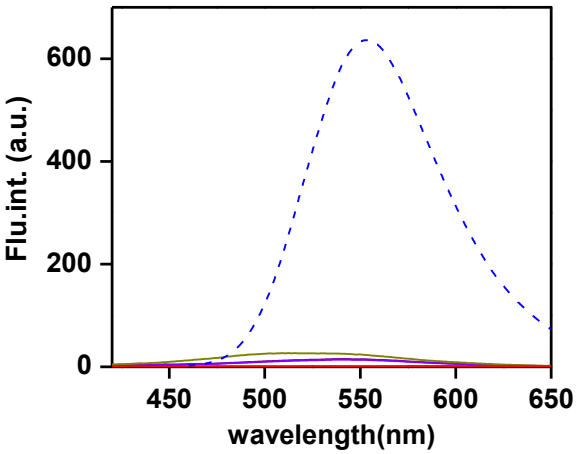

Fig. S13. Fluorescence spectra of **1** (5 μM with respect to L_1) in presence of GSH (dark yellow), Cys (purple) and Hcy (violet) (20 equiv. each) in 20 mM HEPES, pH 7.3 for the excitation at 365 nm. The spectrum in presence of GSH (broken blue) (20 equiv.) for excitation at 440 nm is depicted for comparison.

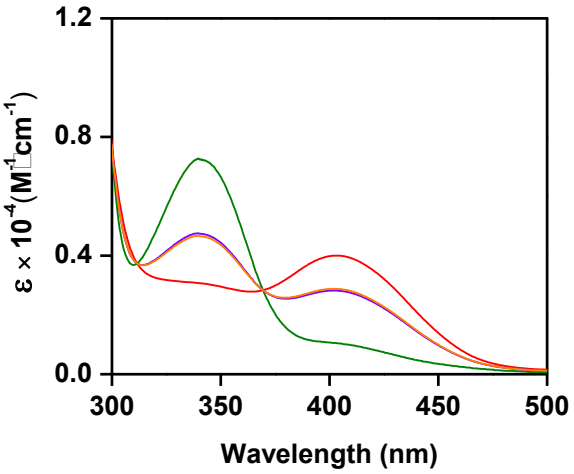

Fig. S14. UV-vis absorption studies of **1** (5 μM with respect to L_1) in absence (red) and presence of Cys (20 equiv., purple) or Hcy (20 equiv., orange). The spectrum of L_1 (green) is depicted for comparison.

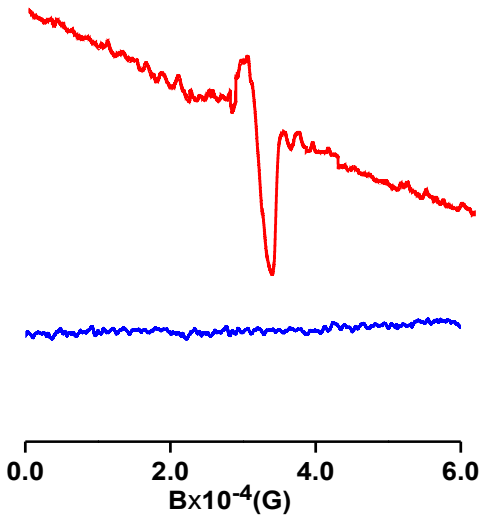

Fig. S15. EPR spectrum of **1** (red) before and (blue) after addition of GSH addition in H_2O-MeCN (1:1) mixture at 77K. (blue) The spectrum was recorded in 40 s after addition of GSH.

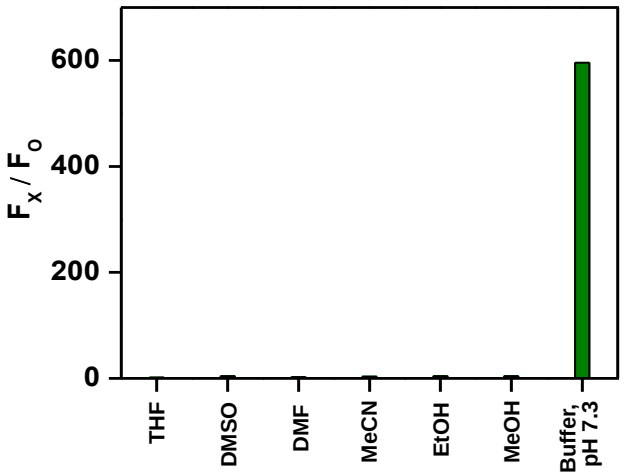

Fig. S16. The extent of 555-nm fluorescence intensity enhancement (F/F_0) due to 440-nm excitation for **1** in presence of GSH (20 equiv.) for different solvent medium are depicted by bar-diagram.

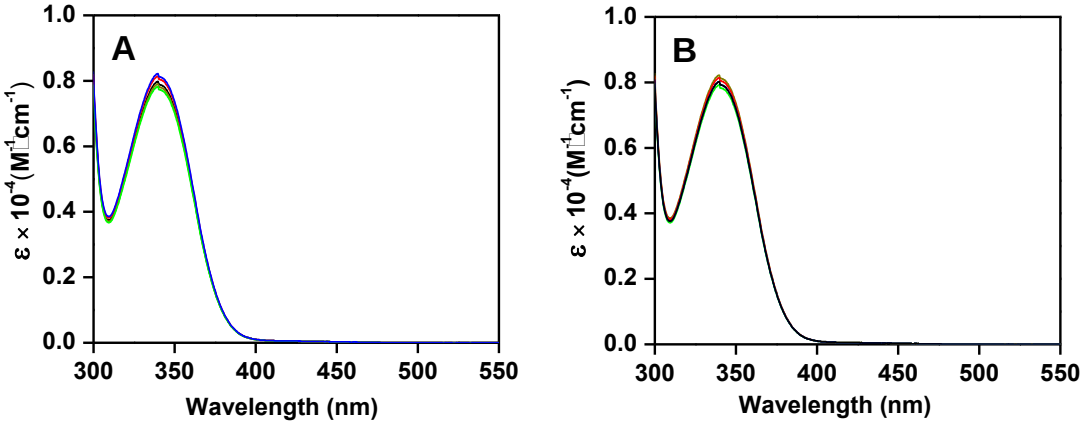

Fig. S17. UV-vis absorption spectrum of **1** (5 μM with respect to L_1) in presence of GSH (20 equiv.) in different solvents (A) without and (B) with saturated oxygenated conditions: black, THF; red, MeCN; blue, DMSO; green, MeOH and dark yellow, DMF.

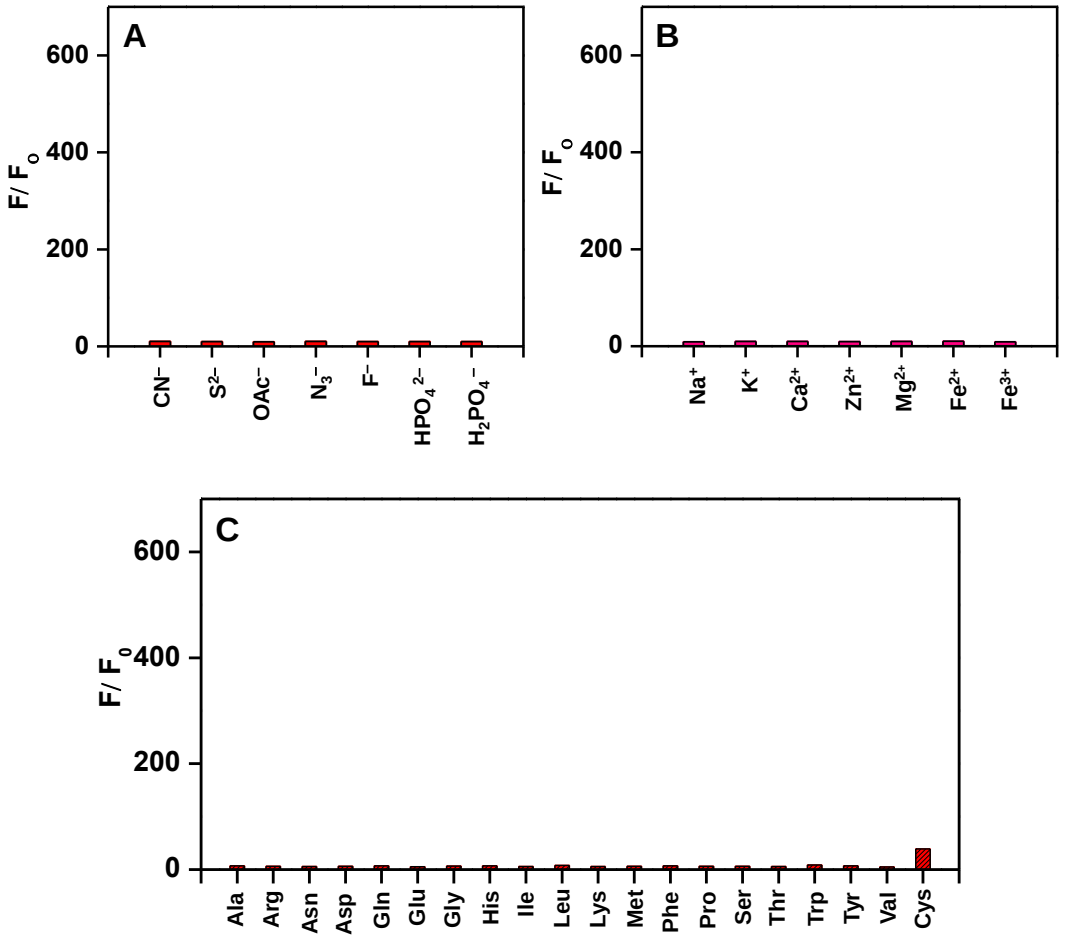

Fig. S18. The extent of 555-nm fluorescence intensity enhancement (F/F_0) due to 440-nm excitation for **1** in presence of various (A) anions (50 equiv. each), (B) biologically important metal ions (50 equiv. each) and (C) amino acids (20 equiv. each) in 20 mM HEPES-NaOH, pH 7.3.

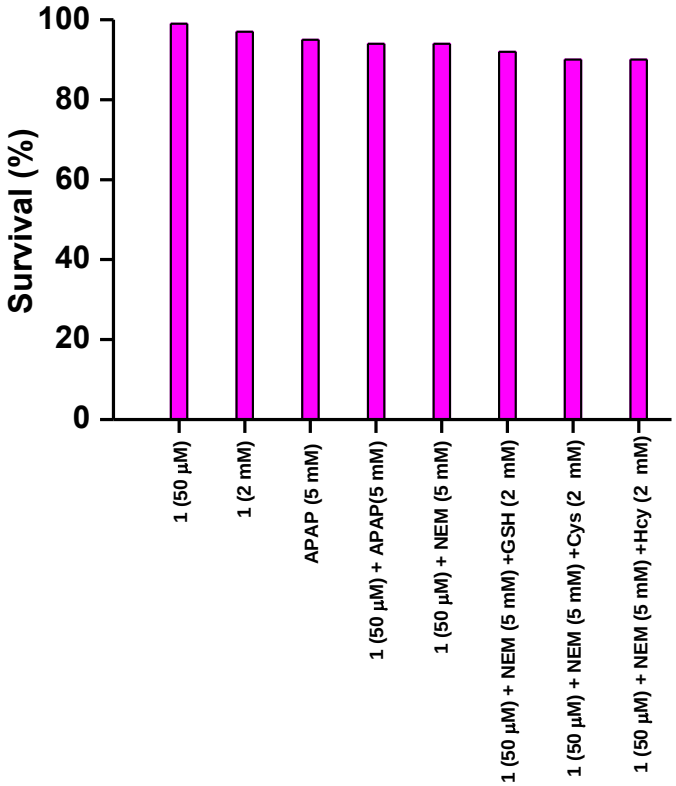

Fig. S19. Assessment of toxicity of the **1** in presence and absence of various analytes after incubation of 30 min. by survival assays in *C. elegans* are depicted by bar-diagram.

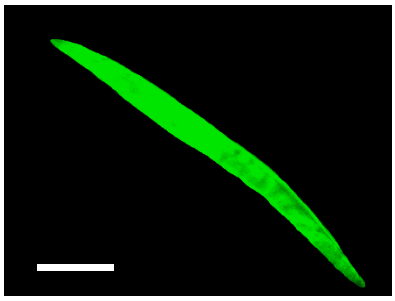

Fig. S20. Fluorescence images of the *C. elegans* exposed to L_1' (40 μM) for 30 min. The scale bars: 40 μm.

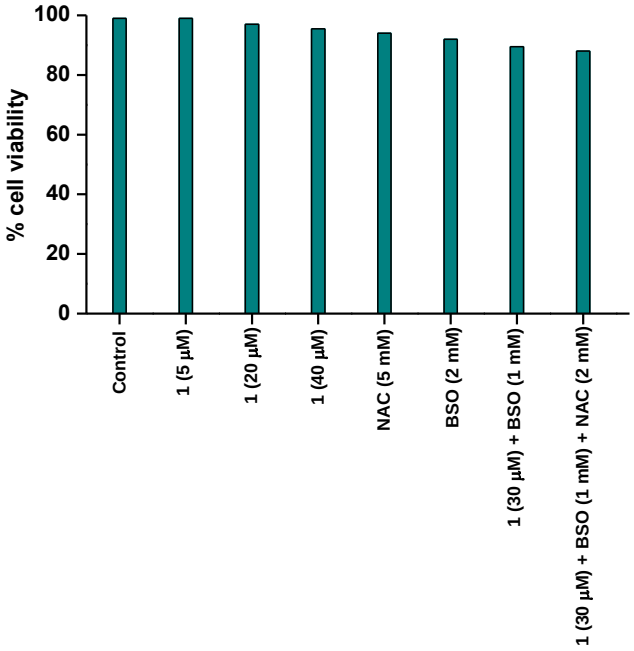

Fig. S21. Percent (%) cell viability of SH-SY5Y cells treated with different analytic concentrations for 12 h determined by MTT assay.