Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Supplementary information

Designing Thiol Specific Fluorescent Probe for a Possible Use as a Reagent for Intracellular Detection and Estimation in Blood Serum: Kinetic Analysis for Probing the Role of Intra Molecular Hydrogen Bonding

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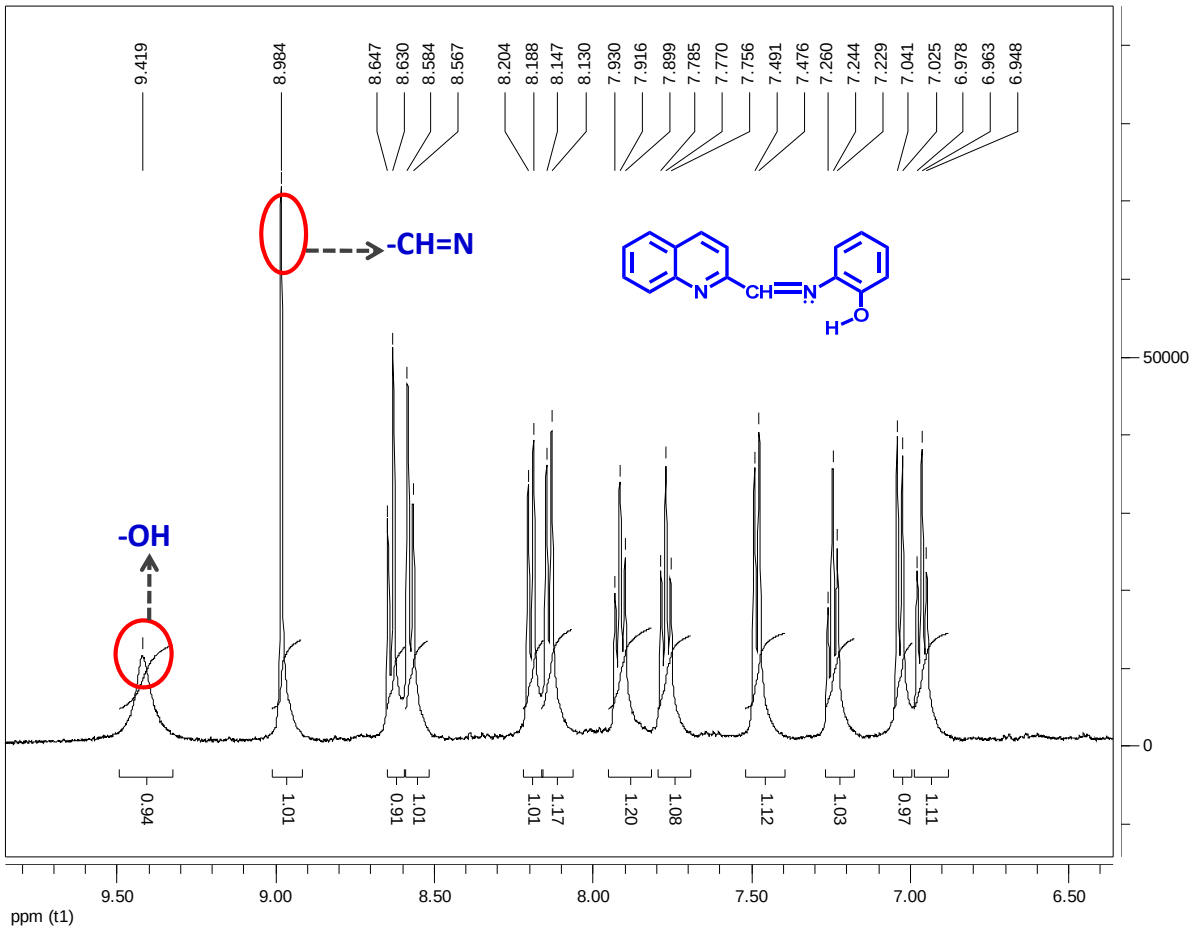
^{1}H NMR spectra of L_1



SI Figure 1: Partial ^{1}H NMR spectra of $\mathbf{L_1}$ in DMSO-d_6 medium.

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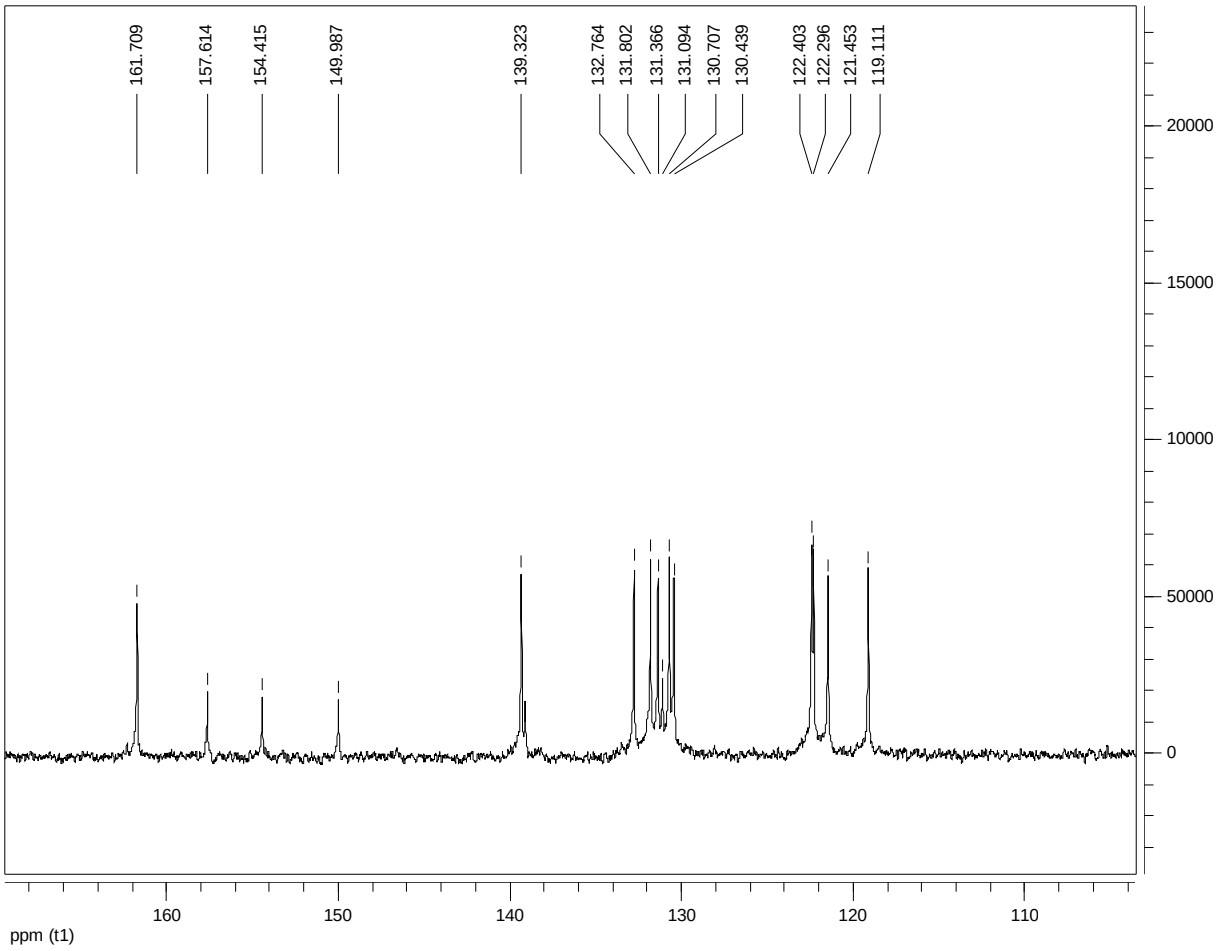
^{13}C NMR spectra of L_1



SI Figure 2: ^{13}C NMR spectra of **L_1** in DMSO-d_6 medium.

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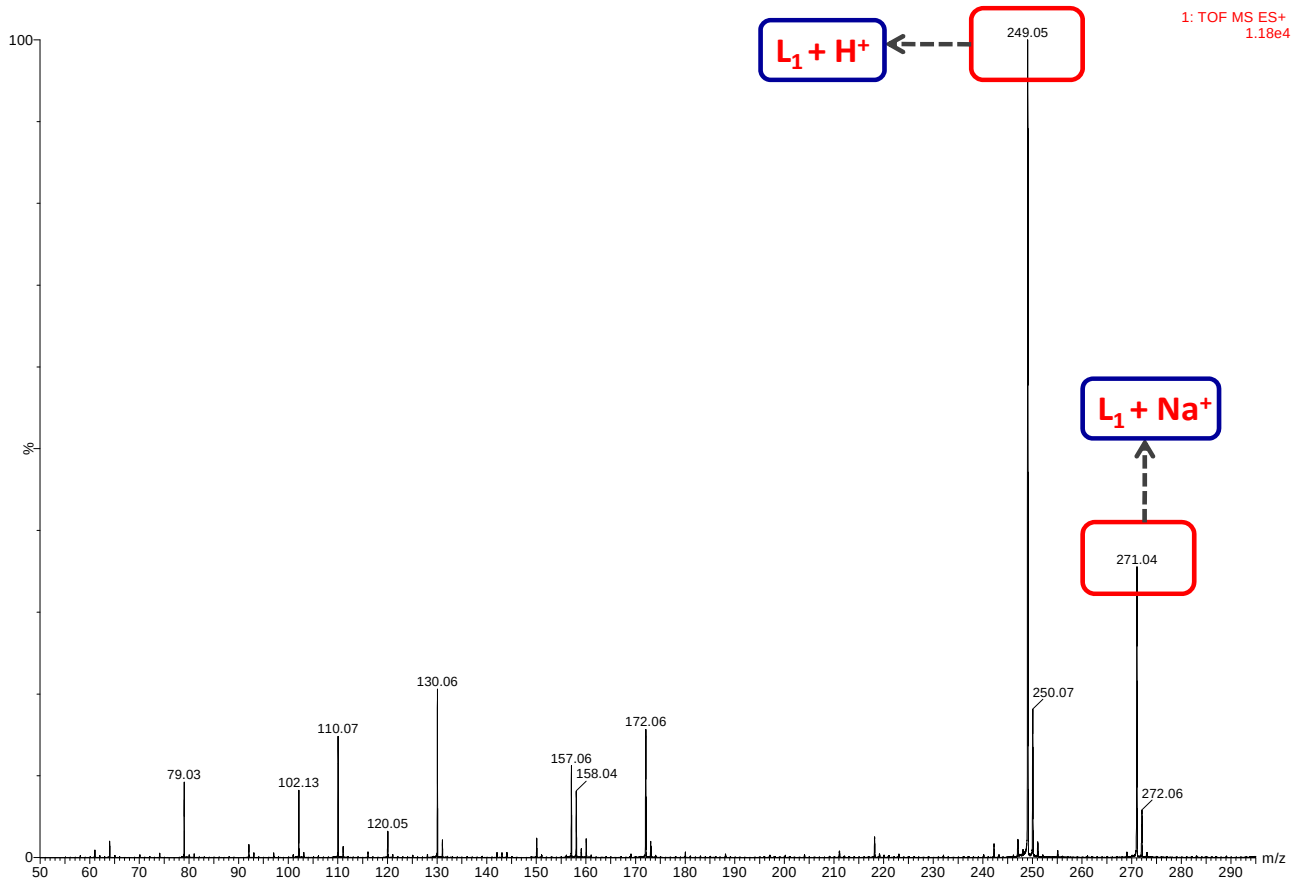
Mass spectra of L_1



SI Figure 3: ESI- Ms spectrum of $\mathbf{L_1}$.

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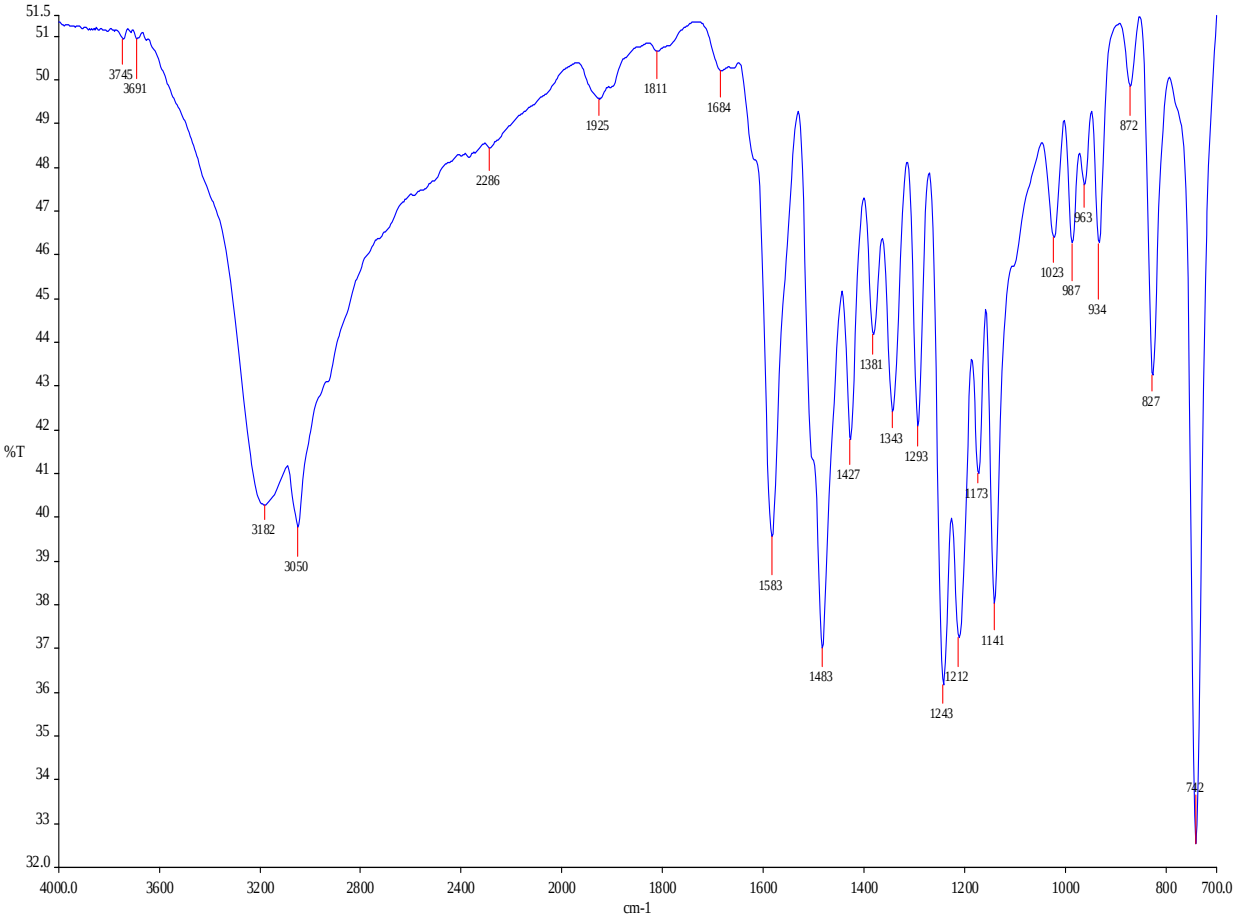
FTIR spectra of L_1



SI Figure 4: FTIR spectra of **L_1** recorded as KBr pellet.

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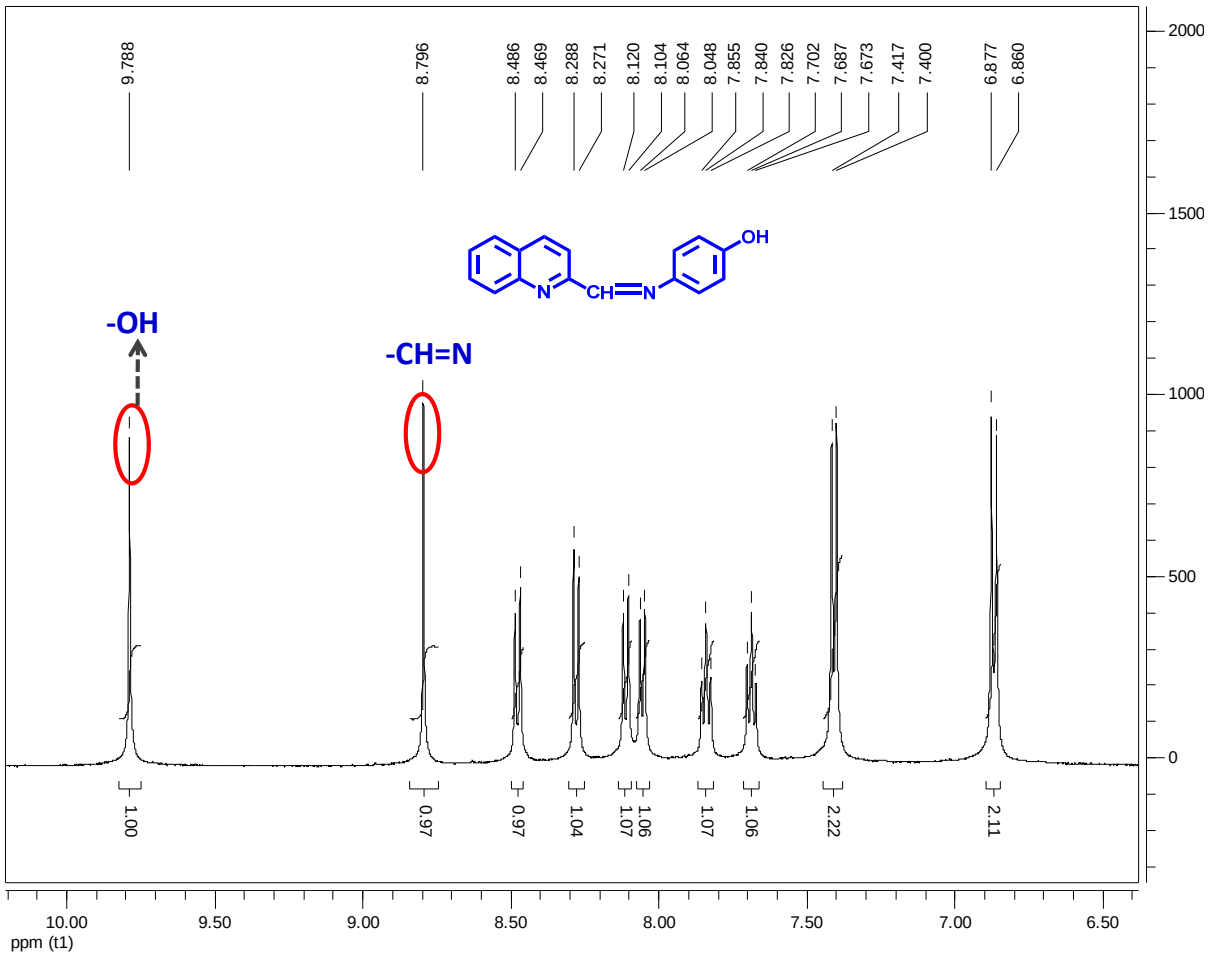
^{1}H NMR spectra of L_2



SI Figure 5: Partial ^{1}H NMR spectra of **L_2** in DMSO-d_6 medium.

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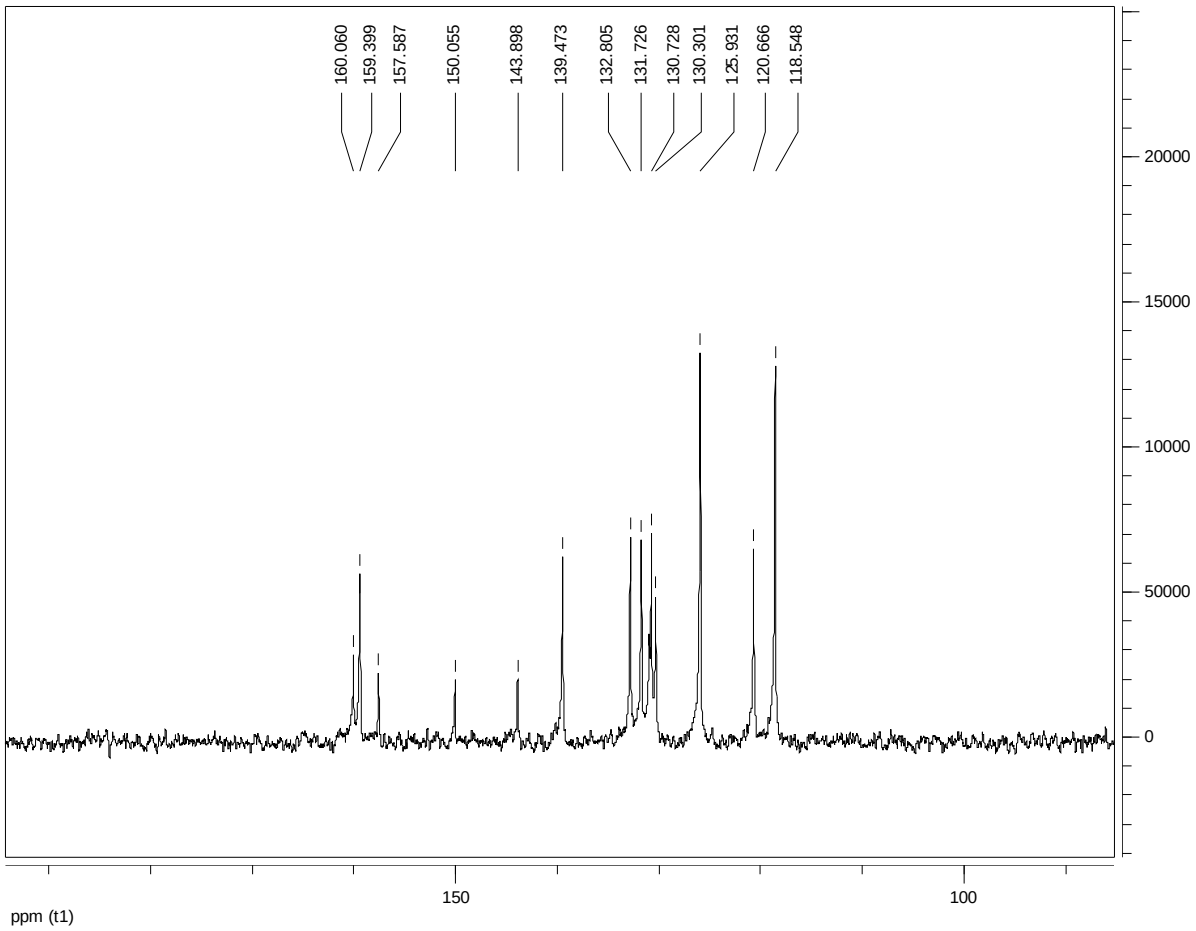
^{13}C NMR spectra of L_2



SI Figure 6: ^{13}C NMR spectra of $\mathbf{L_2}$ in DMSO-d_6 medium.

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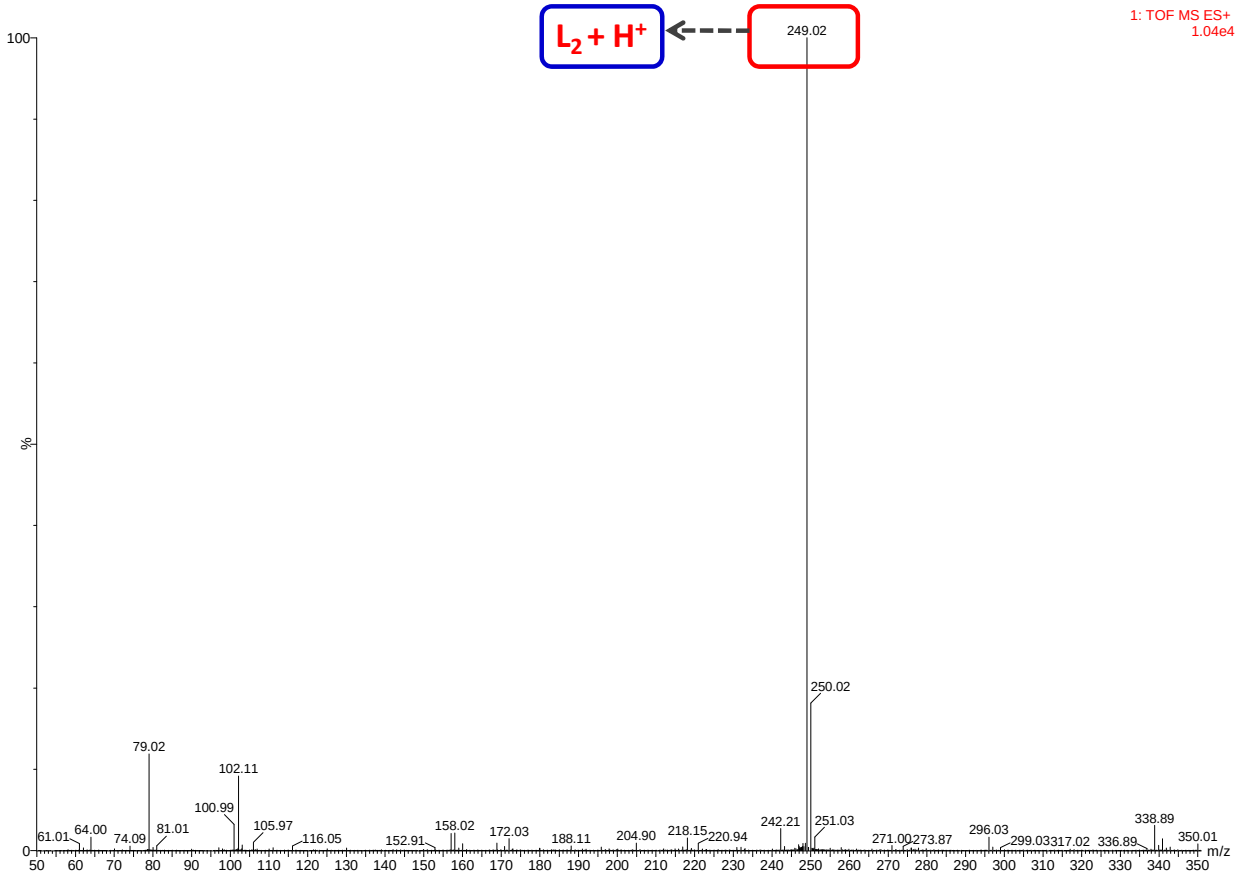
Mass spectra of L_2



SI Figure 7: ESI-Ms spectra of L_2.

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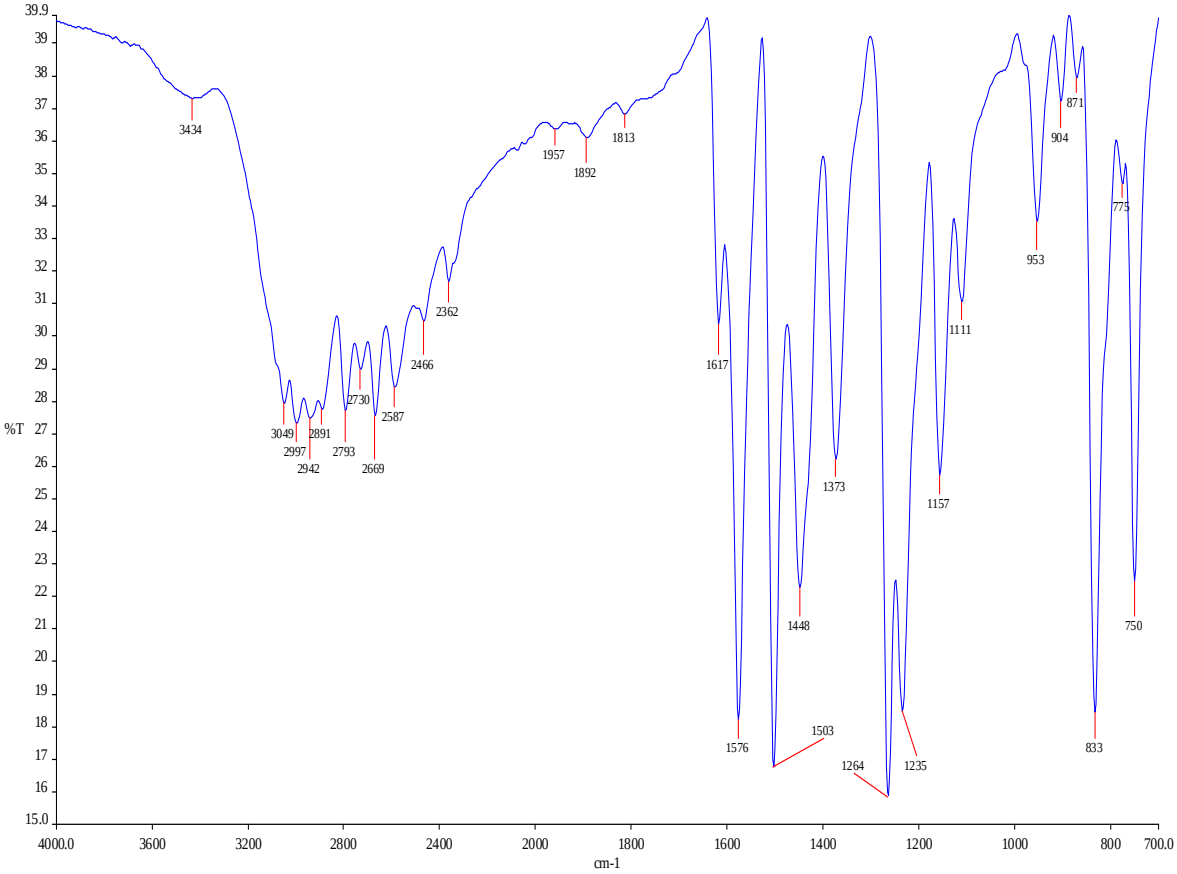
FTIR spectra of L_2



SI Figure 8: FTIR spectra of L_2 recorded as KBr pellet.

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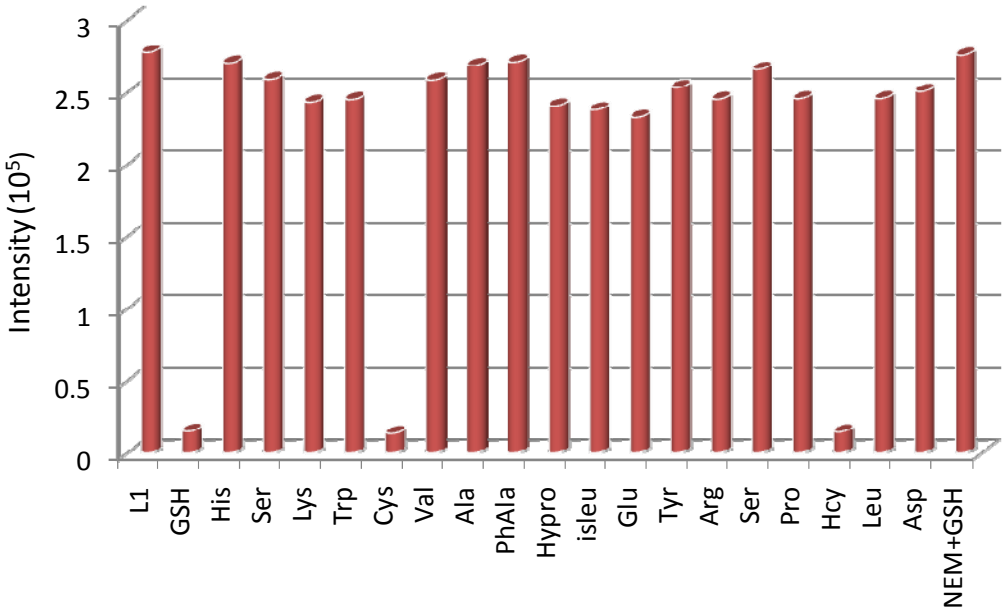
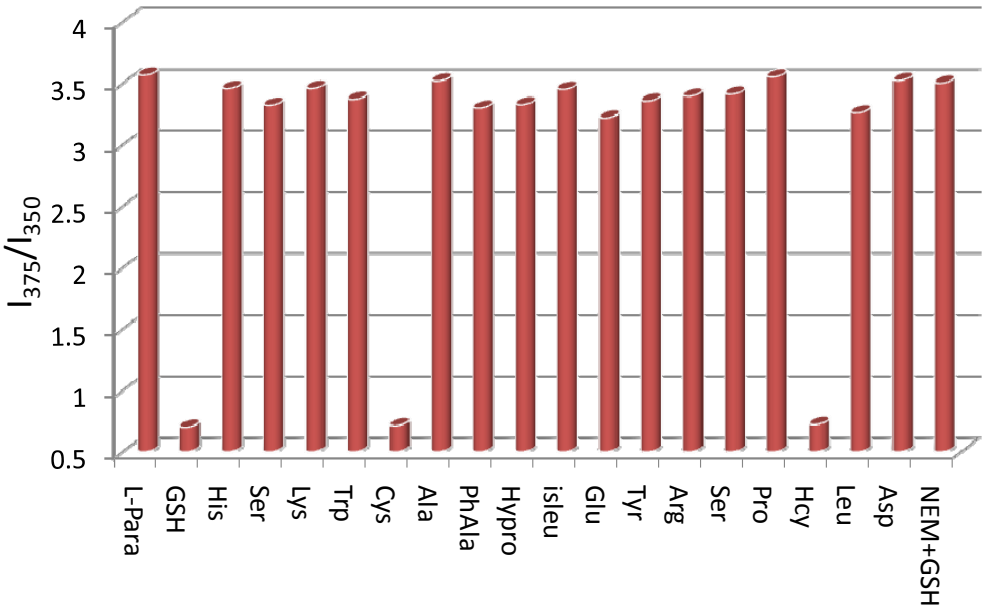
Fluorescence response of L_1 in presence of different amino acids



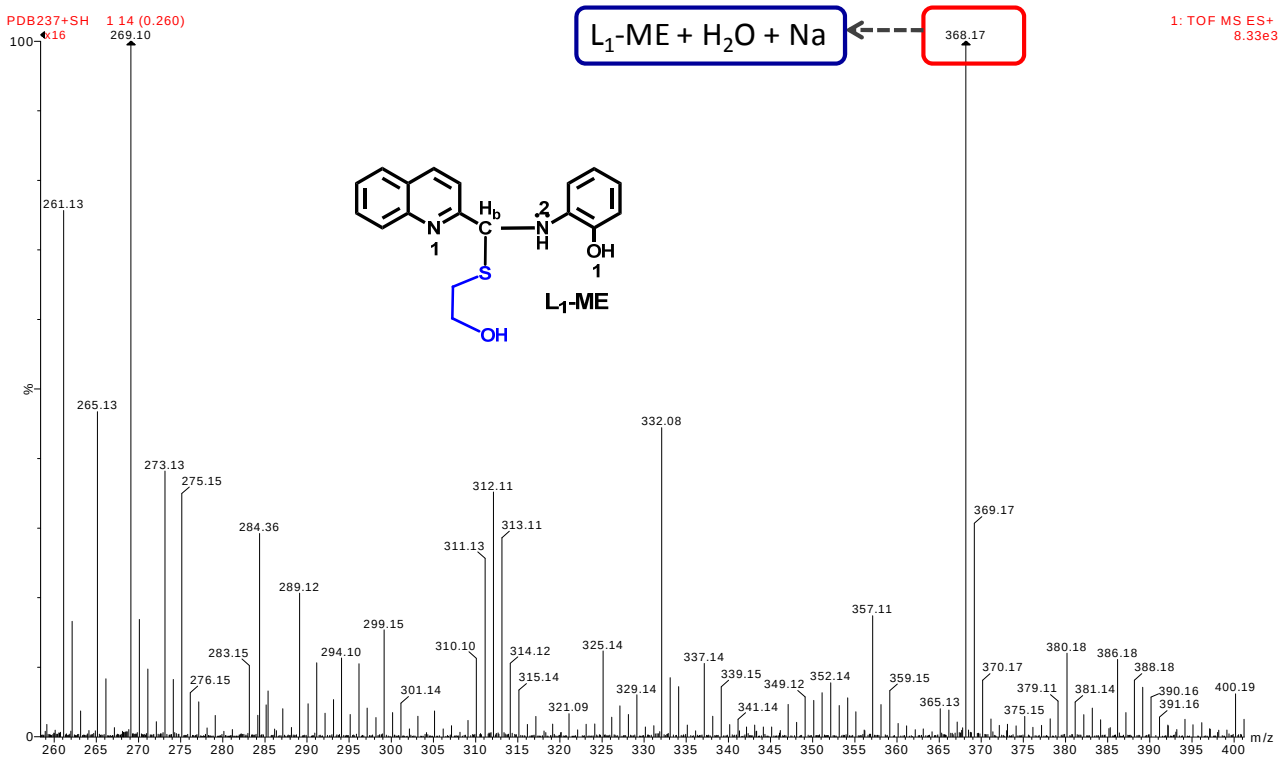
SI Figure 9: Fluorescence responses of $\mathbf{L_1}$ (20 μM) upon addition of various amino acids (20 mM) in DMSO-aq. HEPES buffer (50mM, 7:3 (v/v); pH 7.4). If GSH was pre-treated with *N*-ethylmaleimide (NEM, a scavenger of GSH), the change in fluorescence intensity of $\mathbf{L_1}$ was insignificant owing to the decrease in the effective concentration of GSH, λ_{Ext} = 296 nm, λ_{Mon} = 342nm.

Fluorescence response of L_2 in presence of different amino acids



SI Figure 10: The change in intensity ratio (I_{375}/I_{350}) of $\mathbf{L_2}$ (20 μM) upon addition of various amino acids (20 mM) in DMSO-aq. HEPES buffer (50mM, 7:3 (v/v); pH 7.4). If GSH was pre-treated with *N*-ethylmaleimide (NEM, a scavenger of GSH), the change in luminescence intensity ratio (I_{375}/I_{350}) of $\mathbf{L_2}$ is insignificant owing to the decrease in the effective concentration of GSH, λ_{Ext}=291 nm.

ESI-Ms spectrum of L_1-ME

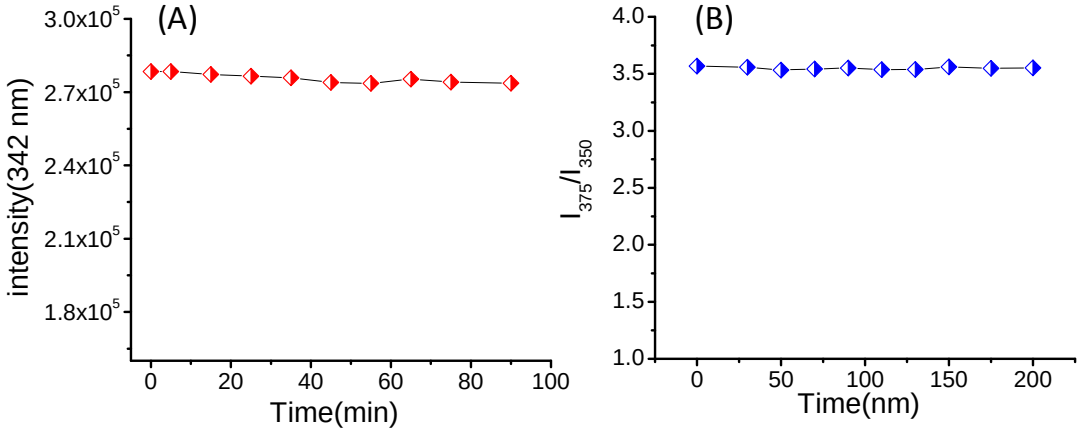


SI Figure 11: ESI-Ms spectra of **L_1-ME**.

Fluorescence Micorographs of only Live HeLa Cells

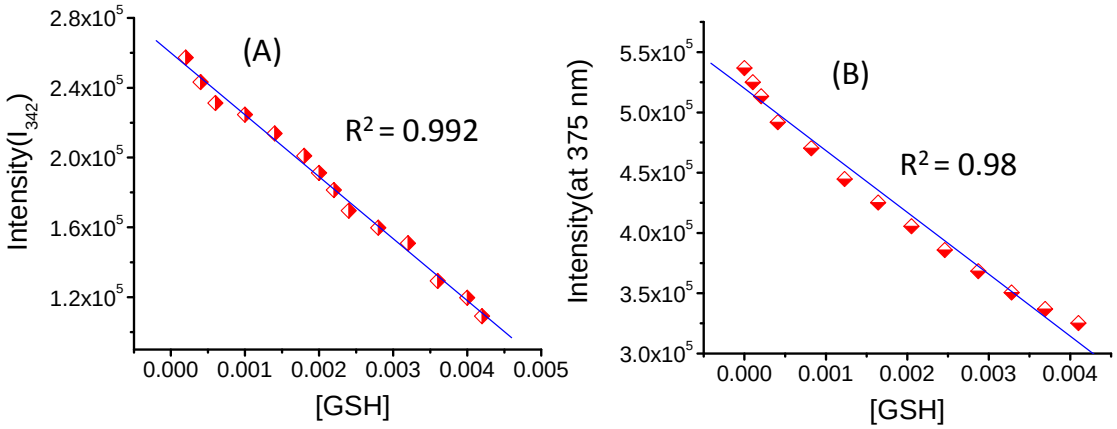
SI Figure. 12. (A) Differential interference contrast, and (B) fluorescence micrographs of HeLa cells in absence of **L_1** and (C) Overlay of the dark field image with a fluorescence phase contrast image. These images reveal that intracellular biothiols in HeLa cells are nonluminescent.

Hydrolytic stability test of L_1 and L_2



SI Figure. 13. (A) A plot of emission intensity of $\mathbf{L_1}$ at 342 nm with time in in DMSO-aq. HEPES buffer (50mM, 7:3 (v/v); pH 7.4) at 25°C, λ_{Ext} = 296 nm. (B) A plot emission intensity ratio (I_{375}/I_{350}) of $\mathbf{L_2}$ in DMSO-aq. HEPES buffer (50mM, 7:3 (v/v); pH 7.4) at 25°C, λ_{Ext} = 291 nm.

Determination of detection limit of L_1 and L_2



SI Figure. 14. (A) Plot of emission intensity of $\mathbf{L_1}$ at 342 nm as a function of various concentrations of GSH in DMSO-aq. HEPES buffer (50mM, 7:3 (v/v); pH 7.4) at 25°C, λ_{Ext} = 296 nm. An assay time of 35 min was chosen for each reaction. (B) Plot of emission intensity of $\mathbf{L_2}$ at 375 nm as a function of various concentrations of GSH in DMSO-aq. HEPES buffer (50mM, 7:3 (v/v); pH 7.4) at 25°C, λ_{Ext} = 291 nm. An assay time of 90 min was chosen for each reaction.