Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Specific receptor for hydrazine: Mapping the in-situ release of hydrazine in live cells and *in vitro* enzymatic assay

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Experimental details:

Materials & Methods:

All commercial reagents were procured from suppliers, were used as received without further purification. Hydrazine hydrate ($NH_2NH_2.H_2O$) (99%) AR Grade was procured from S. D. fine chem. Limited, India. Solvents were dried as per required by using standard procedures. TLC plates, Silica Gel on Aluminum, with layer thickness 0.2 mm were used for solid state studies. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400/500 MHz FT NMR (Model: Advance-DPX 400/500) using TMS as an internal standard. IR spectra were recorded on Bruker Alpha FT IR spectrometer. UV-Vis spectra were recorded using Shimadzu UV-1800 spectrometer. All the Fluorescence measurements were carried out on *PTI* Quanta Master™ Steady State Spectrofluorometer. High-resolution mass spectra were recorded on JEOL JM AX 505 HA mass spectrometer. Confocal images were acquired in Olympus Fluoview microscope. Intermidate compound **L** was synthesised by following literature procedures.[1] Quantum yield was recorded using standard methods[2] and perylene (Φ_f = 0.94) as reference compound in cyclohexane medium. The 3D intensity plots were obtained by using Image*J* software.

General experimental procedure for UV-Vis and Fluorescence studies:

Stock solution of probe $\mathbf{L_2}$ (5×10^{-3} M) was prepared in DMSO and the same solution was used for all the studies after appropriate dilution to 3 ml of 0.4 mM TX100 in HEPES aqueous buffer medium having solution pH 7.2 to make the effective $\mathbf{L_2}$ concentration of 7.8 μM. Unless and otherwise mentioned, 10 mM and pH 7.2 solution of aq. HEPES buffer was used for all spectroscopic studies. All amines solutions of 1×10^{-3} M were prepared in aq-HEPES buffer (pH 7.2). All luminescence measurements were done using λ_{Ext} = 360 nm with an emission slit width of 1 nm. The fluorescence quantum yield was determined according to literature method using perylene (in cyclohexane) as reference.[2]

Preparation of TLC test strips and solid state fluorescence studies:

TLC test strips were prepared by drop-casting 1.5 mM of probe ($\mathbf{L_2}$) solution in DMSO on silica TLC plates. Then it was dried properly and this silica coated plates were used for solid state experiments. These TLC plates were expose to the vapour as well as solution of

hydrazine and other amines for 20 minutes, dried and change of emission colour were recorded using lamp with excitation of 365 nm. Furthermore, silica coated TLC plates treated with different concentration of hydrazine were used for solid state fluorescence measurements using λ_{Ext} = 360 nm with an emission slit width of 1/1 nm.

General procedure for enzymatic study:

N-Acetyl-hydrazine was purchased from commercially available sources. 1×10^{-1} M Acetyl-hydrazine solution was prepared in 10 mM aq-HEPES buffer solution (pH 7.2). Enzyme solution was prepared according to the requirement by dissolving 1 mg/ml in 10 mM aq-HEPES buffer solution (pH 7.2). A fixed concentration of acetyl-hydrazine (200 equiv.) was added to the 7.8 μM of **L_2** in 0.4 mM Triton X 100 solution. Since 1 mg of solid enzyme contains 3301 units of protein and 1 unit can hydrolyse 1 μM of substrate, accordingly enzyme concentration was varied with respect to the substrate concentration.

General procedure for confocal studies:

Hct116 cells were seeded on cover slips placed in 6 well plates. After 24 hours cells were treated with **L_2** (7.8 μM) for 40 minutes. Cells were then washed thrice with Phosphate Buffer Saline (1X PBS) and fixed with 4% PFA for 10 minutes and washed again with Phosphate Buffer Saline (1X PBS). Permeabilization of the cells was done using 0.2% Triton X 100 for 5 minutes. The **L_2**-stained colon cancer cells Hct116 incubated with hydrazine (20 μM) for 40 min. Again three washes were given and then cover slips mounted using mounting medium. Nail paints was used to seal the coverslips mounted on the glass slides. Images were acquired in Olympus Fluoview Microscope.

General procedure for enzymatic study in Hct116 cells:

Hct116 cells were incubated with **L_2** (7.8 μM) for 40 minutes, then cells were washed thoroughly with buffer and acetyl hydrazine (1 mM) was incubated for 40 mins. After that different concentration of Aminoacylase-1 (0, 0.4, 0.7, 1.0 mU) were added to cellular medium for 1h and after the above mentioned treatment, CLMS images were captured.

Drug metabolism assay in live HepG2 liver cancer cells:

For drug metabolism studies, HepG2 cells (3×10^5) were seeded on cover slips placed in 6 well plates. After 24 hours, HepG2 cells were treated with 1 mM of Isoniazid (a well known

drug for Tuberculosis) for 3h at 37˚C in a 5% CO_2 air environment. Cells were then washed thrice with Phosphate Buffer (1X PBS). Then **L_2** (7.8 μM) was incubated for 1h and fixed with 4% PFA for 20 minutes and washed again with 1X PBS. Nail paint was used to seal the cover slips mounted on the glass slides for each well plates. For control experiment HepG2 cells were treated **L_2** (7.8 μM) for 1h. Then cells were washed thrice with media and followed under same conditions. Confocal laser scanning microscopic (CLSM) images were acquired in Olympus Fluoview Microscope with $\lambda_{Ext}/\lambda_{Mon}$ = 352/455 nm.

Synthesis of L_1:

Compound **L** (100 mg, 0.27 mmol) was dissolved in 5 ml of methanol. H_2O (3 ml) and Fe (200 mg, 3.58 mmol) were added and the reaction mixture was heated to reflux. Hydrochloric acid in a methanol solution (2 ml, 0.6 mol L^{-1}) was added drop wise. The solution was refluxed for 2 h until TLC monitoring indicated complete consumption of the starting material. After cooling to room temperature, it was filtered and concentrate at reduced pressure, the crude product was purified by silica-gel column chromatography. Yield: 68%, HRMS (ESI) (m/z) calculated for [$C_{21}H_{15}N_3$+H^+]: 310.1266 , observed: 310.1333 [M + H^+], ^{1}H NMR [500 MHz, DMSO-d6: δ (ppm)]: 8.82 (2H, s), 8.53 (2H, d, *J* = 7.8), 7.99 (2H, d, *J* = 8.5), 7.77 (2H, d, *J* = 14.8), 7.72 (2H, d, *J* = 14.9), 6.83 (2H, d, *J* = 8.5); ^{13}C NMR (125 MHz, DMSO-d_6, δ (ppm)) : 152.89, 149.38, 129.76, 128.44, 128.22, 127.69, 127.18, 124.56, 122.73, 122.00, 114.15, 111.45.

Synthesis probe L_2:

A mixture of **L_1** (280 mg, 0.96 mmol) and phthalic anhydride (331 mg, 2.24 mmol) in acetic acid (15 ml) then heated to reflex and stirred for 8 hours. It was filtered under hot condition and solid was collected washed with excess amount of acetic acid. Yield: 71%. MALDI-Ms (m/z) calculated for [$C_{29}H_{17}N_3O_2$+ H^+] : 440.4642, observed: 440.0731 [M + H^+], ^{1}H NMR [500 MHz, DMSO-d6: δ (ppm)]: 8.93 (2H, d, *J* = 7.8), 8.74 (2H, d, *J* = 7.3), 8.55 (2H, d, *J* = 7.7), 8.02 (2H, s), 7.95 (2H, s), 7.85 – 7.77 (4H, m), 7.75 (2H, d, *J* = 7.0). ^{13}C NMR (125 MHz, DMSO-d6, δ (ppm)): 169.15, 167.31, 148.21, 135.35, 133.99, 133.27, 132.00, 131.25, 128.79, 128.49, 128.12, 127.99, 127.80, 126.66, 124.58, 124.02, 122.75.

^{1}H NMR spectrum of L_1:

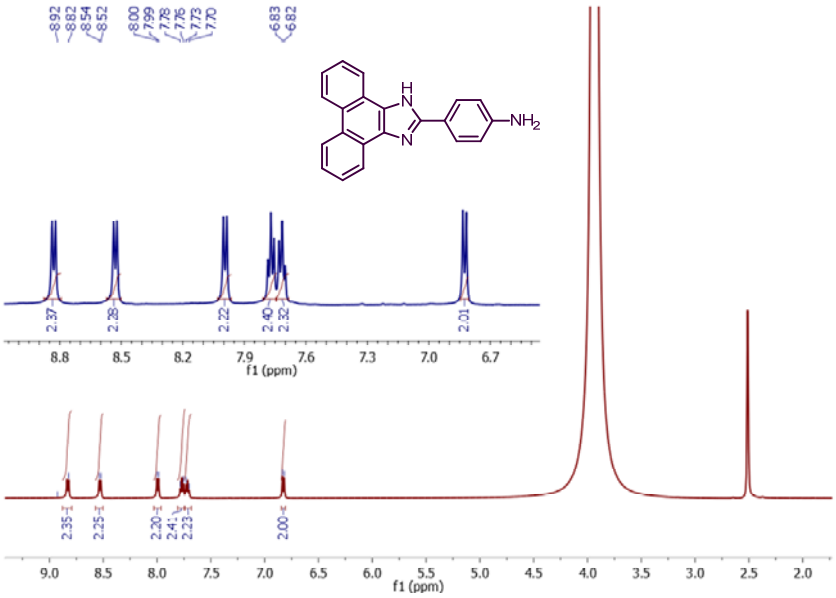

Fig. S 1: ^{1}H NMR of **L_1** in DMSO-d$_6$.

^{13}C NMR spectrum of L_1:

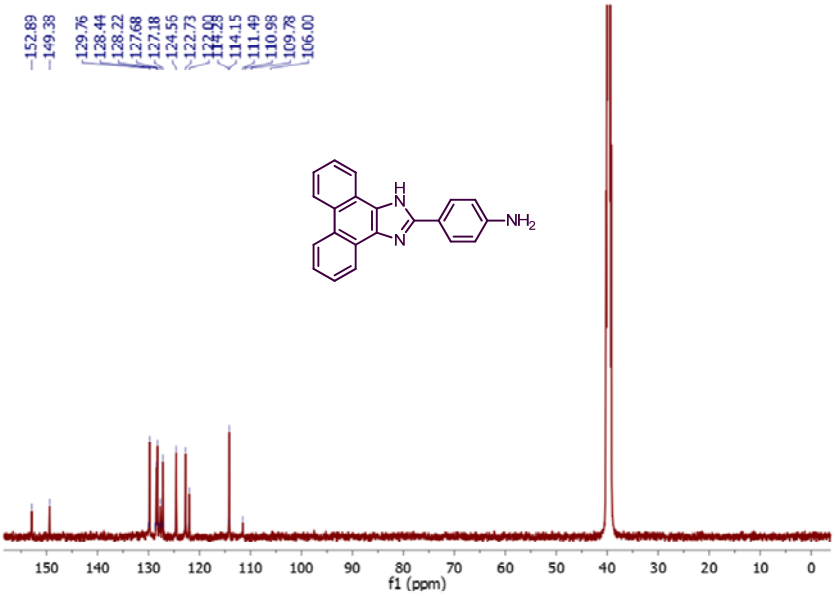

Fig. S 2: ^{13}C NMR of **L_1** in DMSO-d$_6$.

^{1}H NMR spectrum of probe L_2:

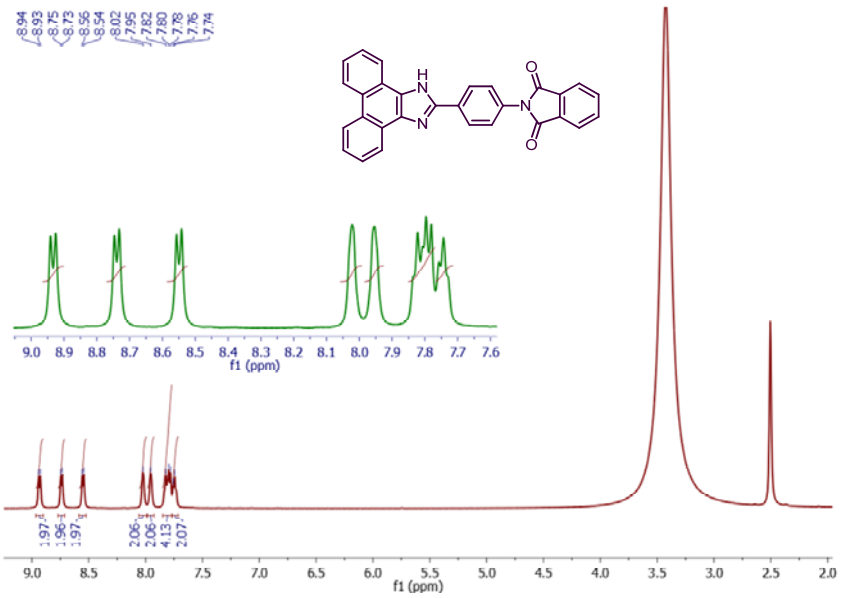

Fig. S 3: ^{1}H NMR of probe **L_2** in DMSO-d_6.

^{13}C NMR spectrum of probe L_2:

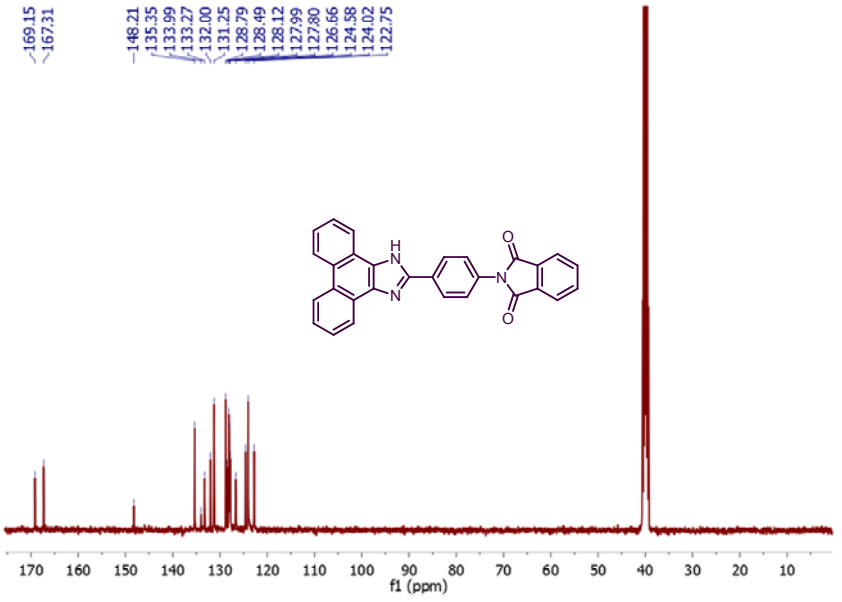

Fig. S 4: ^{13}C NMR of probe **L_2** in DMSO-d_6.

HRMS spectrum of L_1:

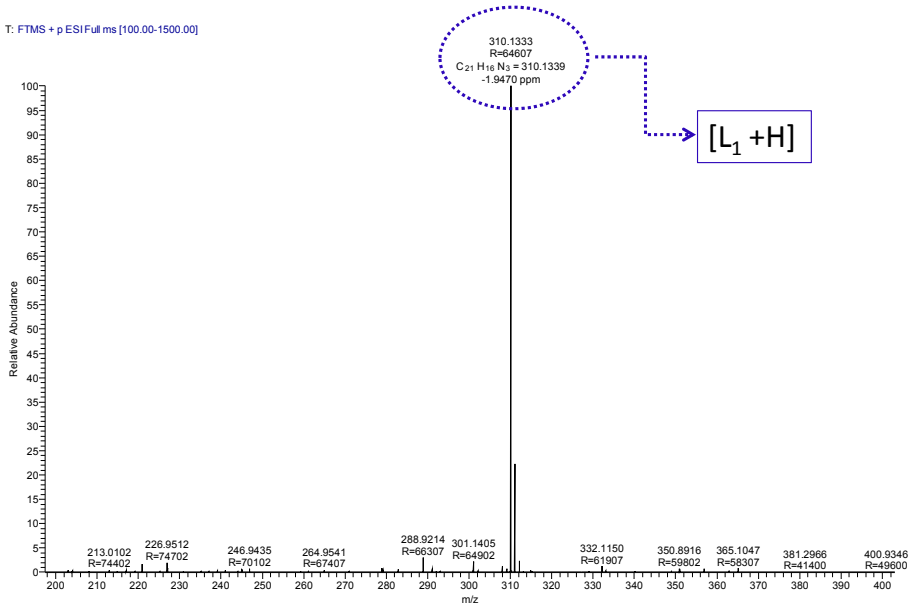

Fig. S 5: HRMS spectrum of $\mathbf{L_1}$ in Methanol.

MALDI Ms spectrum of L_2:

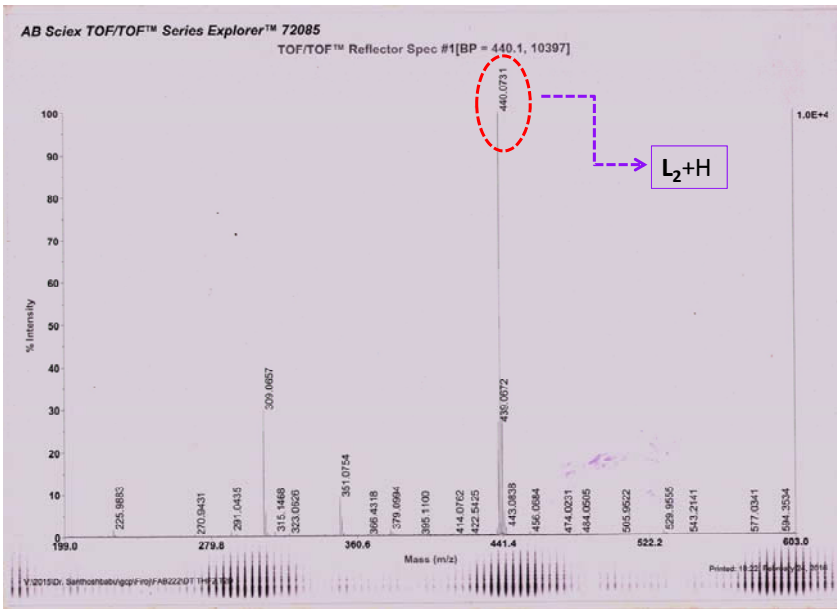

Fig. S 6: MALDI Ms spectrum of probe $\mathbf{L_2}$. Spectra were recorded using Dithranol (1,8-dihydroxy-9,10-dihydroanthracen-9-one) as the inert matrix using instrument AB SCIEX MALDI TOF/TOF™ 5800.

IR spectrum of L_2:

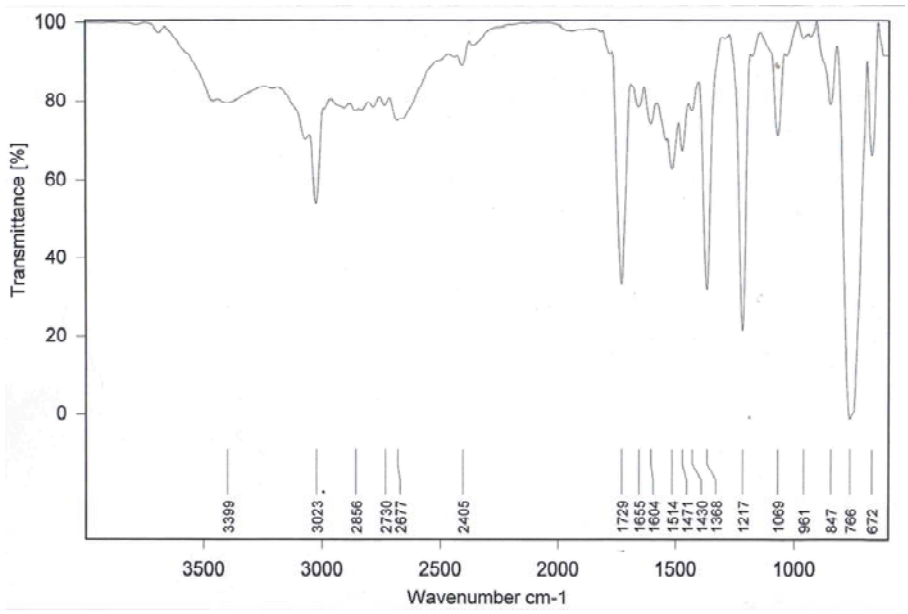

Fig. S 7: IR spectrum of probe L_2.

IR spectrum of L_1:

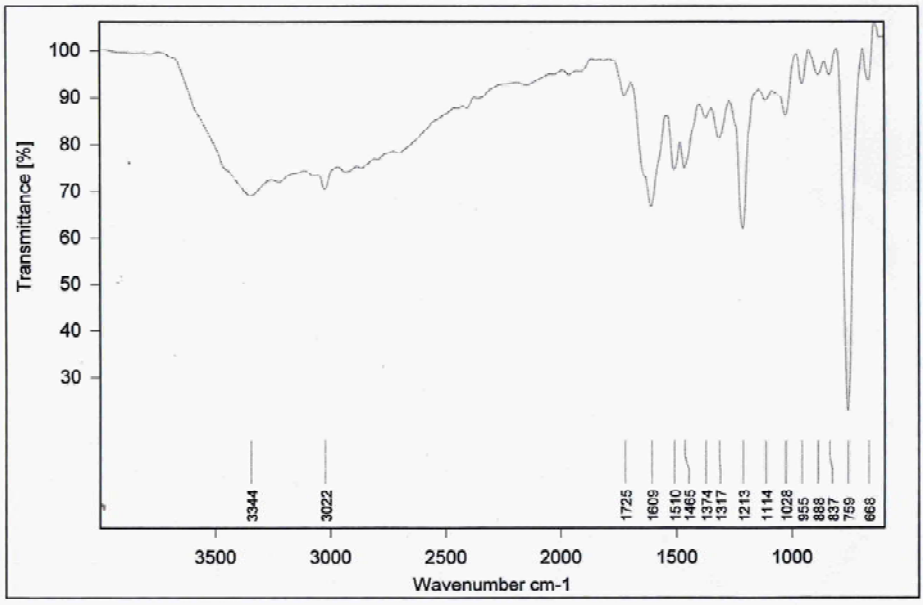

Fig. S 8: IR spectrum of probe L_1.

UV-Vis spectra of L_2 in presence and absence of different amines:

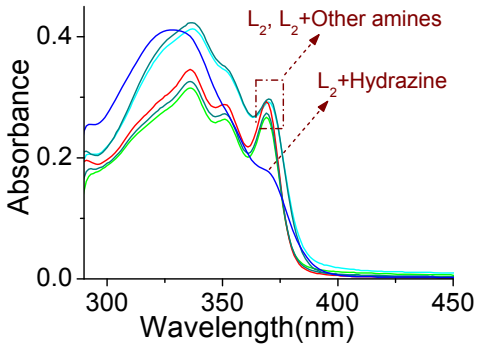

Fig. S 9: Absorbance spectra of **L_2** (7.8 μM) in presence and absence of different amines (2 mM) in 0.4 mM TX100 medium at pH 7.2.

TLC plate images of L_2 in presence and absence of different analytes:

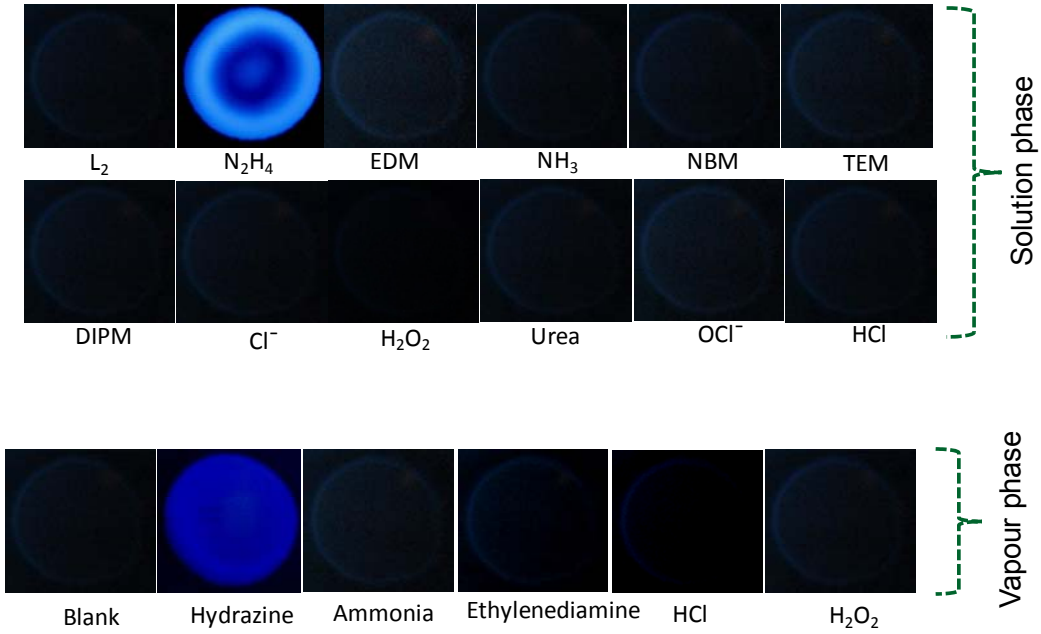

Fig. S 10: Snap shot of the visually detectable changes in fluorescence of silica surface, modified with the probe **L_2** in absence and presence of different analytes (Hydrazine, ethylenediamine, ammonia, butylamine, triethylamine, diisopropyl amine, Cl^-, H_2O_2, urea, OCl^- and HCl) in solution and vapour phase. Fluorescence colour changes were observed using 365 nm UV lamp; [**L_2**] used for the study was 1.5 mM.

Selectivity studies: Scanning with different amines

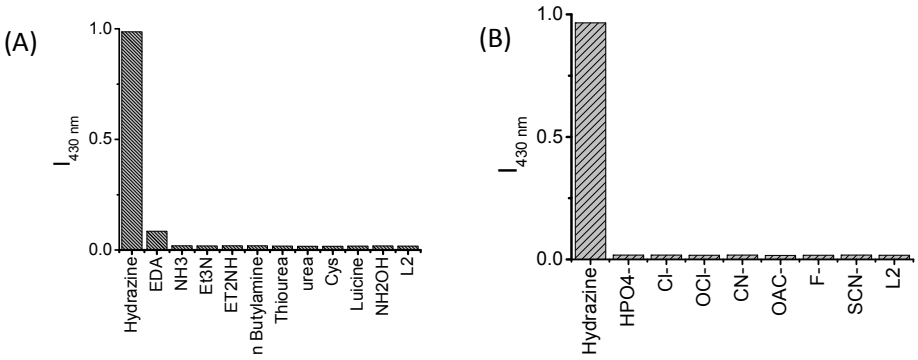

Fig. S 11: Emission response of $\mathbf{L_2}$ (7.8 μM) in the absence and presence of various (A) amines and (B) anionic analytes (200 mole equiv. each) in 0.4 mM TX100 at pH 7.2, λ_{Ext} = 360 nm, λ_{Mon} = 430 nm.

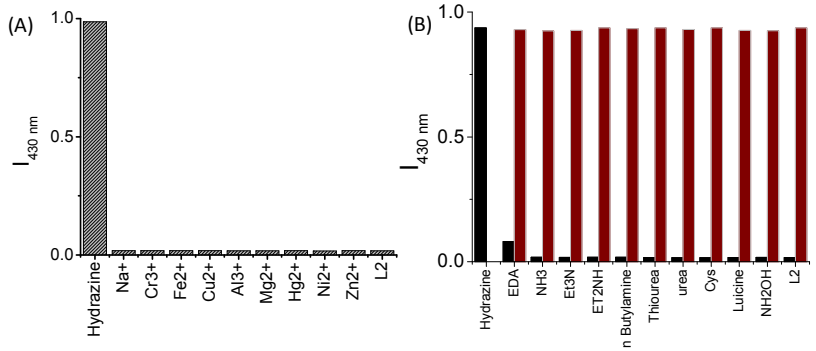

Fig. S 12: Emission response of $\mathbf{L_2}$ (7.8 μM) in the absence and presence of various (A) cationic species and (B) changes in emission intensity of L_2 (7.8 μM) induced by hydrazine (2 mM) in the presence of other (2 mM) amines. Red bar and black bar represent emission response in the presence and absence of hydrazine, respectively in 0.4 mM TX100 at pH 7.2, λ_{Ext} = 360 nm, λ_{Mon} = 430 nm.

Intensity v/s Concentration plot

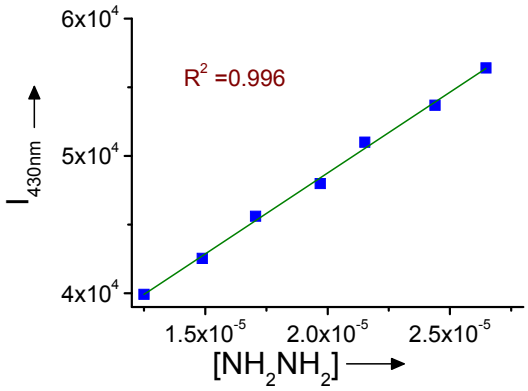

Fig. S 13: Fluorescence intensity of **L_2** at 430 nm upon addition of hydrazine (0-30 μM) in 0.4 mM TX100 at pH 7.2, λ_{Ext} = 360 nm.

Change in emission intensity of probe L_2 with pH:

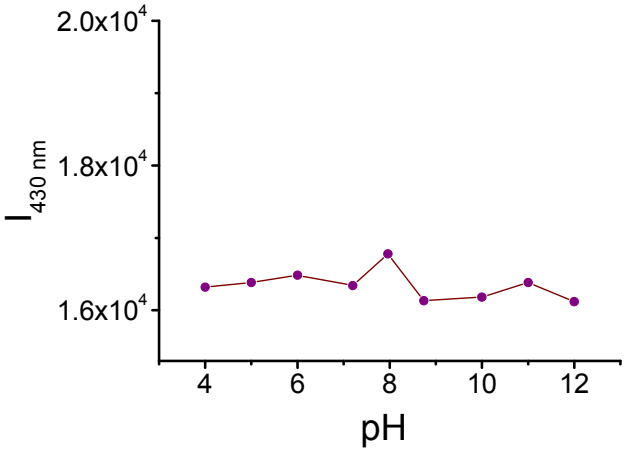

Fig. S 14: Emission spectrum of **L_2** at different pH, λ_{Ext} = 360 nm, λ_{Mon} = 430 nm.

Calculation of detection limit[3]:

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of **L_2** without hydrazine was measured 6 times and the standard deviation of blank measurements was determined. The detection limit (DL) of **L_2** for hydrazine was determined from the following equation:

DL = K * Sb1/S

Where K = 2 or 3 (we took 3 in this case);

Sb1 is the standard deviation of the blank solution;

S is the slope of the calibration curve.

From the graph we get slope = 1.1798×10^7, and Sb1 value is 0.123

Thus using the formula we get the Detection Limit = 1.58 ppb.

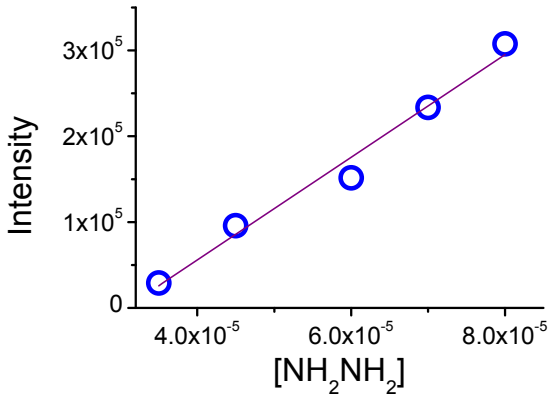

Fig. S 15: Fluorescence intensity at 430 nm upon addition of hydrazine in 0.4 mM Triton X 100, λ_{Ext} = 360 nm.

Kinetic studies[4]:

Time dependent studies of (7.8 μM) **L_2** with different concentration of (0.5, 1.5, 3.0, 6.0 mM) hydrazine were carried out by mixing the reactants and monitored by fluorescence measurements in 0.4 mM TX100 medium of pH 7.2 at 37^0C. λ_{Ext} = 360 nm. λ_{Mon} = 430 nm. Data were collected under pseudo-first-order conditions. The pseudo-first order rate constant for the reaction was determined by fitting the fluorescence intensity changes of the samples to the pseudo first-order equation:

$\ln[(I_{max}-I)/I_{max}] = -k_{obs}\, t$

Where, I and I_{max} represent the fluorescence intensities at times t and the maximum value obtained after the reaction was complete. k_{obs} is the observed rate constant of the reaction.

From the slope we get k_{obs} value for each reaction.

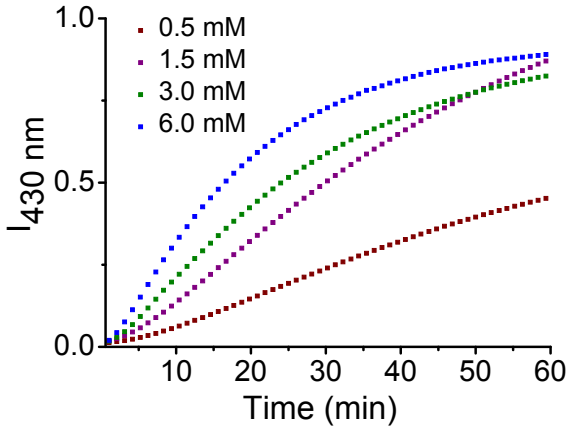

Fig. S 16: Time dependent emission studies of **L_2** (7.8 μM) in absence and presence different concentration of hydrazine in 0.4 mM of TX100 medium of pH 7.2 at 37^0C. λ_{Ext} = 360 nm. λ_{Mon} = 430 nm.

Intensity v/s Concentration plot in Solid TLC plate:

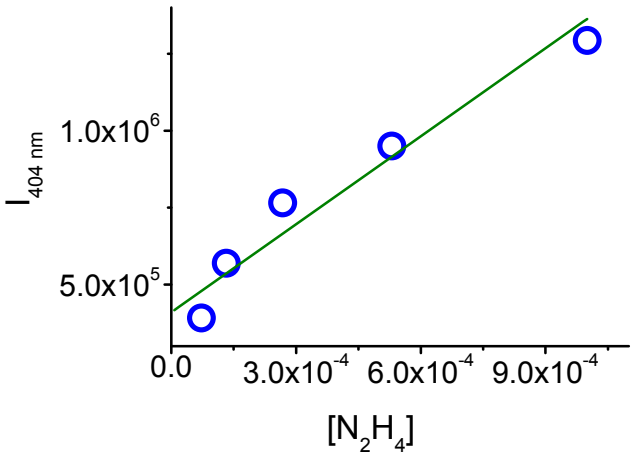

Fig. S 17: Fluorescence intensity of **L_2** at 404 nm upon addition of hydrazine (0-1 mM) in silica TLC plate coated with **L_2**, λ_{Ext} = 360 nm.

MTT assay:

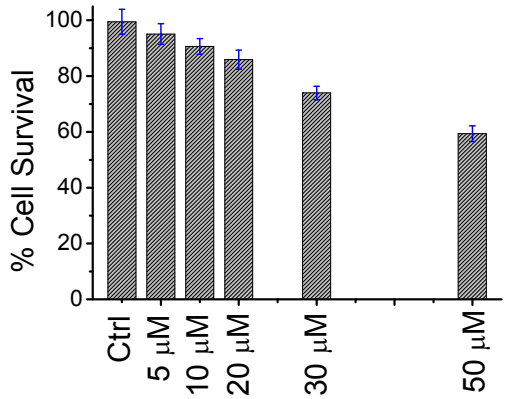

Fig. S 18: MTT assay to determine the cell viability percentage in presence of **L_2** in Hct116 colon cancer cells.

The *in vitro* cytotoxicity of **L_2** on Hct116 cells (Colon cancer cell) were determined by conventional MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Hct116 colon cancer cells (7×10^3) were seeded in each well of a 96 well plate and cultured in a 37°C incubator supplied with 5% CO_2. Cells were maintained in DMEM medium, supplemented with 10% Foetal Bovine Serum and 100 Units of Penicillin Streptomycin antibiotics. After 24 hours the cells were treated with different concentrations of the **L_2** in triplicates for 12 hours. After the treatment, cells were added with 0.5 µg/ml of MTT reagent. The plate was then incubated for 4 hours at 37°C. 100 µl of Isopropyl Alcohol was added to each well. Optical density was measured at 570 nm using Multiskan Go (Thermo Scientific) to find the concentration of the cell inhibition. IC_{50} value has been calculated to be 70 µM.

The formula used for the calculation of the MTT assay for evaluation of the cell viability was as follows:

Cell viability (%) = (Means of absorbance value of treated group/ Means of absorbance value of untreated control) × 100.

Confocal microscopic images of HepG2 cells in presence & absence of hydrazine using L_2 as the imaging reagent:

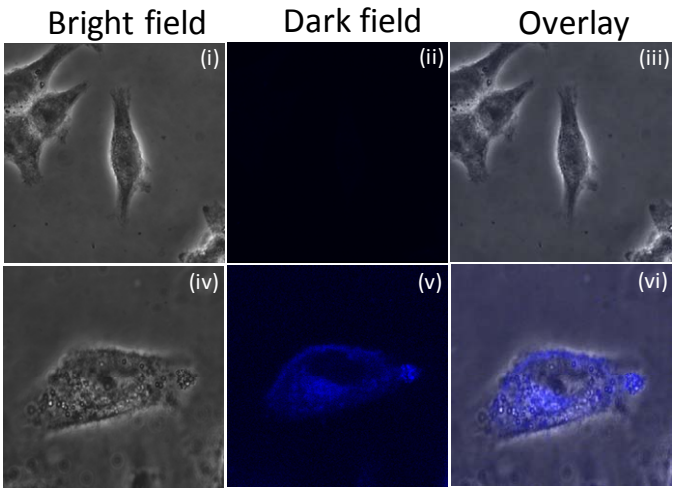

Fig. S 19: (i–iii) Confocal laser scanning microscopic (CLSM) images of HepG2 cells incubated with $\mathbf{L_2}$ (7.8 μM) as control: (i) bright field, (ii) dark field laser and (iii) overlay images of (i) and (ii); (iv–vi) CLSM images of HepG2 cells incubated with $\mathbf{L_2}$ (7.8 μM) for 1h and then further exposed to N_2H_4 (20 μM) for 1h at 37 °C: (iv) bright field, (v) dark field laser and (iv) overlay images of (iv) and (v).

References:

1. Y. Sun, W. Huang, C.-G. Lu and Y.-P. Cui, *Dyes Pigm*., 2009, **81**, 10.
2. F. Ali, S. Saha, A. Maity, N. Taye, M. K. Si, M. Suresh, B. Ganguly, S. Chattopadhyay and A. Das, *J. Phys. Chem. B*, 2015, **119**, 13018.
3. S. Goswami, K. Aich, S. Das, S. B. Roy, B. Pakhira and S. Sarkar, *RSC Adv*., 2014, **4**, 14210.
4. F. Ali, A. H.A., N. Taye, R. G. Gonnade, S. Chattopadhyay and A. Das, *Chem. Commun.,* 2015, **51**, 16932.