Supplementary Information for

Hydrogen sulfide triggered self-immolative fluorescent probe for lysosomes labeling in live cells

Nithya Velusamy, Natesan Thirumalaivasan,KondapaNaidu Bobba,Shu-PaoWu*, Sankarprasad Bhuniya*

[a]Amrita Centre for Industrial Research &Innovation, Amrita School of engineering, Coimbatore, Amrita Vishwa Vidyapeetham, India 641112.

[b]Department of Applied Chemistry, National Chiao Tung University, 1001 Ta Hsueh Road, Hsinchu, Taiwan 300.

[c] Department of Chemical Engineering & Materials Science, Amrita School of Engineering, Coimbatore, Amrita Vishwa Vidyapeetham, India 641112.

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Experimental

Materials, methods and instrumentations

4-Bromo-1,8-naphthalic anhydride (TCI, Japan), 4-(2-Aminoethyl)morpholine (Avra, India), 2-carboxybenzaldehyde (Avra, India), oxalyl chloride (Alfa Aesar, India), potassium carbonate (Avra, India), hydrogen iodide (Avra, India), dichloromethane (Merck, India), ethanol (ChangshuYangyuvan chemical, China), methanol (Finar, India), sodium sulfate (LobaChem, India), lipase (SRL, India), trypsin (Himedia, India) pepsin (Himedia, India) and esterase (Sigma, India) were purchased commercially and used without further purification. Column chromatography was performed using silica gel (100-200 mesh, LobaChem) as the stationary phase. Analytical thin layer chromatography was performed using silica gel 60 (precoated sheets with 0.25 mm thickness).UV/Vis and fluorescence spectra were recorded with a Shimadzu UV-1800 and RF- 6000 spectrophotometer respectively. NMR spectra were collected on a 400MHz spectrometer (Bruker, Germany). Mass spectra were recorded on anionSpecHiResESI mass spectrometer.

Synthesis of FL-1

Compound FL-1 was synthesized according to literature.[44]

Synthesis of LS-1

To a solution of 2-carboxybenzaldehyde (440 mg, 3.19 mmol) in DCM (20 mL) under argon atmosphere, oxalyl chloride (809 mg, 6.38 mmol) was added. The reaction was carried out for 4 h at RT. Then DCM was evaporated under argon atmosphere in a rotary evaporator. The crude reaction mass was diluted with DCM (10 mL), and then few drops of TEA was added. The reaction mixture was cooled to 0 ^{o}C, and then FL-1 (400 mg, 1.23 mmol) was added. The reaction mixture was stirred for 12 h at ambient temperature. After completion of the reaction, the reaction mixture was diluted with water and extracted with DCM. The organic layer was dried over anhydrous sodium sulfate. The organic layer was concentrated, and column chromatography was done using MeOH in DCM (0.5:4.5) as eluent, dried to afford **LS-1** as pale yellow solid. (381 mg; 67.86 %). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.17 (s, 1H); 8.60 (d, *J*=8.40 Hz,1H); 8.53 (dd, *J*=7.2 Hz, 1H); 8.43 (dd, *J*=8.40 Hz, 1H); 8.01 (m, 1H); 7.98 (dd, *J*=7.6 Hz, 1H); 7.83 (m, 3H); 7.66 (s, 1H); 4.19 (t, *J*=6.80 Hz, 2H); 3.53 (t, *J*=4.80 Hz, 4H); 2.58 (t, *J*= 7.2 Hz, 2H); 2.48 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6): 168.10,164.05,157.33, 144.55, 136.06, 132.37, 129.19, 128.65, 126.11,125.80, 123.58, 122.54, 117.42, 110.98, 99.15, 66.70, 62.66, 56.05, 53.95, 49.09, 40.43, 40.10, 39.93, 39.60, 37.35, 25.80. LC-MS *m/z* (M+H): calcd. 459.15, found 459.10.

Absorption and fluorescence studies

All fluorescence and UV-Vis. spectra were obtained with RF- 6000 FL spectrometer with a 1cm standard quartz cell and UV-1800 spectrophotometer, respectively. Sodium sulfide (Na_2S) was used as the source of H_2S. Stock solutions (600 μM) of various analytes (HOCl, H_2O_2, $NaNO_2$, $Cu(OAc)_2$, $Zn(OAc)_2$, $Na_2S_2O_4$, $FeSO_4$, NO, KCl, $CaCl_2$, Na_2CO_3, GSH, ascorbic acid (AA), cysteine, folic Acid, histidine, lysine, pepsine, trypsin, lipase and esterase were prepared in double distilled water. The stock solution of probe **LS-1** (20 μM) was prepared in HEPES buffer (pH = 7.4) with 1% DMSO. Excitation was effected at 450 nm with excitation and emission slit widths as 5 nm each. The Fluorescence experiments (solution test) of **LS-1** (5.0 μM) was

recorded in the presence of increasing concentrations of Na_2S (0–40 eq.) in HEPES buffer (pH = 7.4) with 1% DMSO. **LS-1** was incubated with Na_2S for 30 min at 37 °C.

The Linear range and detection limit.

The detection limit of the probe toward H_2S was calculated by fluorescence titration. The fluorescence emission spectrum of **LS-1** (2.0×10^{-6} M) was measured ten times. Also, the standard deviation of the blank solution was measured. The fluorescence intensity (λ_{em} = 562 nm) of the probe was plotted vs. concentrations of H_2S. The detection limit was calculated by using following equation.

Detection Limit 3σ/k:

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus H_2S concentration.

Cytotoxicity assay

The cellular cytotoxicity of **LS-1** in MCF-7 cells was evaluated by the methyl thiazolyltetrazolium (MTT) assay. MCF-7 cells were grown in a 96-well cell-culture plate and several concentrations (5, 10, 15, 20, 25 μM) of **LS-1** were added to the wells. Then the cells were incubated at 37 ^{0}C under 5% CO_2 for 24 h. After that, each well was incubated with 10 mL MTT (5 mg mL^{-1}) at 37^{o}C under 5% CO_2 for 4 h. The MTT solution was removed, and the yellow precipitates (formazan) was dissolved in 200 mL DMSO and 25 mL Sorensen's glycine buffer (0.1 M glycine and 0.1 M NaCl). The absorbance at 570 nm for each well was measured by Multiskan GO microplate reader. The viability of the cells was calculated by the following equation:

Cell viability (%) = (Mean of absorbance value of treatment group)/ (Mean of absorbance value of control group)

Cell culture and confocal fluorescence imaging of LS-1 in MCF-7 cells

MCF-7 cells were grown in DMEM media with 10% (v/v) FBS (fetal bovine serum) and penicillin/streptomycin (100.0μg/mL) at 37°C in a 5% CO_2 incubator. The cells were treated with 3 μL of 2 mM **LS-1** (final concentration: 3μM) dissolved in DMSO and incubated for 30 min at 37^{o}C. After addition of H_2S (150 μM) to the above cells, the culture medium was removed, and the treated cells were washed with 0.1 M HEPES (2 mL×3) before observation. Confocal fluorescence imaging of cells was performed with a Leica TCS SP5 X AOBS Confocal Fluorescence Microscope (Germany), and a 63×oil-immersion objective lens was used. The cells were excited with UV light below 450 nm and emission was collected at 562 nm.

Colocalization of LS-1 with LysoTracker Red DND-99

MCF-7 cells were grown in DMEM media with 10% (v/v) FBS (fetal bovine serum) and penicillin/streptomycin (100 μg/mL) at 37 °C in a 5% CO_2 incubator and then washed with cell culture medium twice. For the **LS-1** (5μM) and LysoTrackerLysoView^{TM} 633 (2 μM) in cell culture medium was added to pre-washed cells and incubated at 37^{o}C for 45 min. After addition of H_2S (150.0 μM) to the above cells, the culture medium was removed, and the treated cells were washed with 0.1 M HEPES (2 mL×3) before observation. For nuclear staining, cells were incubated with DAPI (4′, 6-diamidine-2′-phenylindole dihydrochloride) (1.0 μM) at 37 °C for 15 min prior to imaging. Confocal fluorescence imaging of cells was performed with a Leica TCS SP5 X AOBS Confocal Fluorescence Microscope (Germany), and a 63×oil-immersion objective

lens was used. The cells were excited with UV light below 450 nm and emission was collected at 562 nm.

Cell culture and confocal fluorescence imaging of LS-1 in HT-29 cells

HT-29 cells were grown in DMEM media with 10% (v/v) FBS (fetal bovine serum) and penicillin/streptomycin (100 mg mL $^{-1}$) at 37 ^{o}C in a 5% CO_2incubator. The cells were treated with 5.0 µL **LS-1**dissolved in DMSO and incubated for 30 min at 37^{o}C. The culture medium was removed, and the treated cells were washed with 0.1 M PBS (2mL × 3) before observation. Confocal fluorescence imaging of cells was performed with a Leica TCS SP5 X AOBS Confocal Fluorescence Microscope (Germany), and a 63 oil-immersion objective lens was used. The cells were excited at 450 nm.Furthermore, we carried out the fluorescence imaging by two photon excitation in order to prove the two-photon effect of **LS-1**. Under the same experimental condition, we could observe green fluorescence emission by using two-photon excitation wavelength at 900 nm. The results clearly demonstrated that **LS-1** was able to sense the H_2S by using two-photon excitation.

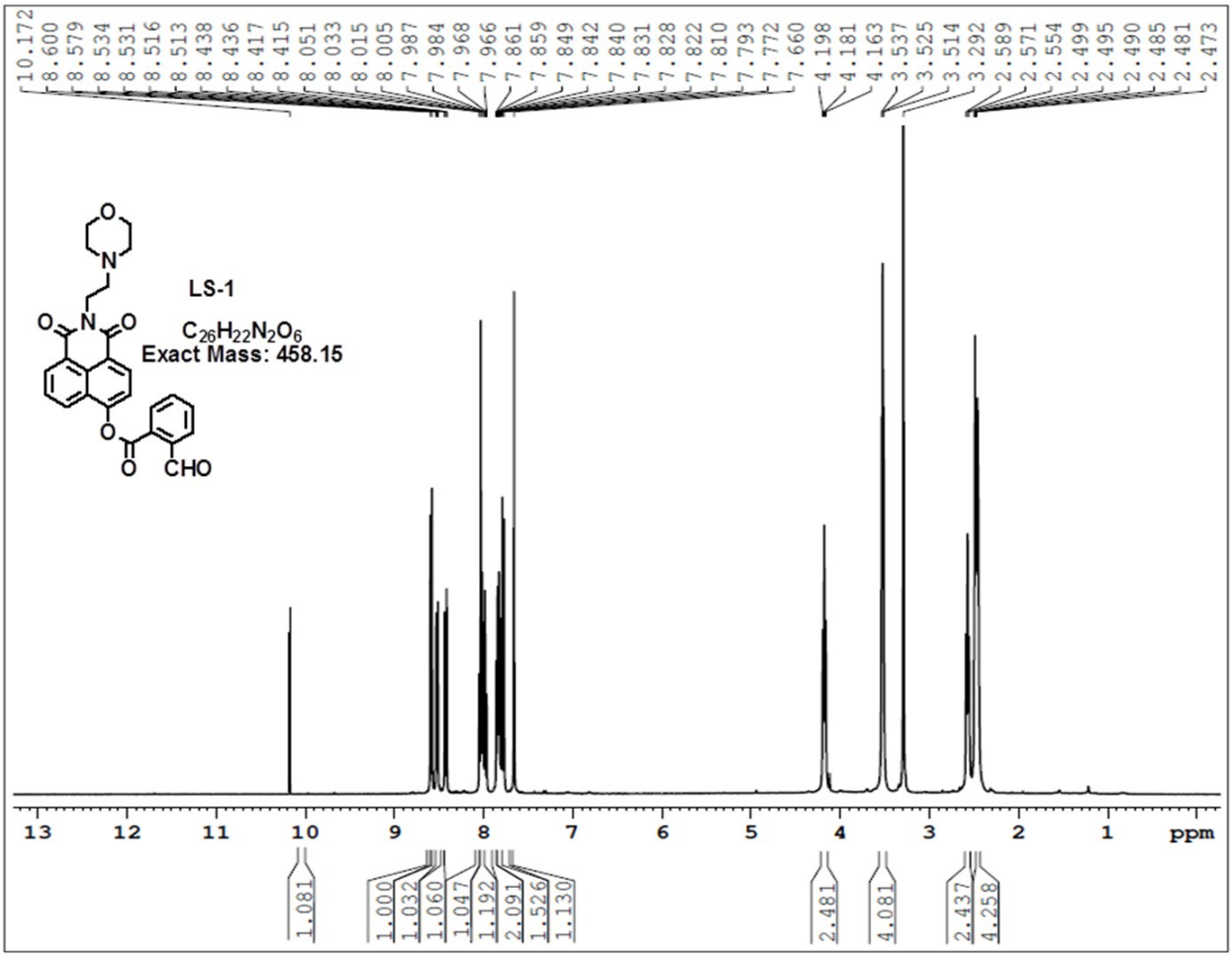

Fig. S1:^{1}H-NMR of **LS-1**in DMSO.

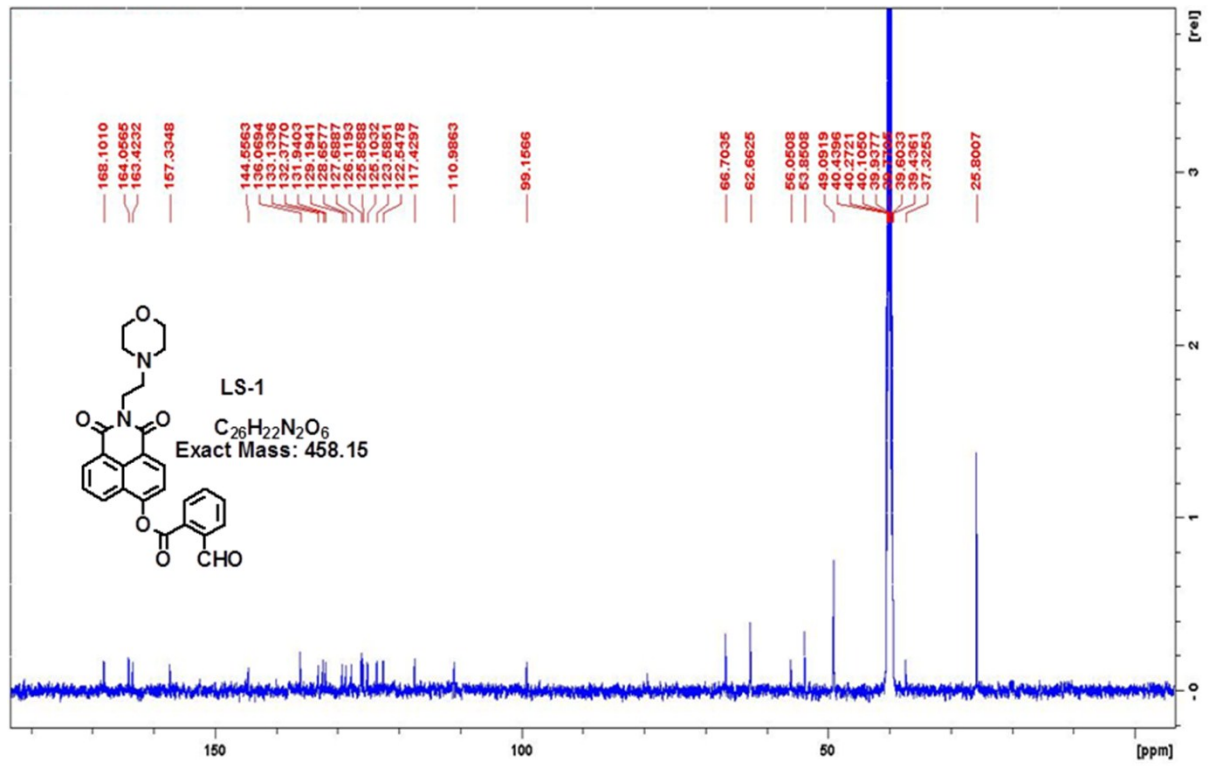

Fig. S2:[13] C-NMR of **LS-1**in DMSO.

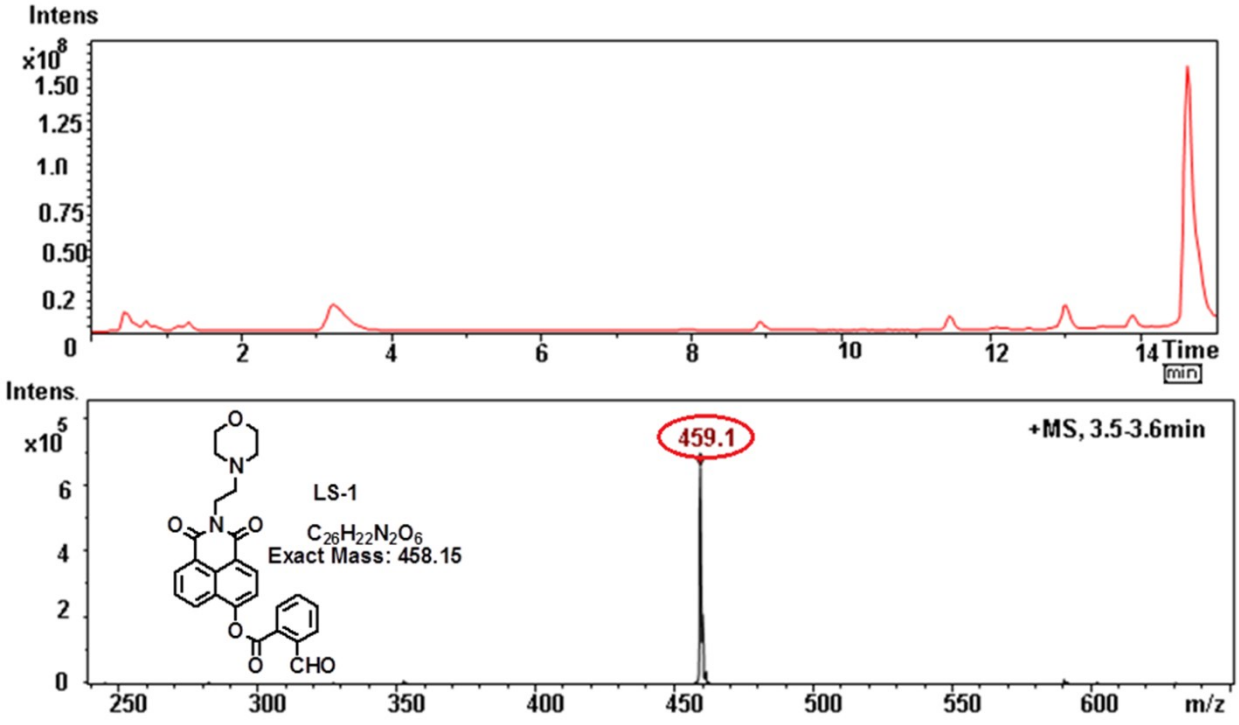

Fig. S3: LCMS of **LS-1.**

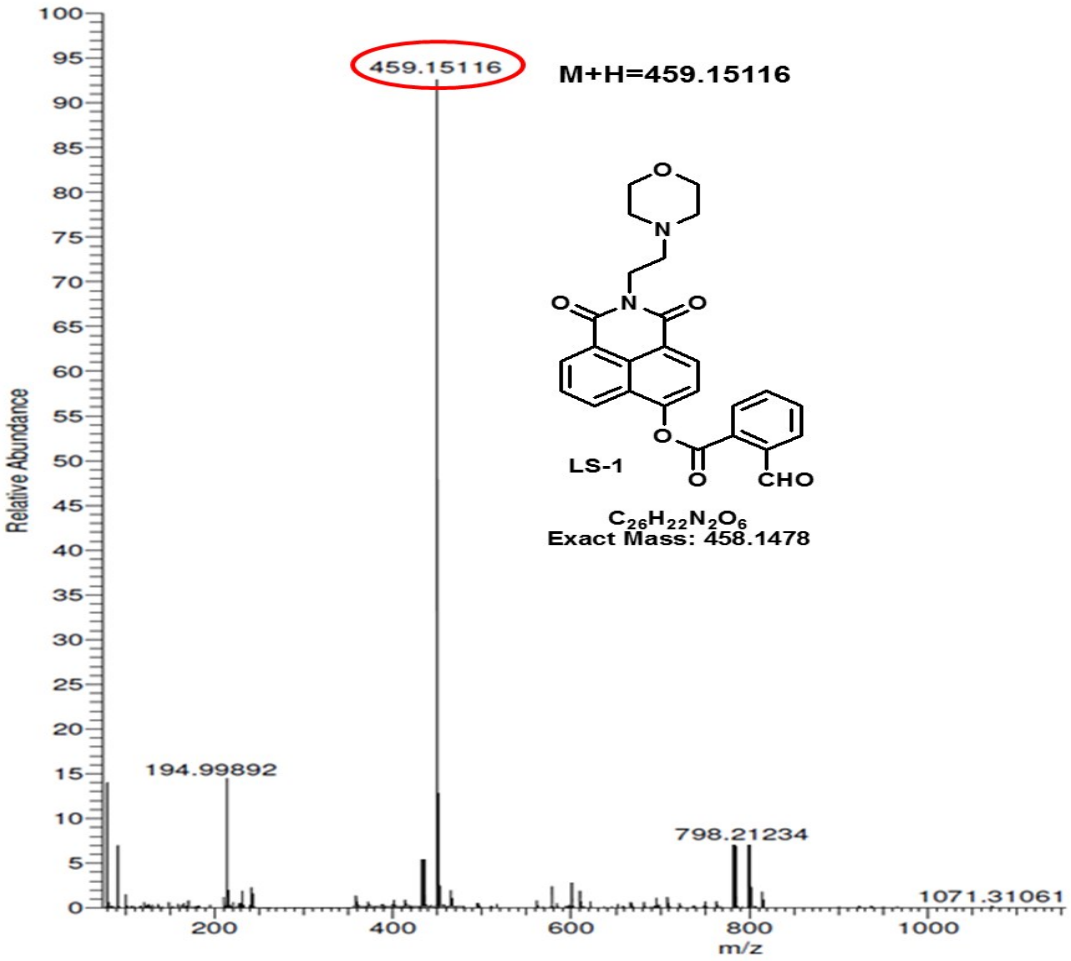

Fig. S4: The High resolution Mass spectrum of **LS-1**

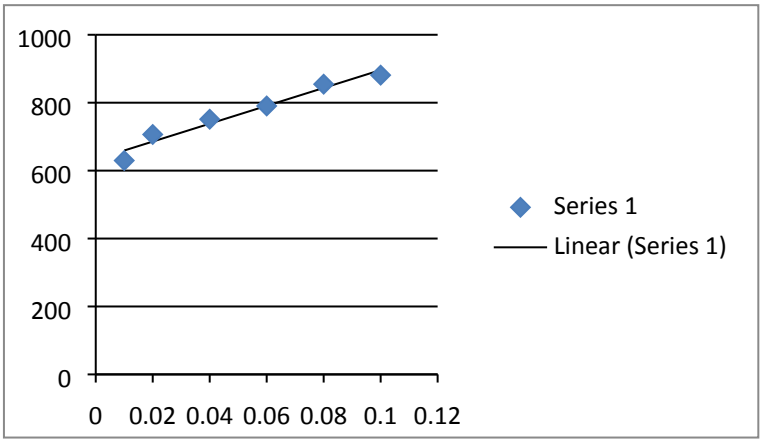

Fig. S5: Thechanges in the fluorescence intensity of **LS-1** (5.0 μM) at 562 nm against varied concentrations of H_2S from 0 to 0.15 μM in DMSO−HEPES buffer (0.01 M, pH 7.4) (V/V= 2:98) with the slit width 3/3 nm.

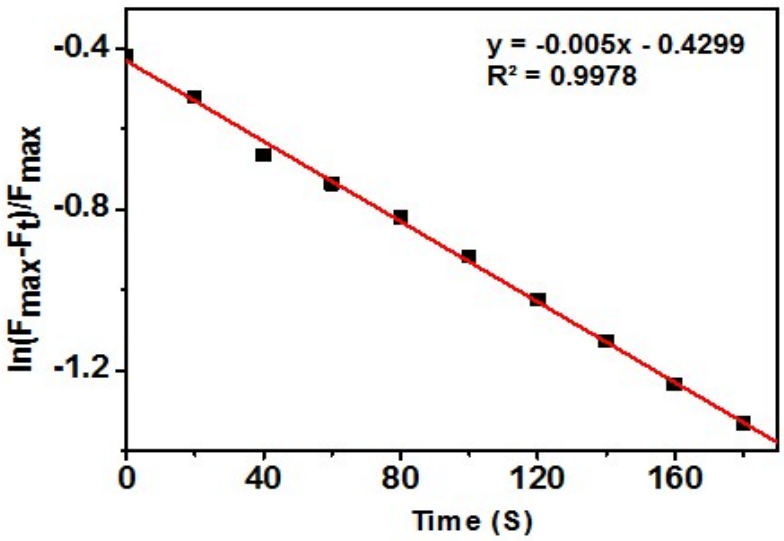

Fig. S6: Kinetic plot of first order reaction of **LS-1** (5 μM) in presence of Na_2S (200 μM) Rate = 5 X 10 $^{-3}M^{-1}s^{-1}$

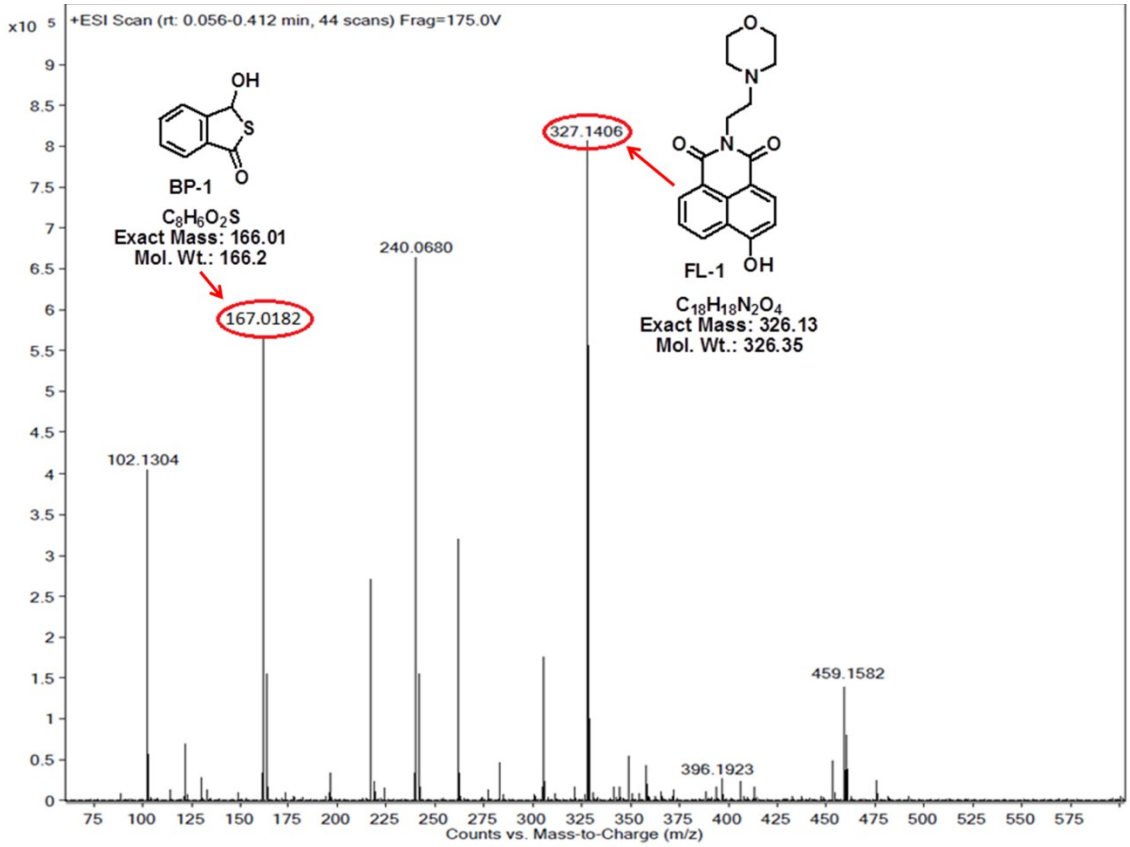

Fig. S7: The High resolution Mass spectrum of **LS-1** with Na_2S after 30 min.

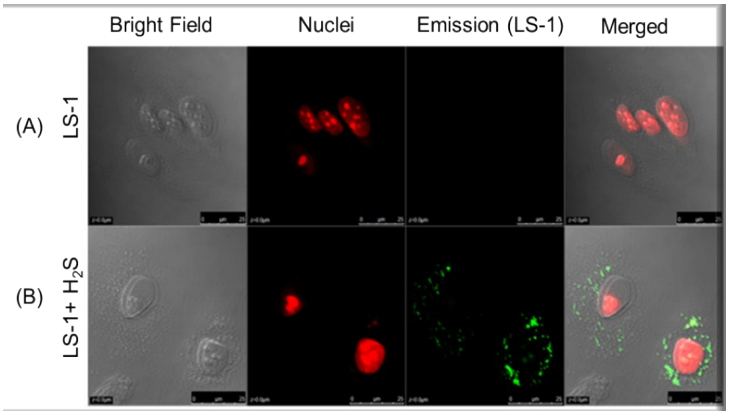

Fig. S8: Confocal fluorescence images of MCF-7 cells. (A) The cells were incubated with **LS-1** (2.5 μM) alone for 30 min. (B) Cells pretreated with **LS-1** (2.5 μM) for 30 min and then incubated H_2S (150 μM) for 30 min. Images were taken at λ_{ex} = 450 nm and emission was collected at 560 nm.