Efficient visualization of H_2S via a fluorescent probe having three electrophilic centres†

Sharad Kumar Asthana,[a] Ajit Kumar,[b] Neeraj,[a] Shweta[a] and K.K. Upadhyay[a]*

[a]*Department of Chemistry, Institute of Science, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India..*

[b]*Department of Applied Sciences & Humanities, National Institute of Foundry & Forge Technology, Ranchi-834003, Jharkhand, India.*

E-mail: *drkaushalbhu@yahoo.co.in; kku@bhu.ac.in, Tel No.: +91-542-6702488*

EXPERIMENTAL

1.1 Materials and Instrumentation:

All solvents and reagents (analytical and spectroscopic grade) were purchased from Sigma-Aldrich and used as received. The solution of hydrogen sulfide was prepared as its sodium salt in double distilled water. The IR Spectra were recorded on JASCO-FTIR Spectrophotometer while ^1H & ^{13}C NMR spectra were recorded on JEOL AL 300 FT NMR Spectrometer and chemical shifts (δ) have been reported in ppm, relative to tetramethylsilane ($Si(CH_3)_4$). UV-vis. absorption spectra were recorded at 25°C using a UV-1800 pharmaspec spectrophotometer while the emission spectra were recorded on JY HORIBA Fluorescence spectrophotometer. Mass spectrometric analysis was carried out on Bruker amaZon SL spectrometer using ultrascan mode (Bruker Daltonics, Bremen, Germany).

1.2 General Methods:

All titration experiments were carried at room temperature. All the anions were used as their sodium salts. The ^1H & ^{13}C NMR spectra were recorded by using tetramethylsilane (TMS) as an internal reference standard. For the ^1H NMR titration spectra of **FLA**, 5×10^{-3} M solutions were prepared in CD_3CN while the stock solution of Na_2S was prepared in D_2O. For UV-visible / fluorescence titration experiments, the solutions of anions were prepared in water. Due to insufficient solubility of **FLA** in water its stock solution of 1.0 mM was prepared in DMSO which was used for fluorescence and absorption titration experiment through dilution in EtOH: HEPES buffer (6:4, v/v) at 1.0 μM and 10.0 μM respectively.

1.3. Synthesis:

Synthesis of Fluorescein-carboxaldehyde (FL-CHO) (1a):

The methanolic solution (3 mL) of fluorescein (2.5 g, 7.75 mmol) was taken in a 100 mL of three-neck round-bottom flask and was heated on an oil bath to maintain 55°C temperature. The 50 mL aqueous NaOH solution (50% by weight) was added to the above solution in a drop wise fashion with constant stirring followed by addition of 2.42 mL $CHCl_3$ (30 mmol) and 0.03 g of 15-crown-5. The reaction mixture was stirred at above temperature for 6h. After cooling at room temperature the reaction mixture was acidified with 5M HCl and a dark yellow solid product was precipitated out. The same was chromatographed on a silica gel column using Ethylacetate / Hexane solvent as eluent. A light yellow solid was obtained.

Spectroscopic characterization data for Fluorescein carboxaldehyde (1a): Yield: 65%; **IR/cm^{-1}:** 3065, 1735, 1598, 1540, 1374, 1239, 1210, 1172, 1115, 851, 756, 659; **^{1}H NMR (300 MHz, DMSO-*d*$_6$, TMS)**: δ = 12.22 (s, 1H, -OH), 10.60 (s, 1H, -OH), 10.19 (s, 1H, -CHO), 7.96 (d, 1H, Ar-H), 7.77-7.69 (dd, 2H, Ar-H), 7.24 (d, 1H, Ar-H), 6.67 (s, 2H, Ar-H), 6.52 (4H, Ar-H); **^{13}C NMR (75 MHz, DMSO-*d*$_6$):** δ = 222.79, 168.81, 159.53, 152.53, 151.89, 135.68, 130.15, 129.08, 126.20, 124.68, 124.06, 112.69, 109.62, 102.35, 83.15; **ESI-MS**: **m/z** Calculated for $C_{21}H_{12}O_6$ [M] = 360.0, found $[M]^+$ = 360.0.

Synthesis of methyl-2-(4-formyl-3-hydroxy-6-methoxy-9H-xanthen-9-yl) benzoate (1b):

The methyl iodide (3.0 mmol) was added in a drop wise fashion with constant stirring to the reaction mixture consisting of 4-formyl fluorescein **(1a)** (1.0 mmol) and K_2CO_3 (3.0 mmol) in 10 ml of DMF in a 100 mL round bottom flask at room temperature. After stirring for ~24 hours at room temperature, the reaction mixture was diluted with distilled water and extracted with ethyl acetate. The organic phase was washed with 1M $NaHCO_3$ and brine followed by drying over anhydrous Na_2SO_4 and finally concentrated under reduced pressure and then used in next step.

Synthesis of 3-hydroxy-6'-methoxy-3-oxo-3H-spiro (isobenzofuran-1, 9'-xanthene)-4' carbaldehyde (Me-FL-CHO) (1c):

10% aqueous solution of NaOH (10 mL, 25.0 mmol) was added in a dropwise fashion with constant stirring to the 10 mL solution of dye **1b** in methanol (3.60 g, 10.0 mmol) taken in a 100 mL round bottom flask at room temperature. After stirring for ~ 4 hours, MeOH was evaporated under reduced pressure in a rotatory evaporator and the resulting reaction mixture

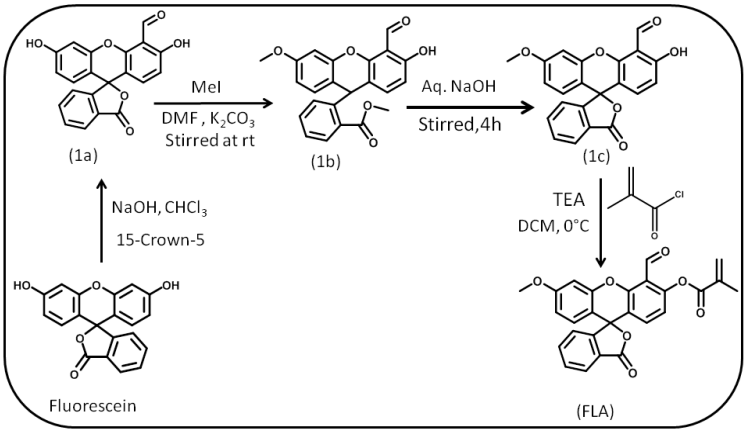
was diluted with water (20 mL). The same was acidified to pH 5-6 with 5 M HCl and finally extracted with ethyl acetate. The organic phase was washed with double distilled water and brine solution, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The obtained light yellow solid compound was chromatographed on a silica gel column using EtOAc / Hexane mixture solvent as eluent. Finally a light yellow solid was obtained.

Spectroscopic characterization data for (1c): Yield: 65%; **IR/cm^{-1}**: 3401, 3102, 2924, 2854, 1938, 1766, 1607, 1583, 1538, 1523, 1490, 1485, 1423, 1405, 1364, 1353, 1109, 1090, 925, 848**; ^{1}H NMR (300 MHz, DMSO-*d*$_6$, TMS)**: δ = 12.20 (s, 1H, -OH), 10.06 (s, 1H, -CHO), 7.94 (d, 1H, Ar-H), 7.79- 7.66 (m, 2H, Ar-H), 7.24 (d, 1H, Ar-H), 6.72 (2H, Ar-H), 6.52 (4H, Ar-H), 4.42 (s, 3H, -OMe); **^{13}C NMR (75 MHz**, **DMSO-*d*$_6$)**: δ = 196.90, 169.80, 169.59, 160.08, 156.88, 150.16, 148.65, 145.66, 145.53, 129.88, 129.80, 129.98, 124.48, 119.99, 115.15, 111.11, 108.01, 105.85, 104.40, 104.98, 98.15, 54.64.

Synthesis of receptor FLA

To a dichloromethane (CH_2Cl_2) solution of **1c** (1.0 mmol) and triethylamine (0.2 mL) in 15 mL of anhydrous dichloromethane at 0°C in a 100 mL round bottom flask the methacryloyl chloride (0.2 mL, mixed with 5.0 mL of CH_2Cl_2) was added dropwise under constant stirring of reaction mixture over a time period of ~ 30 minutes. The reaction mixture was stirred for overnight at room temperature. Finally the content of the flask was diluted with dichloromethane (30.0 mL) and washed with brine (30.0 mL×2) and dried over anhydrous $MgSO_4$ for ~ 3 **-** 4 hrs. The solvent was removed in vacuo to obtain a crude mixture solid. Finally, the target compound **FLA** was isolated by silica chromatography eluting with CH_2Cl_2.

Spectroscopic characterization data for receptor FLA: **Yield**: 60%; **IR/cm^{-1}**: 3401, 3102, 2924, 2854, 1938, 1766, 1607, 1583, 1538, 1523, 1490, 1485, 1405, 1388, 1364, 1257, 1233, 1188, 1109, 1090, 925, 854, 746; **^{1}H NMR (300 MHz, CD$_3$CN, TMS)**: δ = 10.72 (s, 1H, -CHO), 7.93 (d, 1H, Ar-H), 7.72 to 7.66 (m, 2H, Ar-H), 7.46 (s, 1H, Ar-H), 7.17 (d, 1H, Ar-H), 6.69 - 6.52 (m, 4H, Ar-H), 4.86 (s, 1H, =CH), 4.52 (s, 1H, =CH), 4.37 (s, 3H, -OMe), 2.86 (s, 3H, -CH_3); **^{13}C NMR (75 MHz, CD$_3$CN)**: δ = 195.94, 188.98, 185.08, 178.06, 178.50, 169.80, 169.59, 165.58, 160.08, 156.88, 153.38, 150.16, 148.65, 145.66, 145.53, 129.88, 129.80, 129.98, 124.48, 115.33, 110.15, 108.11, 103.31, 99.85, 82.15, 54.64, 49.44; **HRMS**: **m/z** Calculated for $C_{26}H_{18}O_7$ [M] = 442.1053, found $[M-H]^+$ = 441.1049.

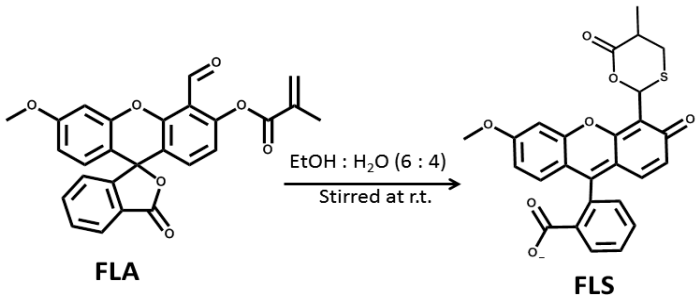


Scheme 1 Synthesis of receptor **FLA**.

Synthesis of Complex FLS

HS^- complex of **FLA** was synthesized by adding a 3 mL aqueous solution of Na_2S (1.5 mmol) slowly to a magnetically stirred 10 mL EtOH: water (3: 2) solution of **FLA** (0.5 mmol). The mixture was further stirred at room temperature for ~ 4 hours where by a yellowish precipitate was formed. The same was filtered and washed several times with diethyl ether and finally dried under vacuum over anhydrous $CaCl_2$.

Spectroscopic characterization data of complex FLS: Yield: 89%; **IR/cm^{-1}**: 3427, 2963, 2876, 2169, 1707, 1634, 1575, 1523, 1486, 1468, 1421, 1387, 1345, 1226, 1209, 1167, 1105, 1033, 882, 740; **^{1}H NMR: (300 MHz, CD_3CN, TMS)**: δ = 7.95 (d, 1H, Ar-H), 7.75-7.63 (m, 2H, Ar-H), 7.17 (d, 1H, Ar-H), 6.69 (d, 2H, Ar-H), 6.62 (m, 2H, Ar-H), 6.55-6.52 (m, 1H, Ar-H), 4.81 (s, 1H, C-H), 4.53 (d, 1H, C-H), 3.94 (s, 2H, -CH_2), 3.49 (s, 3H, -OCH_3), 2.12 (s, 3H, -CH_3); **^{13}C NMR (75 MHz, CD_3CN, TMS)**: δ = 213.85, 191.17, 178.16, 178.50, 169.82, 169.59, 165.28, 160.08, 156.88, 153.38, 150.26, 148.65, 145.66, 145.53, 129.88, 129.82, 129.98, 124.48, 115.95, 111.15, 108.11, 104.01, 100.40, 65.01, 59.44, 54.64, 48.16; **HRMS**: **m/z** Calculated for $C_{26}H_{19}Na_2O_7S$ [M] = 521.0641 found $[M+H]^+$ = 522.0631.



Scheme 2 Synthesis of **FLS**.

Electronic Supplementary Information

TABLE OF CONTENTS

Electronic Supplementary Information

Figure S1: ^{1}H NMR spectrum of FL-CHO (1a) (in DMSO–d_6):

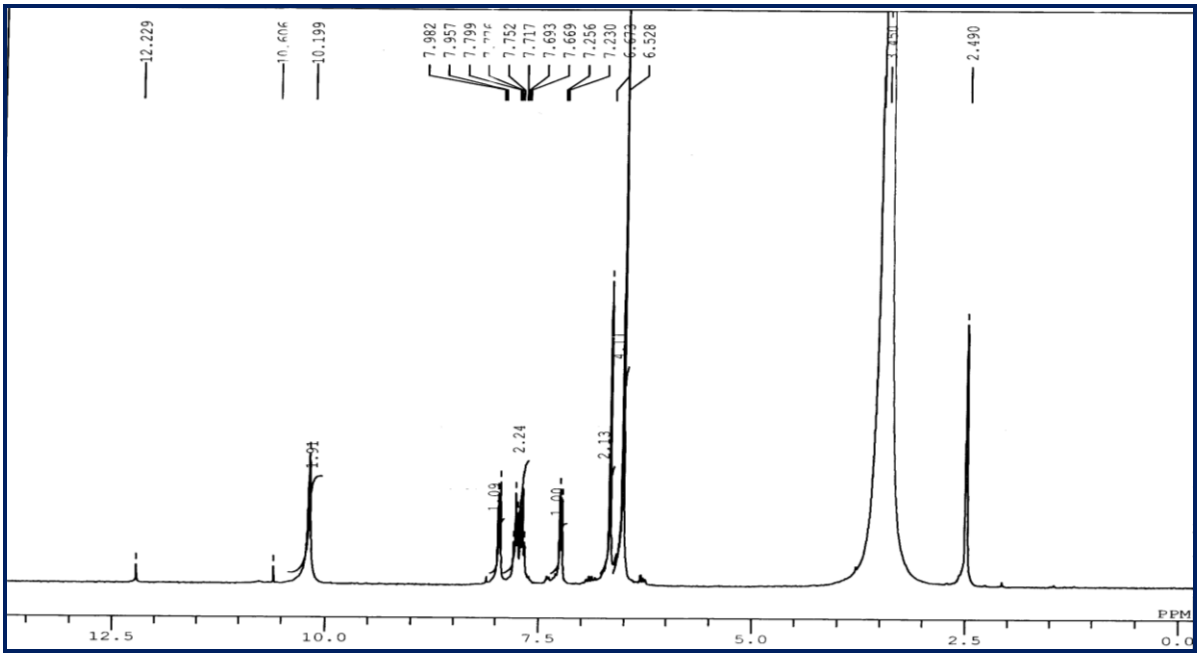


Figure S2: ^{13}C NMR spectrum of FL-CHO (1a) (in DMSO–d_6):

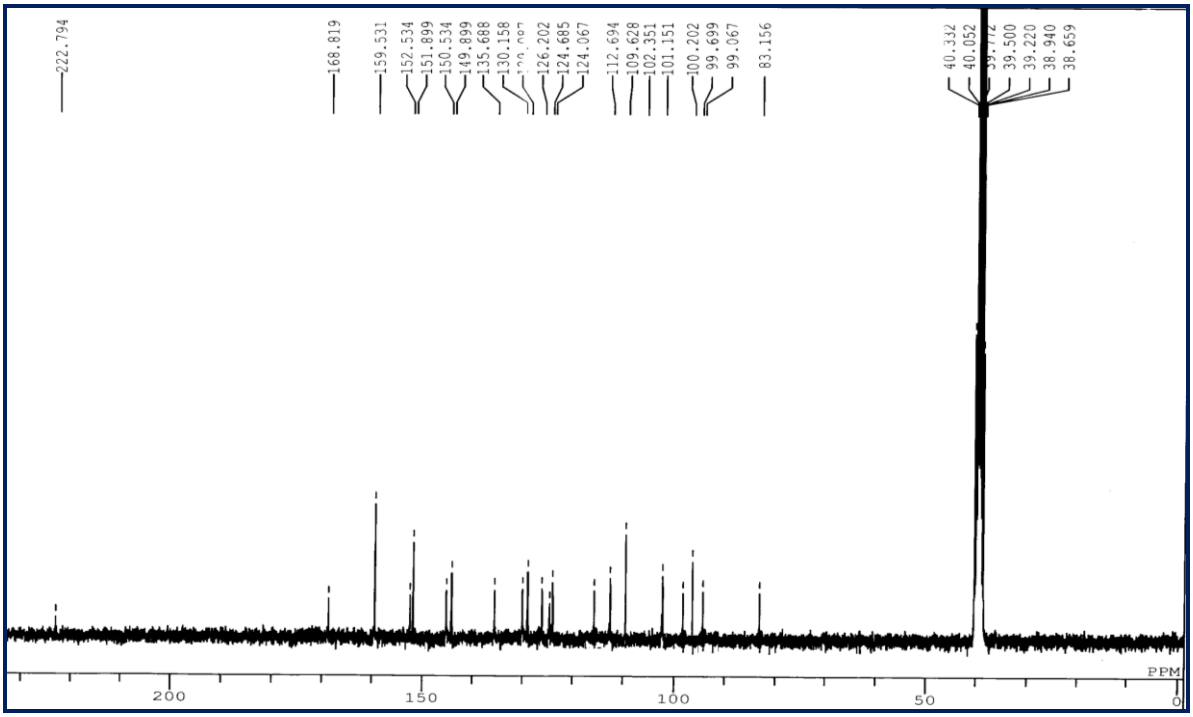


Figure S3: IR spectrum of FL-CHO (1a):

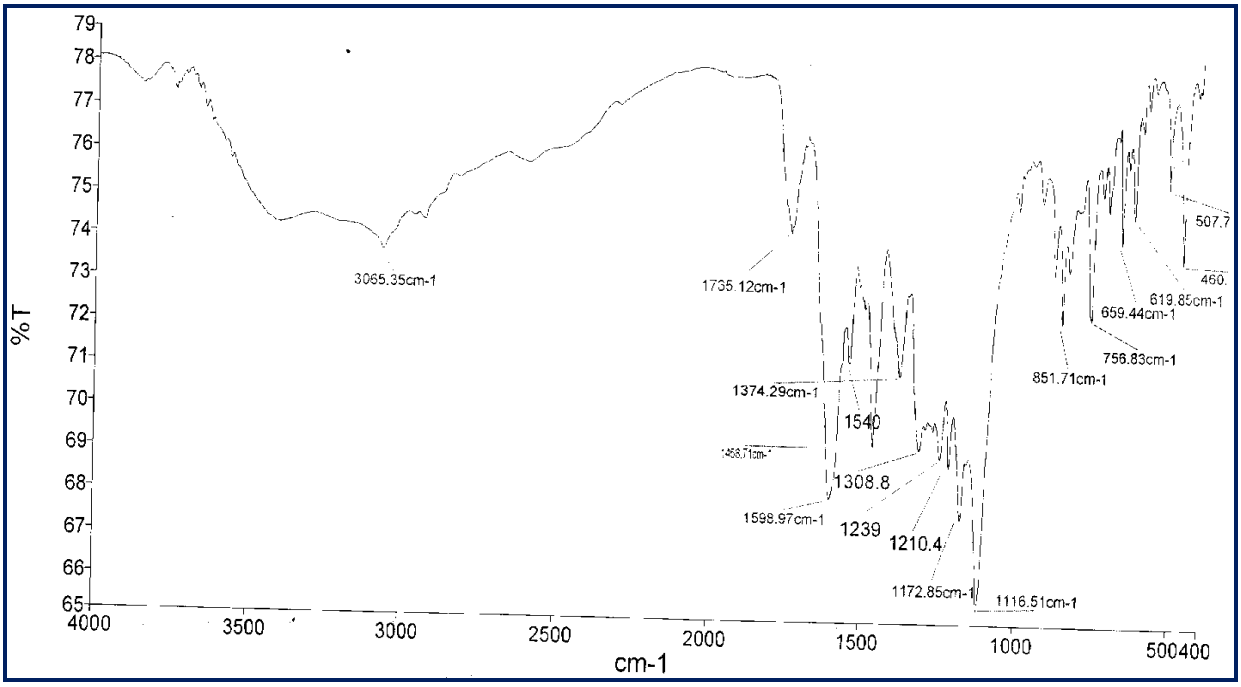


Figure S4: Mass spectrum of FL-CHO (1a):

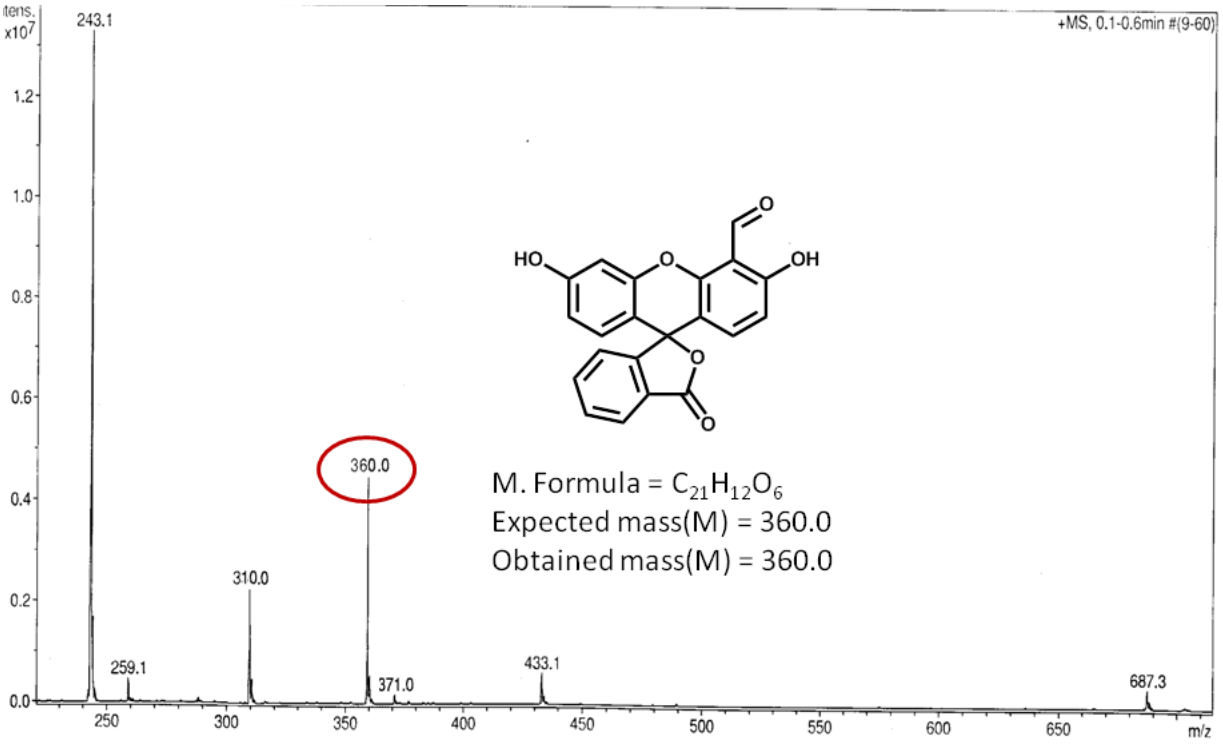


Figure S5: ^{1}H NMR spectrum of Me-FL-CHO (1b) (in DMSO–d_6):

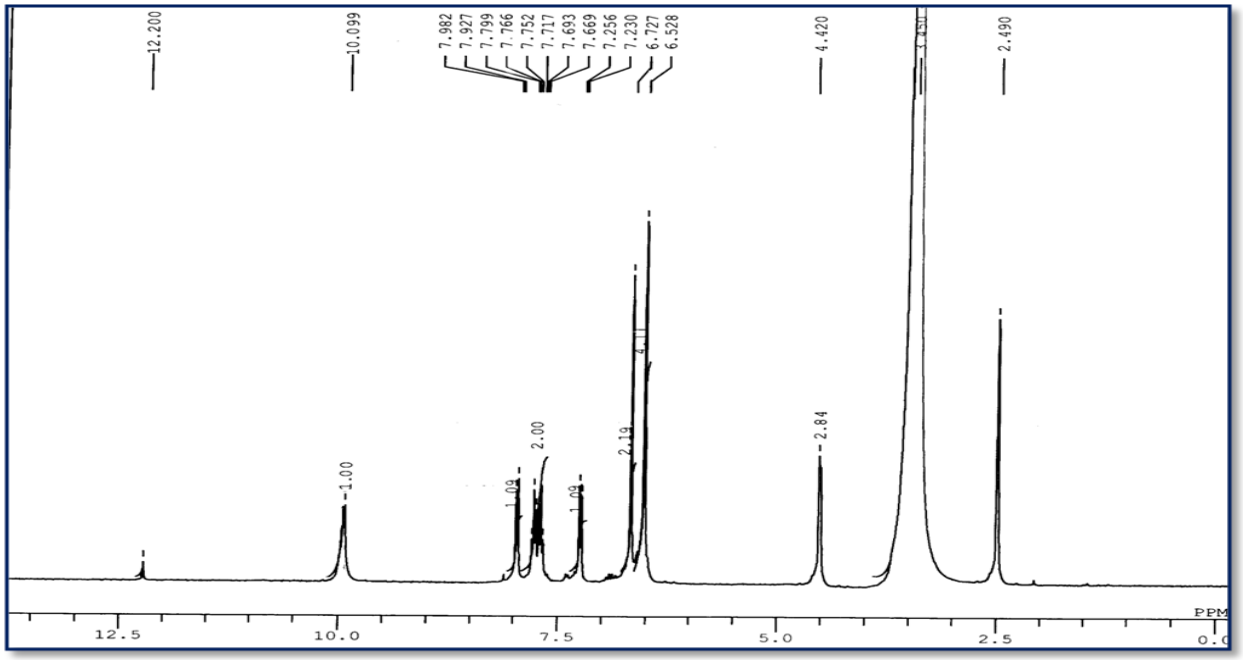


Figure S6: ^{13}C NMR spectrum of Me-FL-CHO (1b) (in DMSO–d_6):

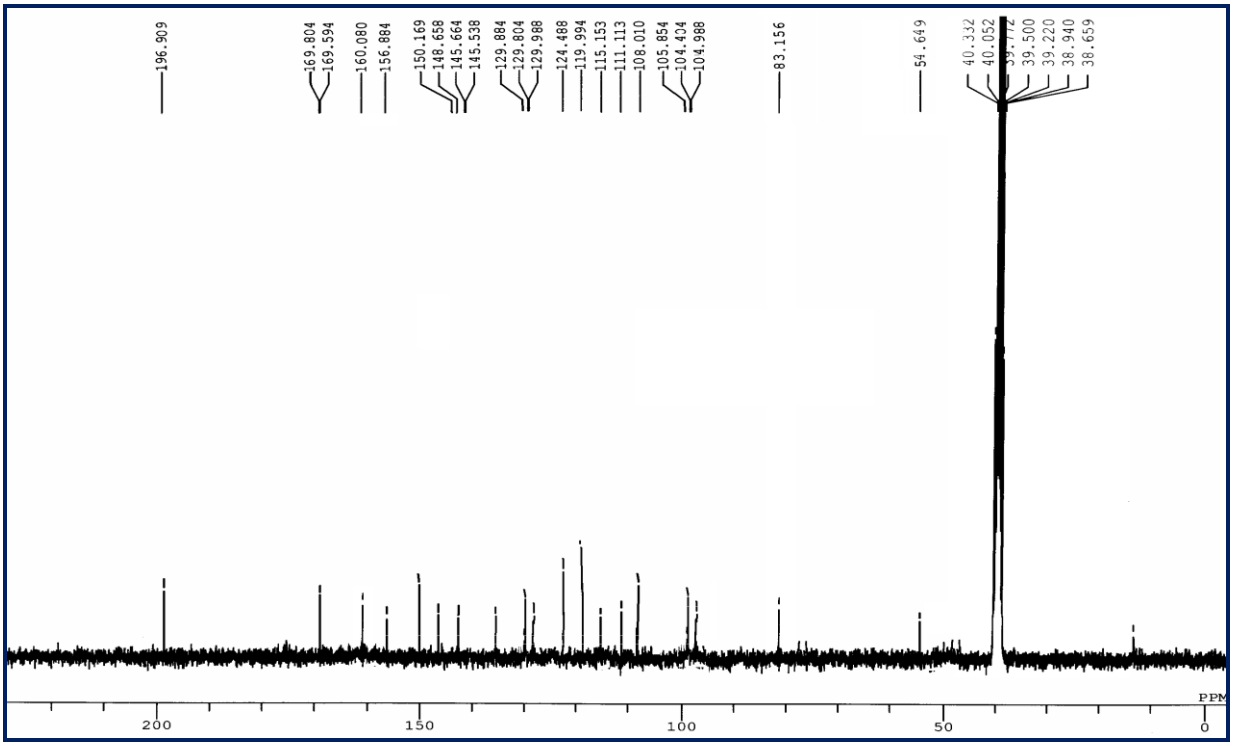


Figure S7: IR spectrum of Me-FL-CHO (1b):

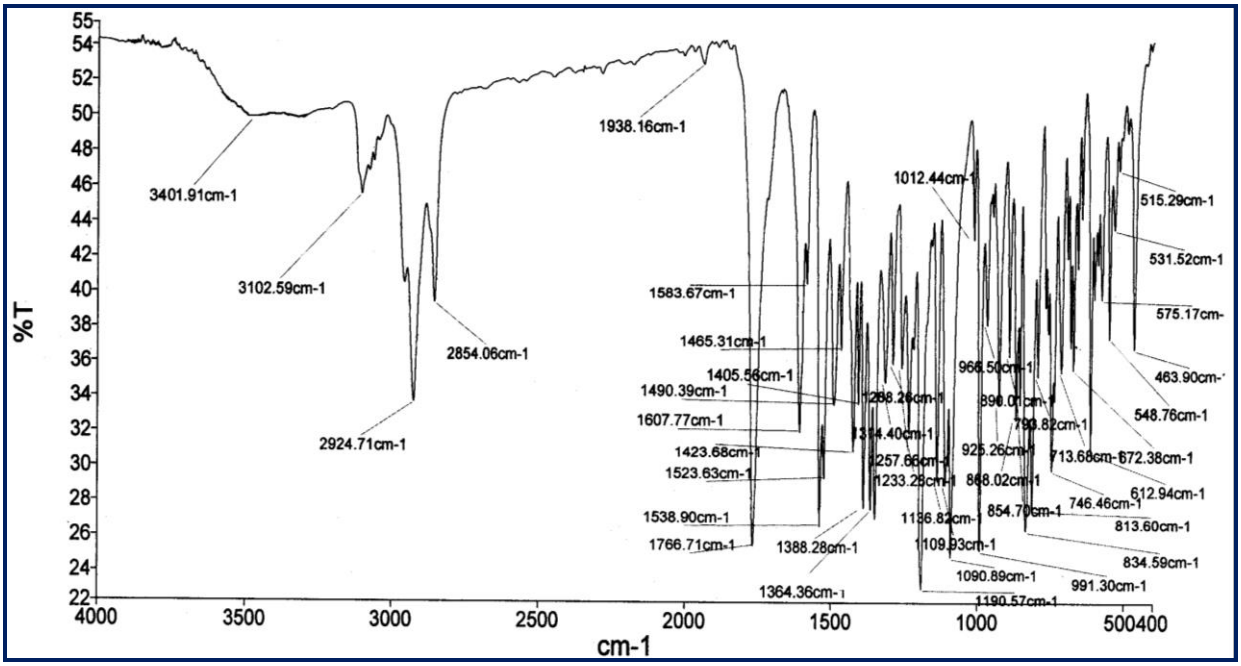


Figure S8: ^{1}H NMR spectrum of **FLA** (in CD_3CN):

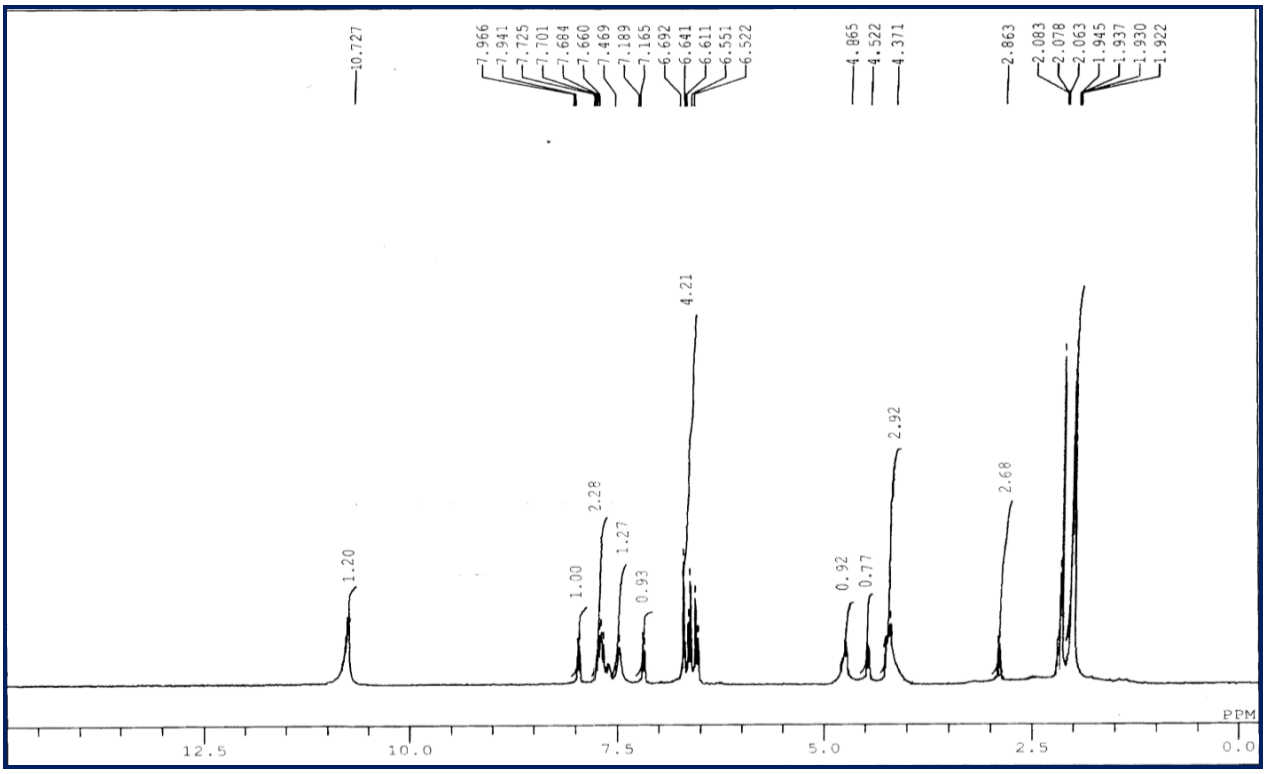


Figure S9: ^{13}C NMR spectrum of **FLA** (in CD_3CN):

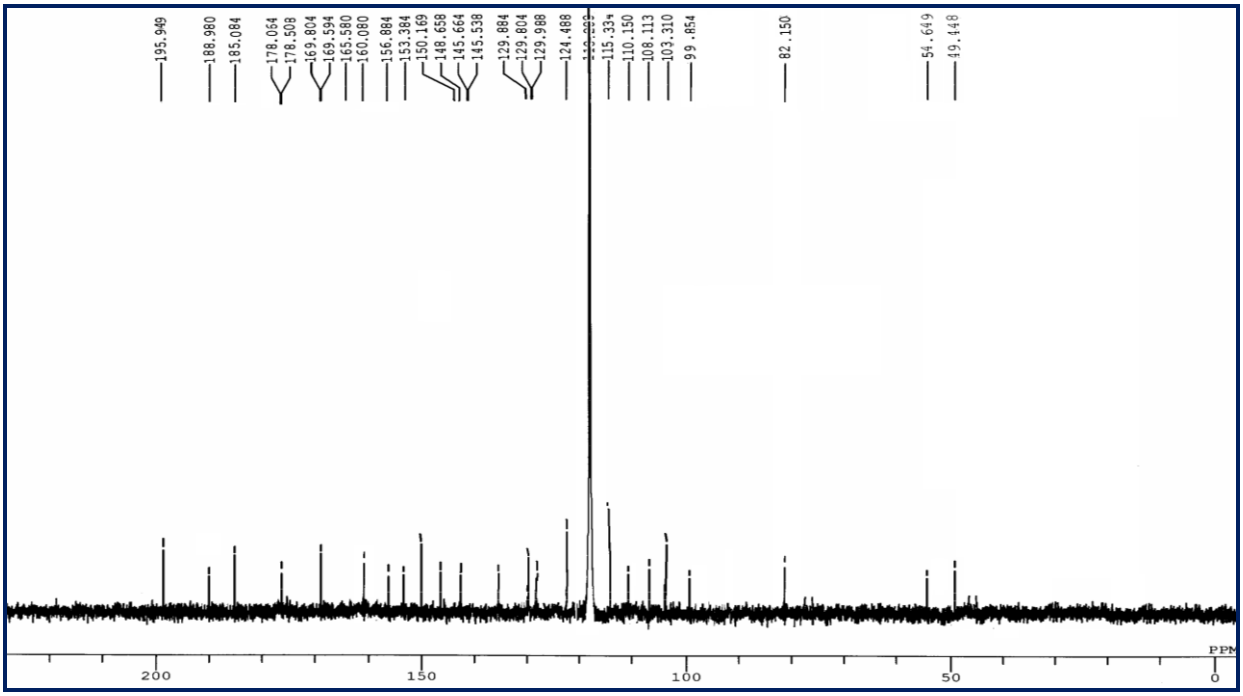


Figure S10: IR spectrum of **FLA**:

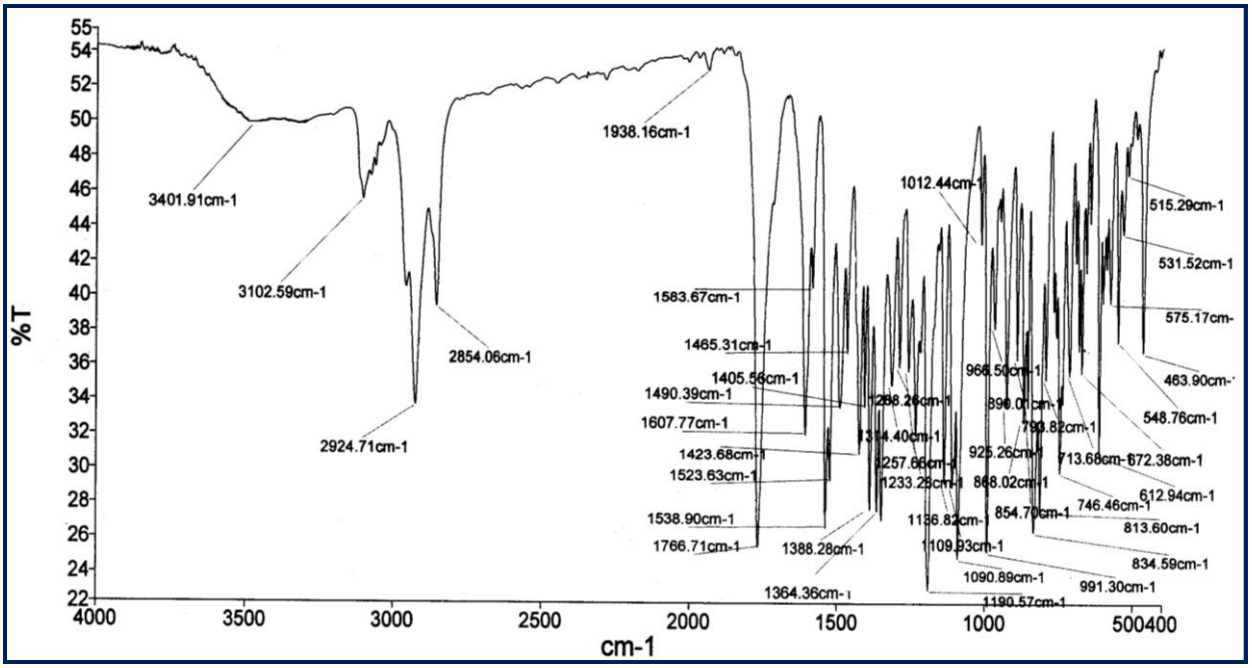


Figure S11: HRMS of **FLS**:

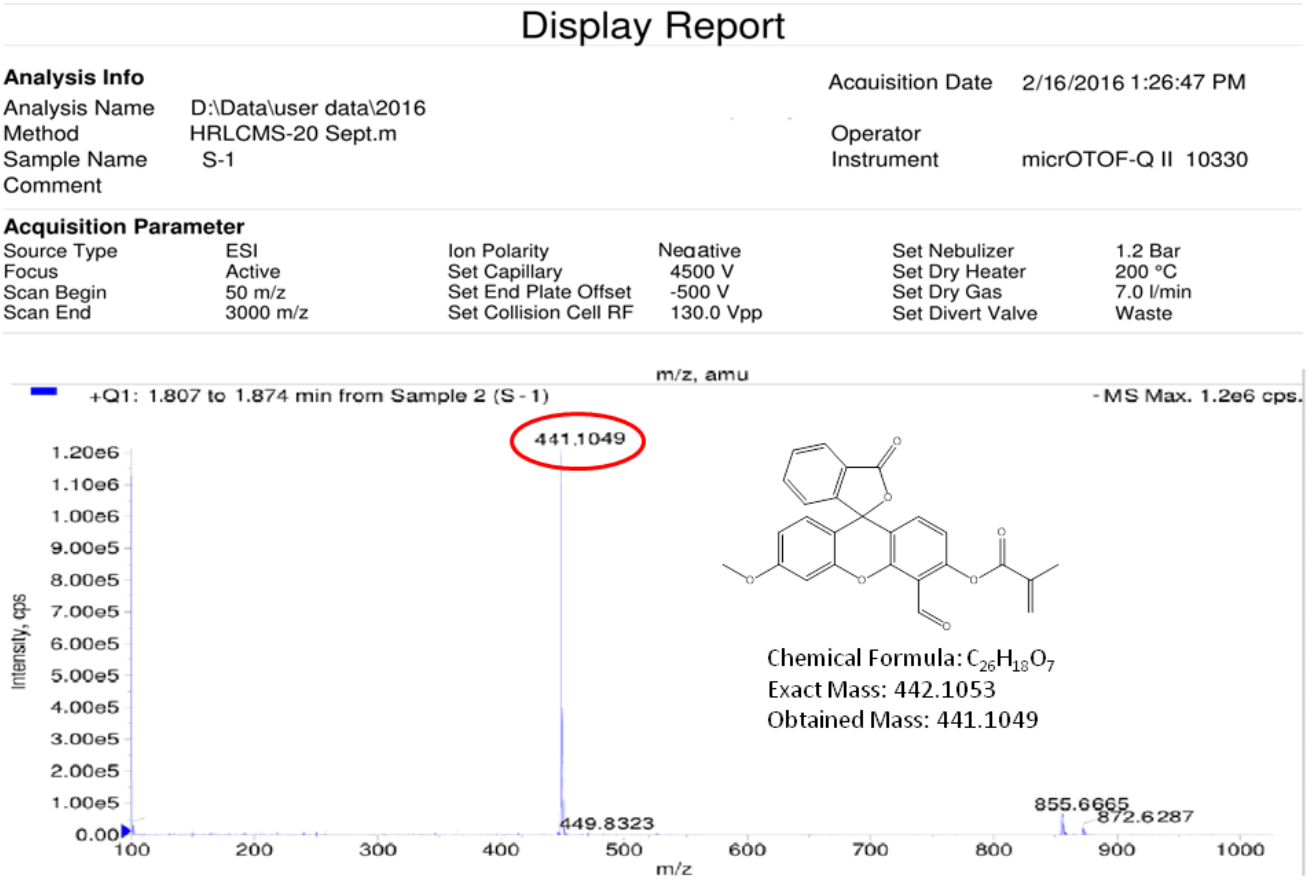


Figure S12: Reaction-time profile changes of emission intensity of **FLA** at 518 nm in the presence of HS^- as a function of time (0-800 second):

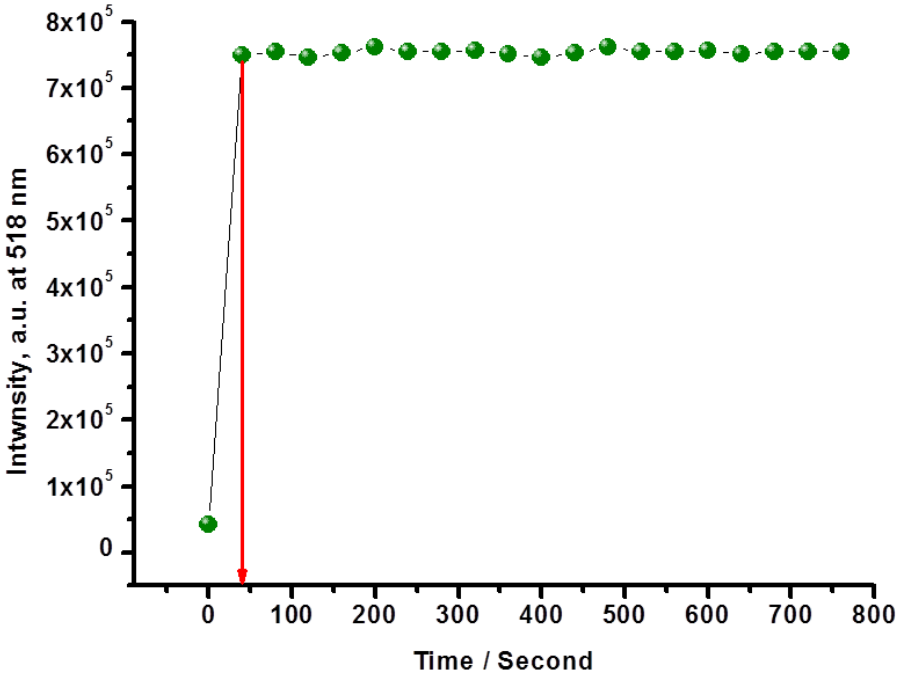


Figure S13: UV-visible bargraph of **FLA** with different anions in EtOH: HEPES buffer (3: 2, v/v):

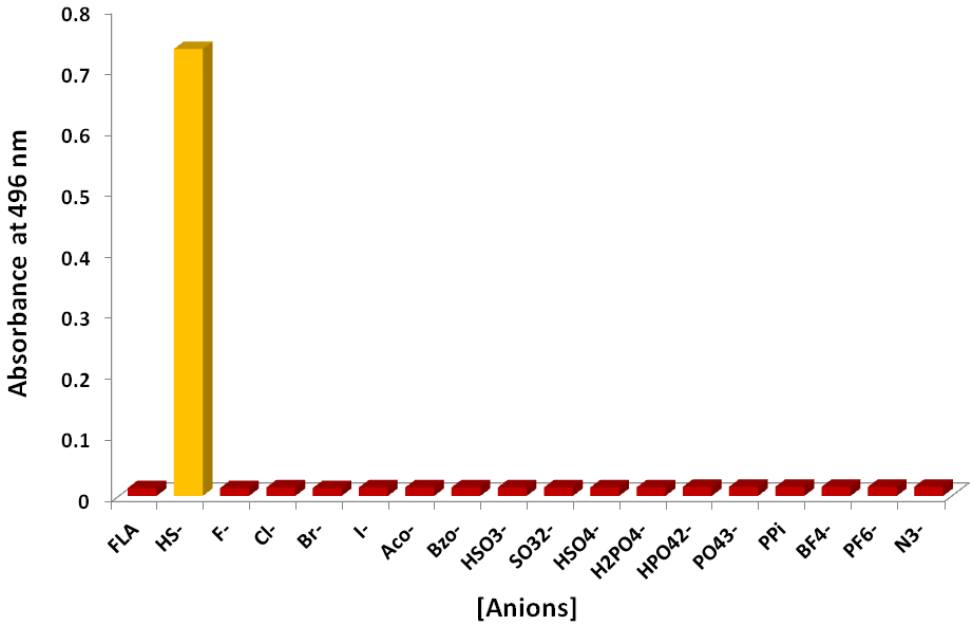


Figure S14: Bar graph representation of Absorption spectrum for competition study; **[yellow bars]** showing response **FLA** in presence of various anions, **[red bars]** showing response of **FLA** in presence of HS^- and HS^- followed by various competing anions:

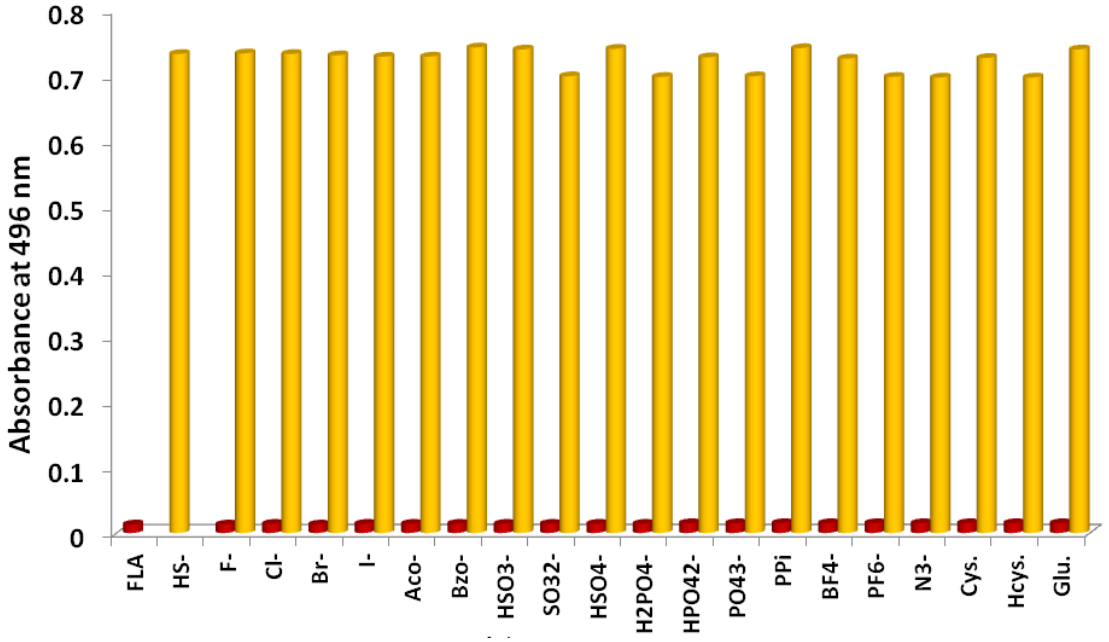


Figure S15: Naked-eye images of **FLA** in the presence of HS^- and various anions (under visible light):

Figure S16: Calibration curve for determination of detection limit of **FLA** for HS^- by using absorption titration data (496 nm):

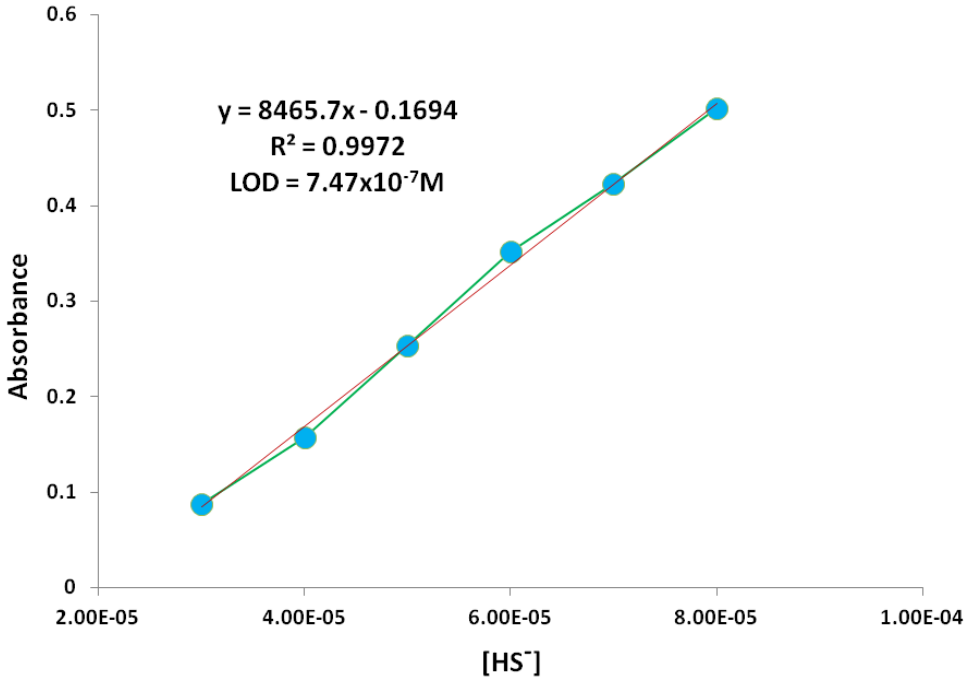


Figure S17: Bar graph representation of Emission spectrum for competition study; **[red bars]** = **FLA** in the presence of various anions, **[green bars]= FLA** + HS^-, followed by various competing anions:

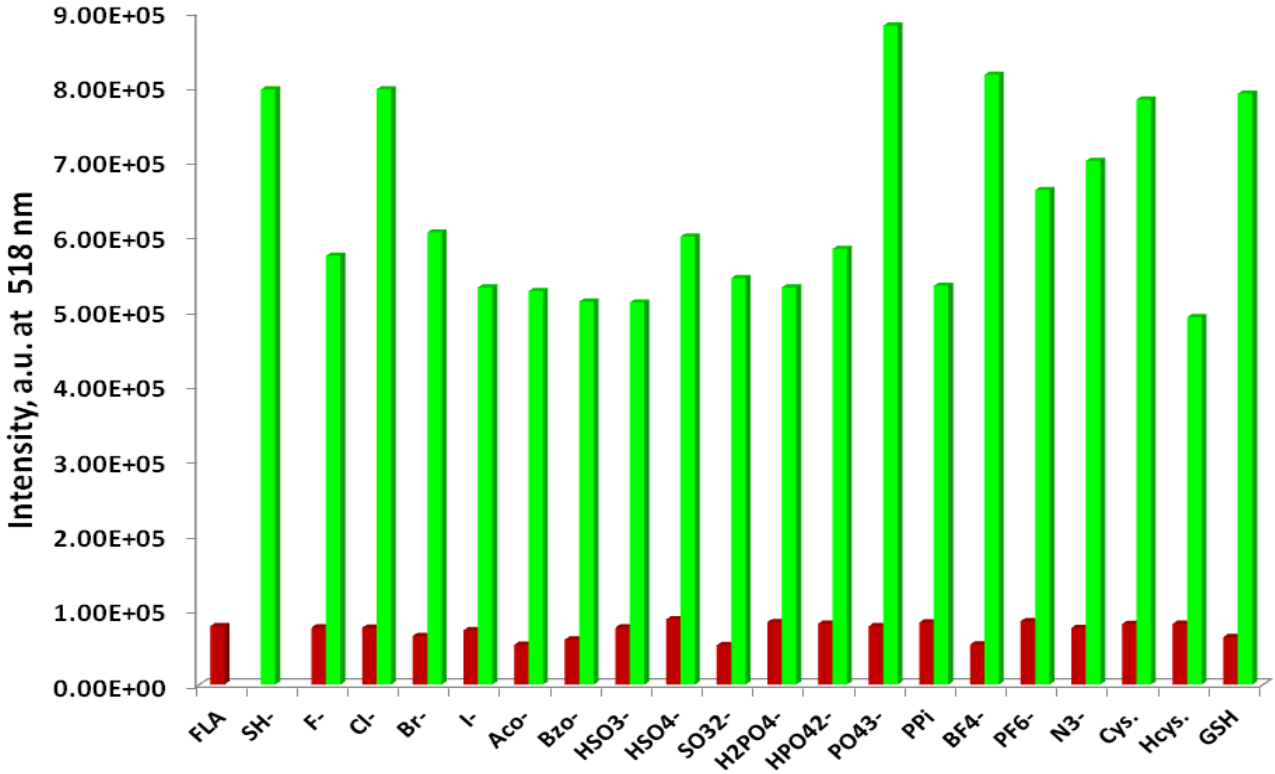


Figure S18: Reaction-time profile: changes of absorbance of **FLA** at 496 nm in the presence of HS^- as a function of time (0-200 second):

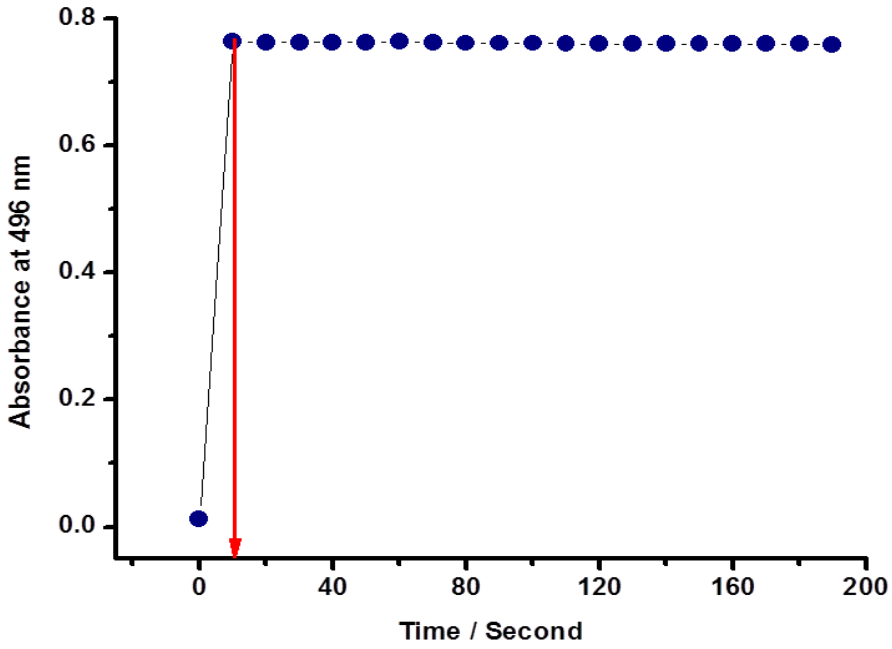


Figure S19A: ^{1}H NMR spectrum of **FLS** (FLA+HS^-) (in CD_3CN):

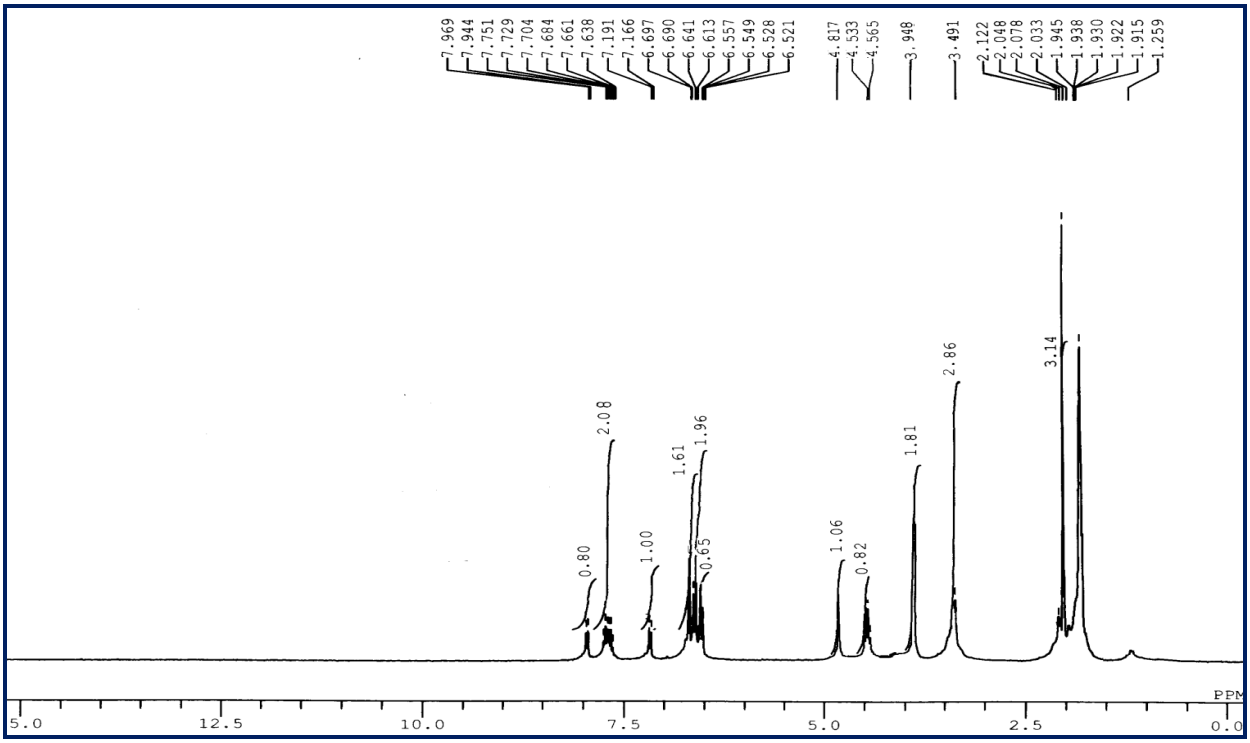


Figure S19B: ^{1}H NMR titration spectrum of **FLA** in presence of HS^- (in DMSO-d_6):

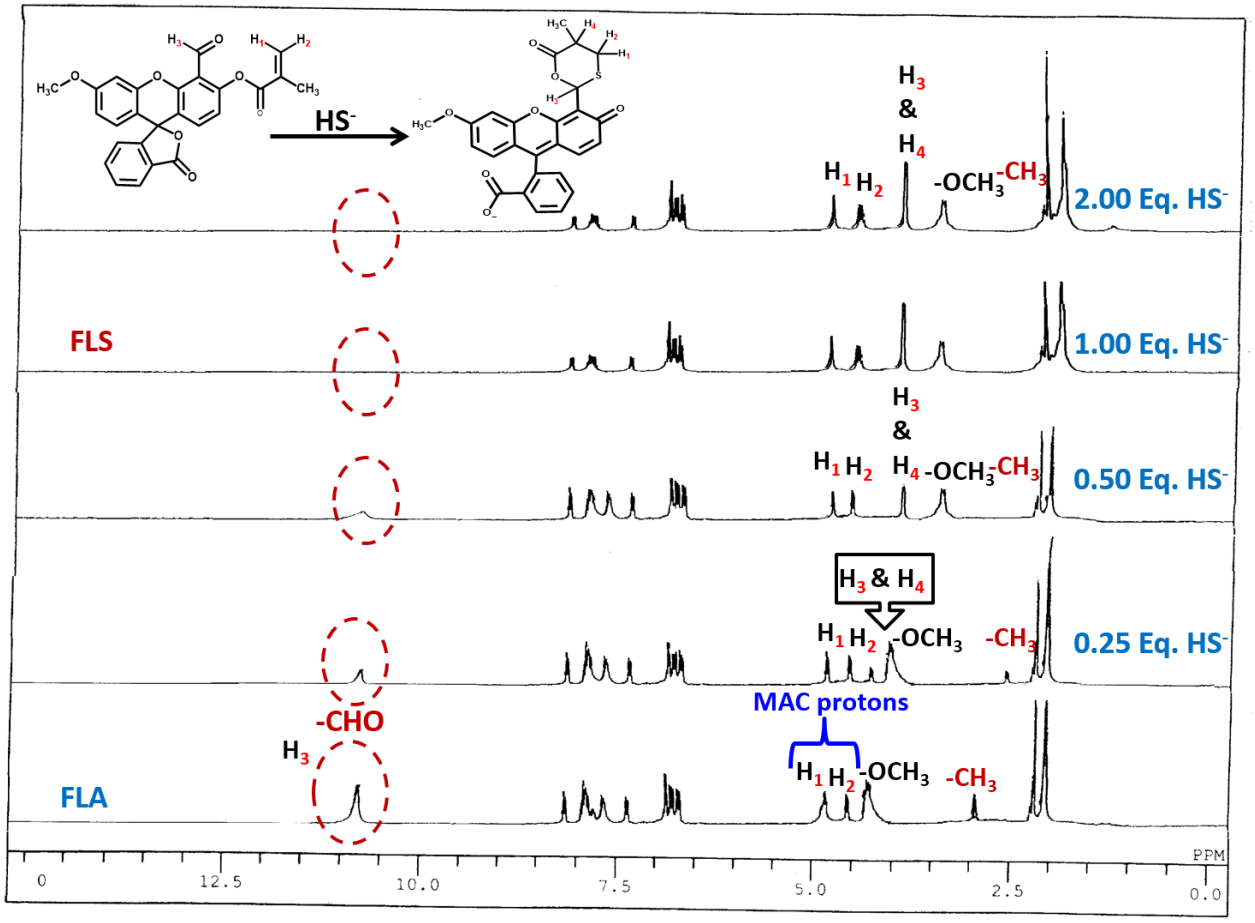


Figure S20: ^{13}C NMR spectrum of **FLS** (FLA+HS^-) in (DMSO-d_6 + CD_3CN):

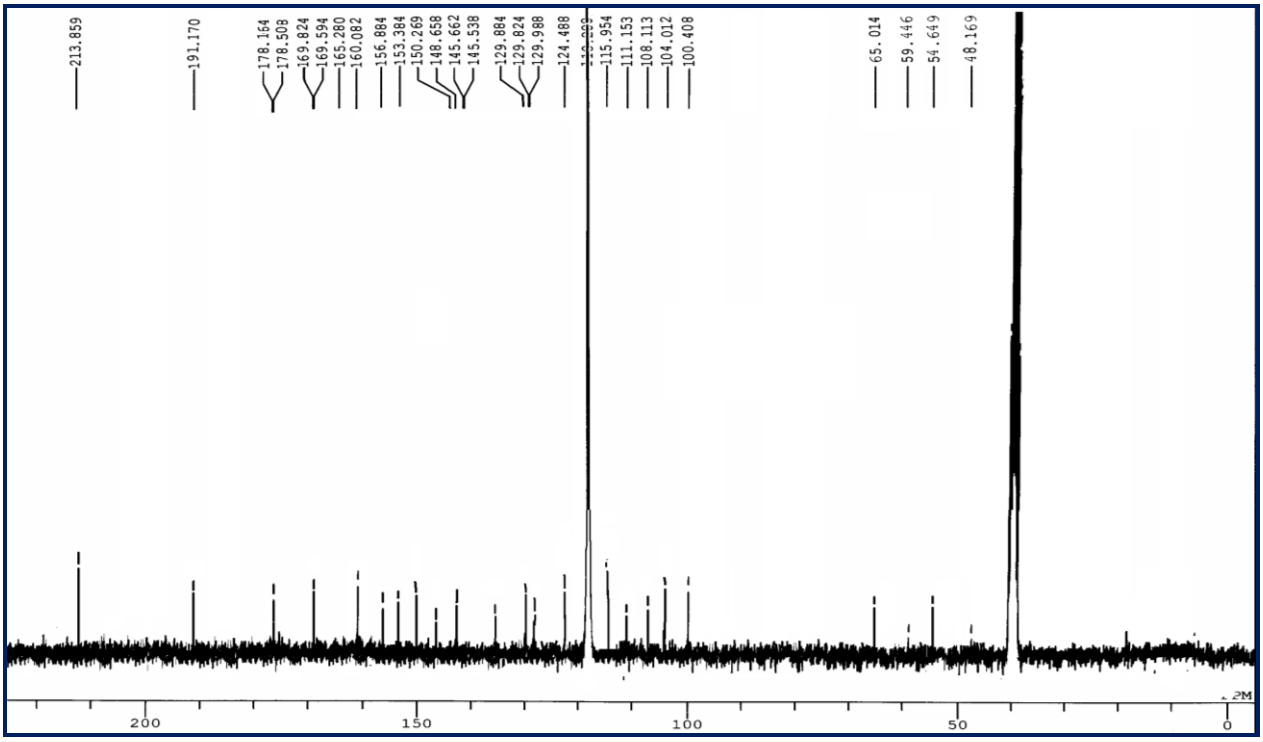


Figure S21: IR spectrum of **FLS** ($FLA+HS^-$):

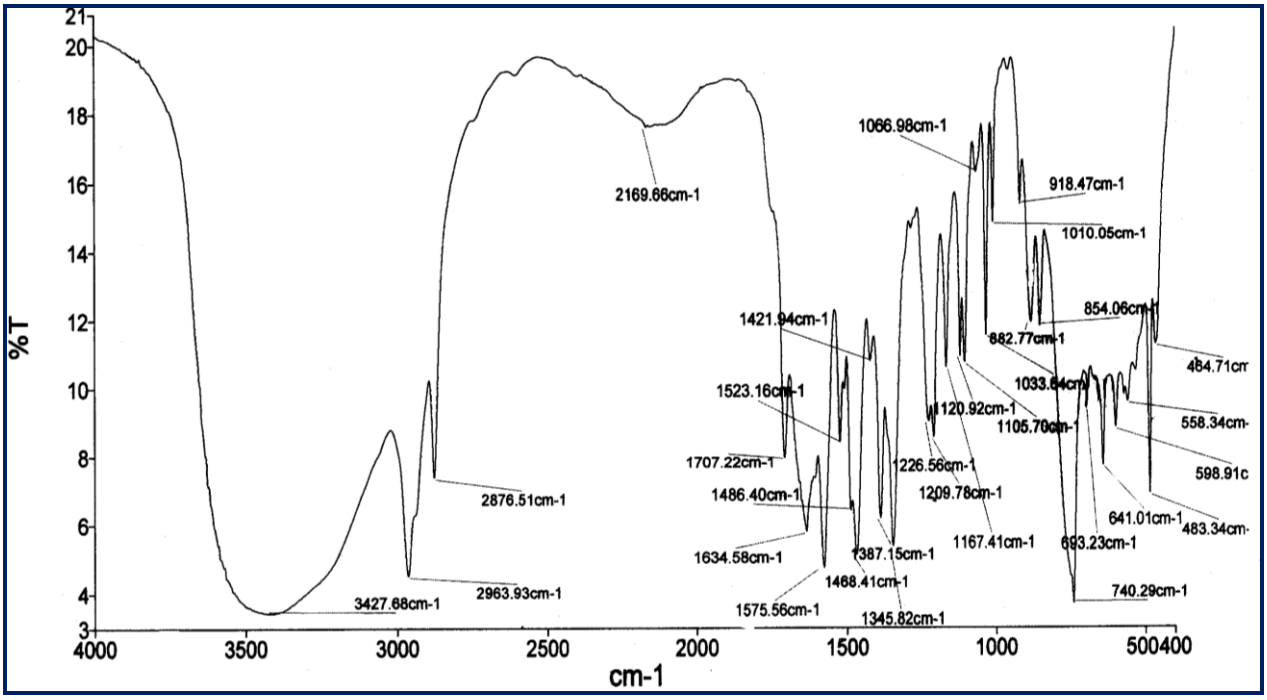


Figure S22: HRMS of **FLS** (FLA+HS$^-$):

Display Report

Analysis Info

Analysis Name	D:\Data\user data\2016	Acquisition Date	2/16/2016 2:16:40 PM
Method	HRLCMS-20 Sept.m	Operator	
Sample Name	S-2	Instrument	micrOTOF-Q II 10330
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.2 Bar
Focus	Active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	7.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	130.0 Vpp	Set Divert Valve	Waste

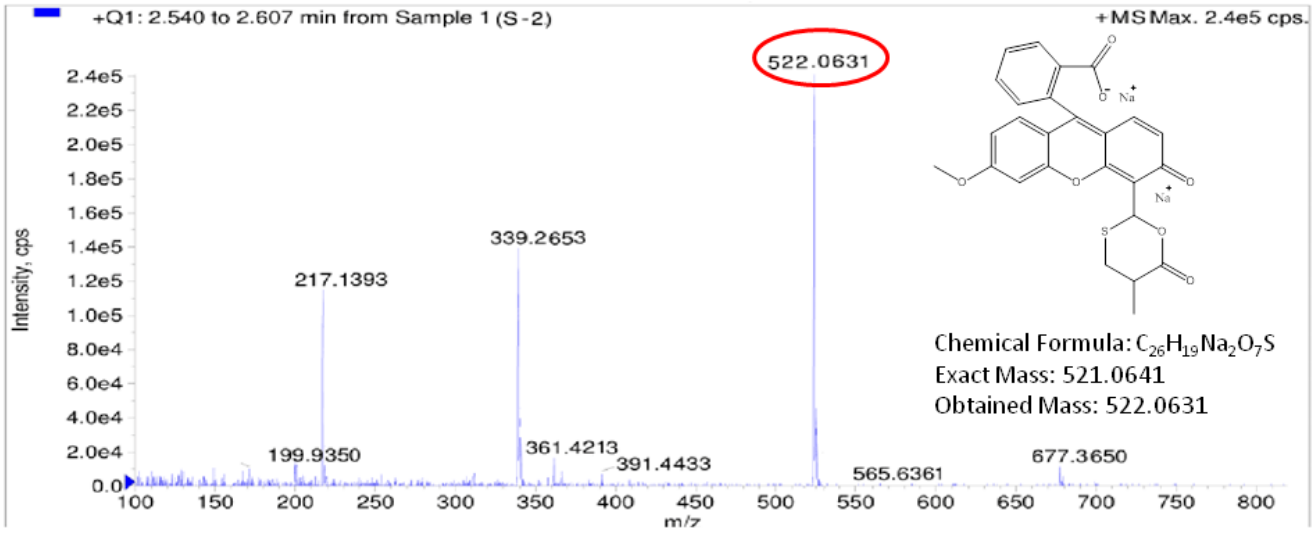


Figure S23: Calibration curve for determination of detection limit of **FLA** for HS^- by using emission titration data (518 nm):