Supplementary Information (SI) for Inorganic Chemistry Frontiers.

Redox-Active Iron Complex as Immunogenic Cell Death-I Inducer with NIR-II Photoacoustic Properties: Potential Theragnostic Agent for "Cold Tumors"

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Experimental details:

1. Physical methods and materials

All the reagents and solvents were procured from commercial sources (such as TCI Chemicals, Alfa Aesar and Sigma-Aldrich) and used without purification unless otherwise mentioned. All hydroxy naphthaldehyde compounds (except 2-hydroxy-1-naphthaldehyde) were synthesized in the laboratory. The solvents $CDCl_3$ and DMSO-d_6 were employed as solvents for conducting ^1H and ^{13}C nuclear magnetic resonance (NMR) experiments on JEOL 400 and 500 MHz spectrometers. The ^1H NMR chemical shifts were referenced using the remaining hydrogen signal of the deuterated solutions. In the context of ^1H and ^{13}C NMR spectroscopy, the chemical shift is expressed as dimensionless quantities and is calibrated with respect to TMS as a frequency reference. The ESI-MS data was collected using the Agilent LCMS Q-TOF mass spectrometer. The UV-visible spectra were acquired utilizing a JASCO V-670 UV-Visible absorption spectrophotometer. The Fourier-transform infrared (FT-IR) spectra were obtained by conducting measurements on a Perkin Elmer Spectrometer within the frequency range of 400-4000 cm^{-1}. These measurements were performed using potassium bromide (KBr) pellets. The analysis employing high-performance liquid chromatography (HPLC) was performed on an Agilent 1200 apparatus. A flow rate of 1 mL/min was employed, and a gradient was applied, commencing with a mixture consisting of 10% MeCN (acetonitrile) and 90% H_2O (water) and progressively progressing to a composition of 100% MeCN within a time span of 15 minutes. The investigation was performed using a diode array detector. The experimental setup involved the utilisation of an Agilent Eclipse XDB-C18 column with dimensions of 4.6 mm × 150 mm and a particle size of 5 μm. The evaluation of purity was conducted by employing the integrated UV chromatogram at a designated wavelength of 254 nm. The chemicals employed for biological evaluation are required to possess a minimum purity level of 95%.

2. Crystal Structure Determinations.

Single-crystal X-ray data were collected on a Bruker SMART APEX CCD diffractometer using graphite-monochromated Mo-Kα radiation (λ = 0.71069 Å) with the SMART suite of programs.[1] All data were processed and corrected for Lorentz and polarization effects with SAINT and for absorption effects with SADABS.[2] Structural solution and refinement were carried out with the SHELXTL suite of programs.[3] The structures were refined (weighted least squares refinement on *F*2) to convergence. All the non-hydrogen atoms in all the compounds were refined anisotropically by full-matrix least-squares refinement. The lattice parameters and structural data are listed at the end of this Supporting Information. All the crystallographic data have been deposited in The Cambridge Crystallographic Data Centre; CCDC-**2369607** [$Fe(L1)_2$], and **2369608** [$Fe(L3)_2$], contain the crystallographic data.

[1]. SMART version 5.628, Bruker AXS Inc., Madison, WI, USA, 2001.
[2]. G. M. Sheldrick, SADABS, University of Gottingen, Gottingen, Germany, 1996.
[3]. SHELXTL version 5.1, Bruker AXS Inc., Madison, WI, USA, 1997.

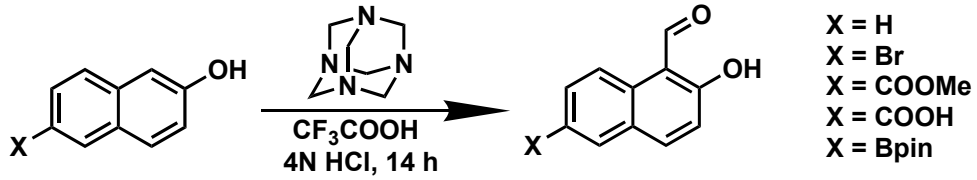
3. Synthesis and Characterization of ligands (HL1-HL5)

Synthesis of *N*-(adamantan-1-yl)-2-aminobenzamide.[1]

In a dry round bottom flask, Isatoic anhydride (979.1 mg, 6 mmol) and Adamantane amine (908.2 mg, 6 mmol) were heated at 80 °C in DMF (50 mL) overnight. After the completion of the reaction, the crude was extracted by ethyl acetate-water mixture. The organic layer was washed thrice with brine solution and dried over sodium sulphate followed by solvent removal using rotavapor to obtain brown powdered product. (Yield: 78 %). ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 7.38 – 7.35 (m, 1H), 7.24 (s, 1H), 7.18 – 7.14 (m, 1H), 6.63 (dd, J = 15.5, 7.8 Hz, 3H), 5.71 (s, 1H), 2.10 (s, 9H), 1.71 (s, 6H).

General Procedure A: Synthesis of Aldehydes.

The aldehydes were synthesized by Duff-reaction according to the reported literature with slight modifications. Briefly, different naphthols (4 mmol) and hexamethylenetetramine, HMTA (6 mmol) were taken in a 100 ml round bottom flask and 15 ml of trifluoroacetic acid was added to it. The reaction was heated at 80 °C for 14 h. After 14 h, the reaction was stopped and allowed to cool at room temperature. Hydrochloric acid (4N, 30 mL) was added to the reaction and stirred the whole reaction for 3 h. The precipitate was filtered in the fume hood and washed with 200-300 mL water to obtain the product. The product was purified by column chromatography.



6-bromo-2-hydroxy-1-naphthaldehyde.

Yield 45%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 13.09 (s, 1H), 10.75 (s, 1H), 8.20 (d, J = 9.0 Hz, 1H), 7.93 (s, 1H), 7.87 (d, J = 9.1 Hz, 1H), 7.67 (dd, J = 9.1, 1.5 Hz, 1H), 7.15 (d, J = 9.1 Hz, 1H). 13C NMR (101 MHz, $CDCl_3$, δ ppm): 192.96, 164.97, 137.97, 132.22, 131.46, 129.13, 120.52, 118.10, 111.33.

Methyl 5-formyl-6-hydroxy-2-naphthoate.

Yield 37%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 13.29 (s, 1H), 10.82 (s,), 8.53 (d, J = 1.8 Hz, 1H), 8.39 (d, J = 8.9 Hz, 1H), 8.20 (dd, J = 8.8, 1.8 Hz, 1H), 8.07 (d, J = 9.1 Hz, 1H), 7.20 (d, J = 9.1 Hz,

1H), 3.98 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, δ ppm): 193.26, 166.60, 140.04, 135.69, 132.1, 128.86, 127.05, 126.30, 120.32, 118.86, 111.37, 52.39.

5-formyl-6-hydroxy-2-naphthoic acid

Yield 33%. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 13.00 (s, 1H), 12.12 (s, 1H), 10.76 (s, 1H), 9.00 (d, *J* = 8.9 Hz, 1H), 8.50 (d, *J* = 1.4 Hz, 1H), 8.27 (d, *J* = 9.1 Hz, 1H), 8.06 – 8.01 (m, 1H), 7.29 (d, *J* = 9.1 Hz, 1H).

2-hydroxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-naphthaldehyde.

This compound was synthesized using the above synthesized 6-bromo-2-hydroxy naphthaldehyde. 6-bromo-2-hydroxy naphthaldehyde (1mmol, 251.07 mg), bispinacolato diborane (B_2Pin_2; 1.2 mmol, 304.7mg), powdered KOAc (3 mmol, 294.4 mg), and $Pd(dppf)_2Cl_2$ (0.12 mmol, 96.9 mg) were taken in a 100 ml round bottom flask and dissolved these in 20 ml of 1,4-dioxane. The reaction mixture was refluxed for 6 h. After the completion of the reaction, the crude product was separated in DCM-water mixture and dried over Na_2SO_4. The organic solvent was removed in rota-vapour and purified by column chromatography.

Yield 41%. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 13.22 (s, 1H), 10.81 (s, 1H), 8.32 (d, J = 8.5 Hz, 1H), 8.28 (s, 1H), 8.01 (d, J = 9.1 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 9.1 Hz, 1H), 1.38 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$, δ ppm): 193.44, 165.83, 139.89, 137.48, 134.89, 134.23, 127.27, 119.20, 117.80, 111.38, 84.17, 24.99.

General Procedure B: Synthesis of Ligands.

N-(adamantan-1-yl)-2-aminobenzamide and aldehydes (1:1 ratio) were taken and dissolved in ethanol (15-20 mL). The reaction was refluxed for 6 h in the presence of a catalytic amount of glacial acetic acid. Afterwards, the ethanol was removed *via* rotavap and the crude product was washed with diethyl ether to obtain a pure product.

HL1

Yield 67%. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 14.39 (s, 1H), 9.39 (s, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 9.1 Hz, 1H), 7.84 (dd, *J* = 7.6, 1.1 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.55 (s, 1H), 7.51 (s, 1H), 7.39 (s, 1H), 7.35 (d, *J* = 6.9 Hz, 1H), 7.22 – 7.18 (m, 1H), 6.09 (s, 1H), 2.07 (s, 9H), 1.67 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): δ 165.64, 159.40, 136.45, 131.50, 129.83, 129.45, 128.32, 126.61, 123.92, 120.68, 120.06, 119.43, 52.85, 41.48, 36.41, 29.54. ESI-MS$^+$ (m/z): $[M+H]^+$ calcd for $[C_{28}H_{29}N_2O_2]$, 425.2229 found, 425.2220.

HL2

Yield 60%. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 14.51 (s, 1H), 9.30 (s, 1H), 7.99 (d, *J* = 9.0 Hz, 1H), 7.89 (d, *J* = 1.7 Hz, 1H), 7.79 – 7.73 (m, 2H), 7.59 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.49 (d, *J* = 0.9 Hz, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 7.20 (t, *J* = 9.0 Hz, 2H), 5.93 (s, 1H), 2.06 (s, 9H), 1.65 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 166.33, 158.32, 145.23, 135.25, 131.73, 131.50, 131.13, 130.83, 129.52, 129.07, 126.72, 122.23, 121.19, 119.86, 117.24, 109.46, 52.89, 41.50, 36.40, 29.54. ESI-MS$^+$ (m/z): $[M+H]^+$ calcd for $[C_{28}H_{28}BrN_2O_2]$, 503.1334 found, 503.1328.

HL3

Yield 55%. ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 14.83 (s, 1H), 9.33 (s, 1H), 8.46 (s, 1H), 8.15 – 8.08 (m, 2H), 7.91 (d, *J* = 9.2 Hz, 1H), 7.73 (dd, *J* = 7.6, 1.2 Hz, 1H), 7.51 (s, 1H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.27 (s, 1H), 7.19 (d, *J* = 9.2 Hz, 1H), 5.88 (s, 1H), 3.96 (s, 3H), 2.07 (s, 9H), 1.66 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 169.23, 167.01, 166.21, 157.71, 144.50, 137.48, 136.21, 132.01, 131.46, 130.74, 129.40, 128.05, 126.79, 125.39, 122.52, 119.75, 119.37, 109.41, 52.95, 52.29, 41.51, 36.38, 29.53. ESI-MS$^+$ (m/z): $[M+H]^+$ calcd for $[C_{30}H_{31}N_2O_4]$, 483.2284 found, 483.2282.

HL4

^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 9.45 (s, 1H), 8.48 (d, *J* = 8.9 Hz, 1H), 8.37 (d, *J* = 1.3 Hz, 1H), 8.01 (d, *J* = 9.3 Hz, 1H), 7.95 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.87 (s, 1H), 7.80 (d, *J* = 8.1 Hz, 1H), 7.55 – 7.49 (m, 1H), 7.46 (d, *J* = 6.8 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 6.98 (d, *J* = 9.2 Hz, 1H), 2.05 – 1.96 (m, 9H), 1.59 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$, δ ppm): 172.72, 167.77, 155.78, 149.44, 142.08, 138.16, 131.62, 131.29, 130.65, 120.55, 126.74, 122.26, 124.07, 120.71, 116.55, 109.22, 52.30, 40.10, 36.61, 29.42, 21.46. ESI-MS$^+$ (m/z): $[M+H]^+$ calcd for $[C_{29}H_{29}N_2O_4]$, 469.2127 found, 469.2109.

HL5

Yield 69%. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 14.54 (s, 1H), 9.35 (s, 1H), 8.24 (s, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.89 (s, 1H), 7.87 (s, 1H), 7.80 (d, *J* = 7.0 Hz, 1H), 7.49 (d, *J* = 1.4 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 7.9 Hz, 1H), 7.15 (d, *J* = 9.1 Hz, 1H), 6.00 (s, 1H), 2.05 (s, 9H), 1.64 (s, 6H), 1.37 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$, δ ppm): 167.27, 166.02, 158.88, 145.47, 137.33, 135.14, 133.38, 131.48, 130.53, 129.70, 127.18, 126.57, 120.90, 119.99, 118.51, 109.49, 84.05, 52.87, 41.48, 36.40, 29.54, 24.99. ESI-MS$^+$ (m/z): $[M+H]^+$ calcd for $[C_{34}H_{40}BN_2O_4]$, 551.3081 found, 551.3108.

4. Synthesis of Iron Complexes ($Fe(L1)_2$-$Fe(L5)_2$)
General Synthesis of Iron complexes.

In a 25 ml round bottom flask, ligand **HL1-HL5** (0.1 mmol) was dissolved in THF (10 ml). Afterwards, a solution of $Fe(ClO_4)_3$•xH_2O (0.12 mmol) in THF (5 ml) was added to the ligand solution in dropwise and stirred it for 3 h at room temperature. A sudden colour transition from yellow to deep green was observed. The solvent was then removed through rotavapor and the crude green/brown solid was then dissolved in DCM and filtered via cotton plug and the filtrate was kept for the crystallization.

$[Fe(L1)_2](ClO_4)$.

(Yield: 76%) UV−Vis (DMSO) λ_{max} (ε), 262 nm (33053 mol^{-1} L cm^{-1}), 318 nm (23167 mol^{-1} L cm^{-1}), 600 nm (621 mol^{-1} L cm^{-1}). ESI-MS$^+$ (m/z): $[M+H]^+$ calcd for $[C_{56}H_{54}FeN_4O_4]$, 902.3494; found, 902.3492. FT-IR (KBr, cm^{-1}): 500, 620, 743, 834, 987, 1098, 1180, 1297, 1391, 1447, 1528, 1555, 1587, 2845, 2906, 3070, 3302.

$[Fe(L2)_2](ClO_4)$

(Yield: 56%) UV−Vis (DMSO) λ_{max} (ε), 260 nm (31780 mol^{-1} L cm^{-1}), 314 nm (16358 mol^{-1} L cm^{-1}), 600 nm (932 mol^{-1} L cm^{-1}). ESI-MS$^+$ (m/z): $[M]^+$ calcd for $[C_{56}H_{52}Br_2FeN_4O_4]$, 1058.1705; found, 1058.1716. FT-IR (KBr, cm^{-1}): 561, 622, 748, 875, 1083, 1308, 1358, 1525, 1566, 1590, 2845, 2909, 3387.

$[Fe(L3)_2](ClO_4)$

(Yield: 63%) UV−Vis (DMSO) λ_{max} (ε), 279 nm (53153.6 mol^{-1} L cm^{-1}), 312 nm (27233 mol^{-1} L cm^{-1}), 594 nm (1172 mol^{-1} L cm^{-1}). ESI-MS$^+$ (m/z): $[M]^+$ calcd for $[C_{60}H_{58}FeN_4O_8]$, 1018.3604; found, 1018.3609. FT-IR (KBr, cm^{-1}): 568, 620, 750, 828, 1095, 1174, 1198, 1268, 1356, 1453, 1535, 1597, 1713, 2845, 2900, 3313.

$[Fe(L4)_2](ClO_4)$

(Yield: 71%) UV−Vis (DMSO) λ_{max} (ε), 278 nm (27976 mol^{-1} L cm^{-1}), 310 nm (21729 mol^{-1} L cm^{-1}), 570 nm (1313 mol^{-1} L cm^{-1}). ESI-MS^+ (m/z): $[M]^+$ calcd for $[C_{58}H_{54}FeN_4O_4]$, 990.3291; found, 990.3282. FT-IR (KBr, cm^{-1}): 620, 761, 1092, 1306, 1450, 1532, 1620, 2853, 2909, 3406.

$[Fe(L5)_2](ClO_4)$

(Yield: 59.5%) UV−Vis (DMSO) λ_{max} (ε), 261 nm (32637 mol^{-1} L cm^{-1}), 322 nm (13886 mol^{-1} L cm^{-1}), 593 nm (513 mol^{-1} L cm^{-1}). ESI-MS^+ (m/z): $[M]^+$ calcd for $[C_{68}H_{76}B_2FeN_4O_8]$, 1154.5199; found, 1154.5160. FT-IR (KBr, cm^{-1}): 617, 699, 752, 822, 1089, 1142, 1332, 1367, 1452, 1529, 1593, 1622, 2851, 2906, 3461.

5. CV analysis

Cyclic voltammograms (CV) were carried out with a Autolab Pgstat-30 potentiostat employing a glassy carbon working electrode, platinum wire auxiliary electrode and Ag/AgCl reference electrode (0.01 M AgNO3, 0.1 M (n-Bu_4N)(PF_6)). Measurements were conducted at ambient temperature under an nitrogen atmosphere in MeCN containing 0.1 M (n-Bu_4N)(PF_6) (triply recrystallized) as an auxiliary electrolyte. Sample concentrations of $Fe(L1)_2$ was 0.5 mM. The voltammograms were recorded from a scan rate of 50-450 mV/s. All electrochemical data were referenced internally to the Ag/AgCl couple.

6. DNA Binding

The DNA binding assay was performed using circular dichroism (CD) spectroscopy. The experiment was performed using a JASCO J-815 spectropolarimeter having 1-mm path-length quartz cuvette. The CD spectra was recorded at various concentrations of $Fe(L1)_2$ keeping fixed DNA concentration. All solutions were made in 1x tris buffer. The spectra were recorded from 320 nm to 220 nm, scan speed 100 nm/min, and three accumulations.

7. Biological profile

Cell lines and culture conditions[2, 3]. Breast adenocarcinoma cell lines MCF-7 and MDA-MB-231, Human lung adenocarcinoma cell line A549, Human prostate cancer cells PC-3, Human cervical cancer cell line HeLa and Human embryonic kidney cell line HEK-293 was obtained from National Centre for Cell Science (NCCS Pune, India). The cell lines were maintained in RPMI 1640 (MDA-MB-231, MCF-7, PC-3 and A549) or DMEM medium (HEK-293), supplemented with 10% fetal bovine serum, 100 Units/mL penicillin and 100 µg/mL streptomycin, at 37 °C humidified atmosphere with 5% CO2. All stocks of the compound were prepared in DMSO.

8. Resazurin assay.

The resazurin assay determined the cytotoxicity of the compounds. The cells were harvested from culture flasks by trypsinization and seeded into 96-well microculture plates at the seeding density of 2500 cells per well. After the cells were allowed to resume exponential growth for 24 h, they were exposed to drugs at different concentrations in media for 72 h. Cisplatin (10 µM) and DMSO (0.5-0.2% of the media) were used as a positive and negative control, respectively. After the exposure of 72 h, the cells were washed once with 1 x PBS (100 µL/well) and then treated with resazurin solution (150 µL/well, 0.02 mg/mL) for 4 h in the dark and then fluorescence was taken at emission wavelength 590 nm (λ_{ex} = 520 nm) by SpectraMax M5^e microplate reader. All procedures were carried out in a triplicate. IC_{50} valves were calculated using GraphPad Prism software, and the results were presented as a mean ± SD.

9. Cell death pathway determination.

The cell viability of MCF-7 cells was measured by resazurin assay after 24 h co-culture with a variety of inhibitors and **$Fe(L1)_2$** complex. MCF-7 cells were seeded in 96-well plate, 2500 cells/well. Cells were allowed to adhere overnight. The cells were then pre-incubated for 1 h with the inhibitors 3-MA (100 µM), cycloheximide (0.1 µM), Necrostatin-1 (50 µM), Ferrostatin-1 (50 µM), and Z-vad-fmk (15

μM) and then treated with **Fe(L1)$_2$** for 24 h. Resazurin assay was then performed to determine the % cell viability in the presence of inhibitors.

10. Intracellular reactive oxygen species (ROS) measurement.
MCF-7 cells (2.5 x 10^4 cells/well) were plated in 96 well plate and allowed to adhere overnight. Afterwards, the cells were treated with **Fe(L1)$_2$**. Hydrogen peroxide (H_2O_2) was used as a positive control, whereas DMSO-treated cells were used as a negative control in this experiment. After 3 h of incubation, the media containing compounds was removed and the cells were washed with PBS. Then, a solution of 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA; 10 μM, 100 μL) in PBS was added to the cells and incubated in the dark at 37 °C for 30 min. The cells were then washed thrice with PBS to remove excess H_2DCF-DA and fluorescence microscopy images were captured immediately using BIO-RAD ZOE™ fluorescent cell imager.

11. TMRM assay.
MCF-7 cells (1.5 x 10^4 cells/well) were plated in 96 well plate and allowed to adhere overnight. Afterwards, the cells were treated with Fe-complex **Fe(L1)$_2$**. CCCP was used as a positive control, whereas DMSO-treated cells were used as a negative control in this experiment. After 3 h of incubation, the media containing compounds was removed, and the cells were washed with PBS. Then, a solution of Tetramethyl rhodamine methyl ester perchlorate (TMRM; 10 nM, 100 μL) in RPMI-1640 media was added to the cells and incubated in the dark at 37 °C for 30 min. The cells were then washed thrice to remove excess TMRM and fluorescence microscopy images were captured immediately using a BIO-RAD ZOE™ fluorescent cell imager.

12. Mitosox Staining.
MCF-7 cells (1.5 x 10^4 cells/well) were plated in 96 well plate and allowed to adhere overnight. Afterwards, the cells were treated with Fe-complex **Fe(L1)$_2$**. After 3 h of incubation, the media containing compounds was removed, and the cells were washed with PBS. Then, a solution of MitoSox (600 nM) in RPMI-1640 media was added to the cells and incubated in the dark at 37 °C for 30 min. The cells were then washed thrice to remove excess MitoSox dye and fluorescence microscopy images were captured immediately using a BIO-RAD ZOE™ fluorescent cell imager.

13. mPTP Opening assay.
MCF-7 cells (1.5 x 10^4 cells/well) were plated in 96 well plate and allowed to adhere overnight. The cells were treated with Fe(L1)$_2$ with 2 x IC_{50} concentration for 4 h in two different sets. Untreated cells were used as control in each sets. Afterwards, cells were washed with PBS and stained with Calcein-AM (1 μM) and mitotracker deep red (300 nM). After 30 min of incubation, washed 3 times to remove excess dye and added 1 mM solution of $CoCl_2$ solution in one of the sets. Washed 3 times with PBS and then imaged the cells using BIO-RAD fluorescent cell imager.

14. GSH-Glo™ Glutathione Assay.
A luminescence-based assay was performed to identify and quantify intracellular glutathione (GSH) level, known as the GSH-Glo™ Glutathione Assay. The experiment was performed according to the manufacturer protocol. In the 96-well plate, 2500 cells of MCF-7 were seeded and allowed to adhere overnight. The cells were treated with four different concentrations of **Fe(L1)$_2$** and incubated for 8 h. Afterwards, media was removed and cells of each well were treated with 100μl of prepared 1 x GSH-Glo™ Reagent and incubated for 30 minutes at room temperature. Afterwards, 100μl of reconstituted Luciferin Detection Reagent was added to each well and incubated for 15 minutes again and then recorded the luminescence using plate reader.

15. ATP assay.
ATP production was measured using the Cell Titer-Glo luminescence cell viability assay kit (Promega) according to the manufacturer's instructions. MCF-7 cells were cultured in an opaque-walled 96-well plate and treated with **Fe(L1)$_2$** at the indicated concentrations for 12 h. 100 μL Cell Titer-Glo reagent

was added to each well. The plate was incubated at room temperature for 15 min. Luminescence intensity was measured in a microplate reader.

16. Lipid peroxidation assay.
MCF-7 cells (1.5 x 10^4 cells/well) were plated in 96 well plate and allowed to adhere overnight. Afterwards, the cells were treated with Fe-complex **$Fe(L1)_2$** with its 5-times and 10-times IC_{50} values, respectively. DMSO-treated cells were used as a negative control in this experiment. After 2 h of incubation, the media containing compounds was removed, and the cells were washed with PBS. Then, a solution of C11-BODIPY in media was added to the cells and incubated in the dark at 37 °C for 30 min. The cells were then washed thrice with PBS to remove excess C11-BODIPY, and fluorescence microscopy images were captured immediately using a BIO-RAD ZOE™ fluorescent cell imager.

17. Endocytosis mechanism.
MCF-7 cells were stained with $Fe(L1)_2$ complex under different conditions by varying the temperature (4 °C and 37 °C) as well as the 1 h pretreatment of metabolic inhibitor (CCCP), ATP inhibitor NaN_3 and endocytic inhibitors (chloroquine and NH_4Cl). After 8 h of incubation, the culture medium was aspirated. Cells were trypsinized, counted and washed three times with PBS. Then, cells were centrifuged at 3000 rpm at 4 °C for 10 min digested in HNO_3 for two days. Each sample was diluted to 10 mL before test. The amount of Fe in cells revealed the uptake of $Fe(L1)_2$, which was determined by inductively coupled plasma mass spectrometry (ICP-MS) technique.

18. Cellular Uptake.
MCF-7 cells were plated in 6-well plate (1×10^6 cells/well). After 24 h incubation, the medium was replaced by medium containing iron complex $Fe(L1)_2$ and $FeClO_4.xH_2O$. After 8 h incubation, the culture medium was aspirated. Cells were trypsinized, counted and washed three times with PBS. Then, cells were centrifuged at 3000 rpm at 4 °C for 10 min digested in HNO_3 for two days. Each sample was diluted to 10 mL before test. The amount of Fe in cells revealed the uptake of $Fe(L1)_2$, which was determined by inductively coupled plasma mass spectrometry (ICP-MS) technique.

19. Molecular Docking.
Autodock Vina has been used to investigate the interactions of the Complex $Fe(L1)_2$ with SIRT1 protein (pdb-id: 4KXQ). Before starting the molecular docking studies, the receptor protein (pdb id 4KXQ) was ready for docking by eliminating excess water and co-factors. The macromolecule SIRT1 was conserved fixed whereas the complex $Fe(L1)_2$ was allowed to span several conformations. The grid size was 25 × 25 × 25 whereas the (x, y, z) coordinates were fixed at (22, -26, -3). Discovery Studio Visualizer was used to visualize the interactions between the protein and the ligand.

20. Cell cycle arrest by Flow cytometry.
MCF-7 cells were cultivated and seeded overnight before being treated to different doses of $Fe(L1)_2$ for a 24-hour period. The cells were collected, centrifuged, washed, and incubated overnight on ice and then with PI/RNase staining buffer solution for 0.5 h. Afterall, flow cytometry was used to find out the proportions of MCF-7 cells that were arrested at various phases.

21. Annexin/PI staining.
Apoptotic cells were detected using an Annexin V-FITC and PI dual-staining assay by flow cytometry. 1×10^5 cells of MCF-7 cells were seeded on a 6-well plate for 48 h. Subsequently, the media were changed, and the cells were treated with different concentrations of drug solution of $Fe(L1)_2$ for 24 h. The cells were then harvested and washed twice with cold 1× PBS, and finally resuspended in an Annexin V binding buffer. The cells were then incubated with both Annexin V-FITC and PI for 15 min under dark conditions at 25 °C. The data were analysed by flow cytometer within 1 h of sample preparation.

22. Immunofluorescence.

MCF-7 cells (1.5 x 10^4 cells/well) were plated in 96 well plates and allowed to adhere overnight. Afterwards, the cells were treated with Fe-complex **$Fe(L1)_2$** for 24 h. DMSO-treated cells were used as a control in this experiment. After 24 h, cells were washed with PBS and fixed with 4% PFA solution. Cells were permeabilized by 0.01% triton-X solution. After permeabilization, the cells were washed thrice with PBS. Cells were incubated with blocking buffer for an hour and then primary antibody solutions were added at 4 °C overnight. Cells were washed again with PBS and samples were incubated with secondary antibody solutions for 1 h. After three times wash with PBS, DAPI (4 μM) for 30 min and cells were imaged by BioRad fluorescent cell imager.

23. Western Blot.

Cell protein extracts were prepared for western blotting. The protein mixtures were separated on SDS-PAGE gel (10%) and then transferred to a PVDF membrane (G-Biosciences, USA). Afterward, the membrane was blocked in 5% BSA for 1 h and incubated with primary antibodies [CST: PERK (D11A8)], [CST: BiP (C50B12)], and [CST: GPX4(E5Y8K)] overnight at 4 °C. The samples were subsequently incubated with β-actin antibody [CST: β-Actin (D6A8) rabbit mAb #8457] as a loading control. The membranes were washed three times (5 min each) with TBST and incubated for 2 h with a secondary antibody. After incubation, the membranes were washed again with TBST three times (5 min each) and visualized Supersignal™ West Pico Plus Chemiluminescent Substrate (Thermo scientific) and a chemiluminescence imaging machine (ChemiDoc Touch Imaging System, BioRad). The level of expression for each protein was analyzed using Bio-Rad Image Lab Software.

24. *In vivo* experiment.

The animal experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Indian Institute of Technology Kanpur with an approval number IEC Communication Number: IITK/IEC/2023-24/I/10 and llTK/IAEC/2023/1186. 2 months old female BALB/c mice were purchased from Central Experimental Animal Facility (CEAF), IIT Kanpur. After about 8 days of inoculation, 6 x 10^5 cells of the 4T1 cell line were injected into the mammary pad of the mice. After tumor establishment (day 0), mice were randomly divided into two groups of three mice each. The control group (i.e. vehicle) and the $Fe(L1)_2$ treated group were intravenously injected with 3% DMSO containing aqueous solution (100 μL) and $Fe(L1)_2$ (10 mg/Kg dose, 100 μL, dissolved in 3% DMSO/H_2O). The first dose was recorded as day 1. The body weight, length and width of tumors were recorded every second day before injection. The tumor volume was calculated by the formula $0.5\times\text{length}\times(\text{width})^2$. On the 8th day, the mice were sacrificed, the blood was collected, and the morphology of erythrocytes was captured. The tumors were stripped out for photograph and weight. Other organs (heart, liver, kidney, lungs, intestine, and spleen) were also harvested and fixed in 4% paraformaldehyde for H & E staining. The organs were digested in aqua-regia to determine the Fe content by ICP-MS.

References.

[1]. G. C. Senadi, V. S. Kudale, J.-J. Wang, *Green Chem.* **2019**, 21, 979.

[2]. A. Kumar, A. Chaudhary, B. Agrahari, K. Chaudhary, P. Kumar, R. G. Singh, *Chem. Commun.* **2023**, 59, 14305.

[3]. A. Kumar, A. Chaudhary, H. Sonker, S. Subhadarshini, M. K. Jolly, R. G. Singh, *ACS Org. Inorg. Au* **2024**, 4, 319.

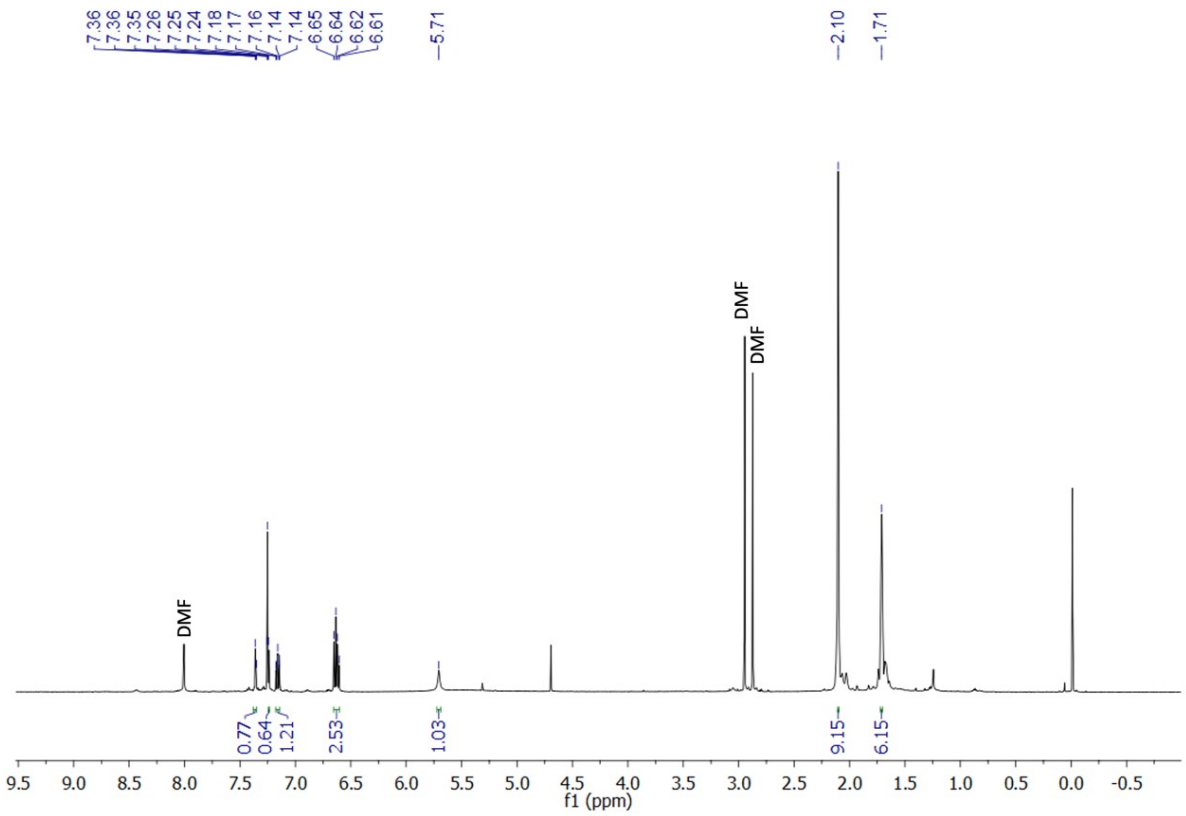
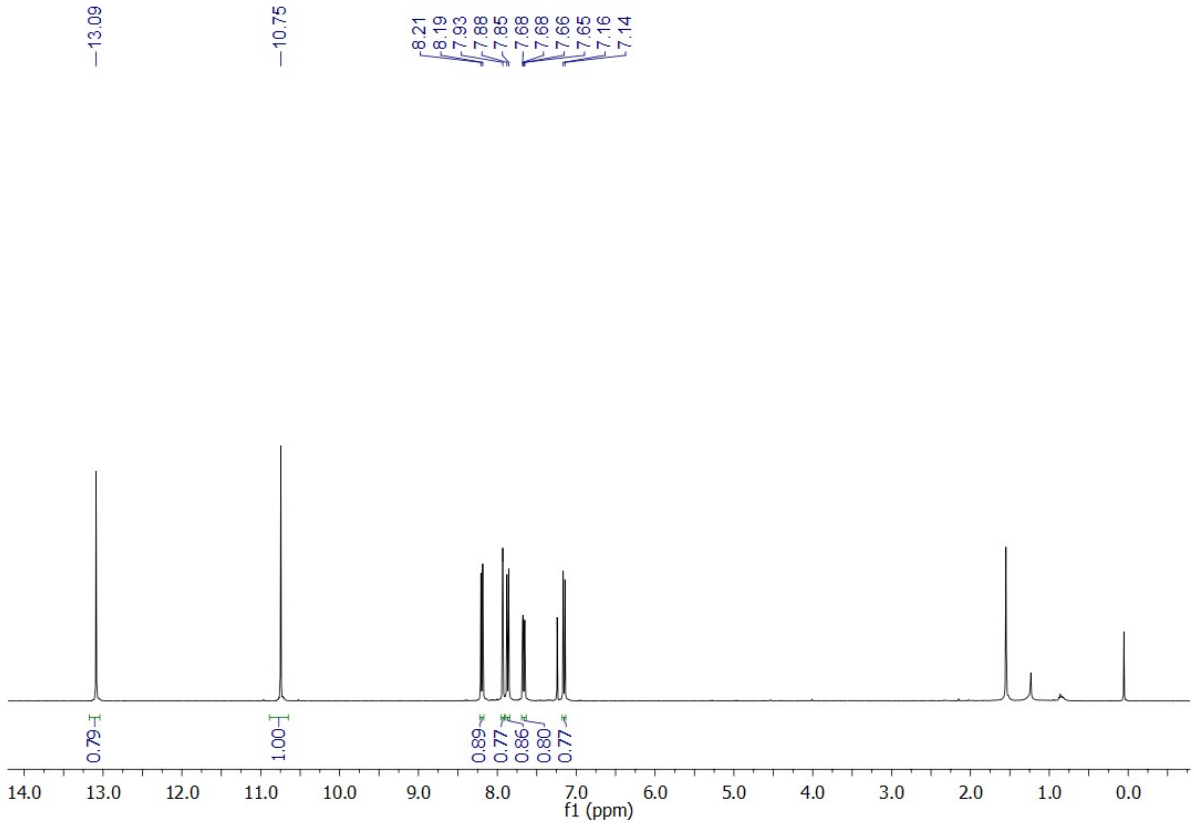


Fig S1. [1]H NMR of *N*-(adamantan-1-yl)-2-aminobenzamide.



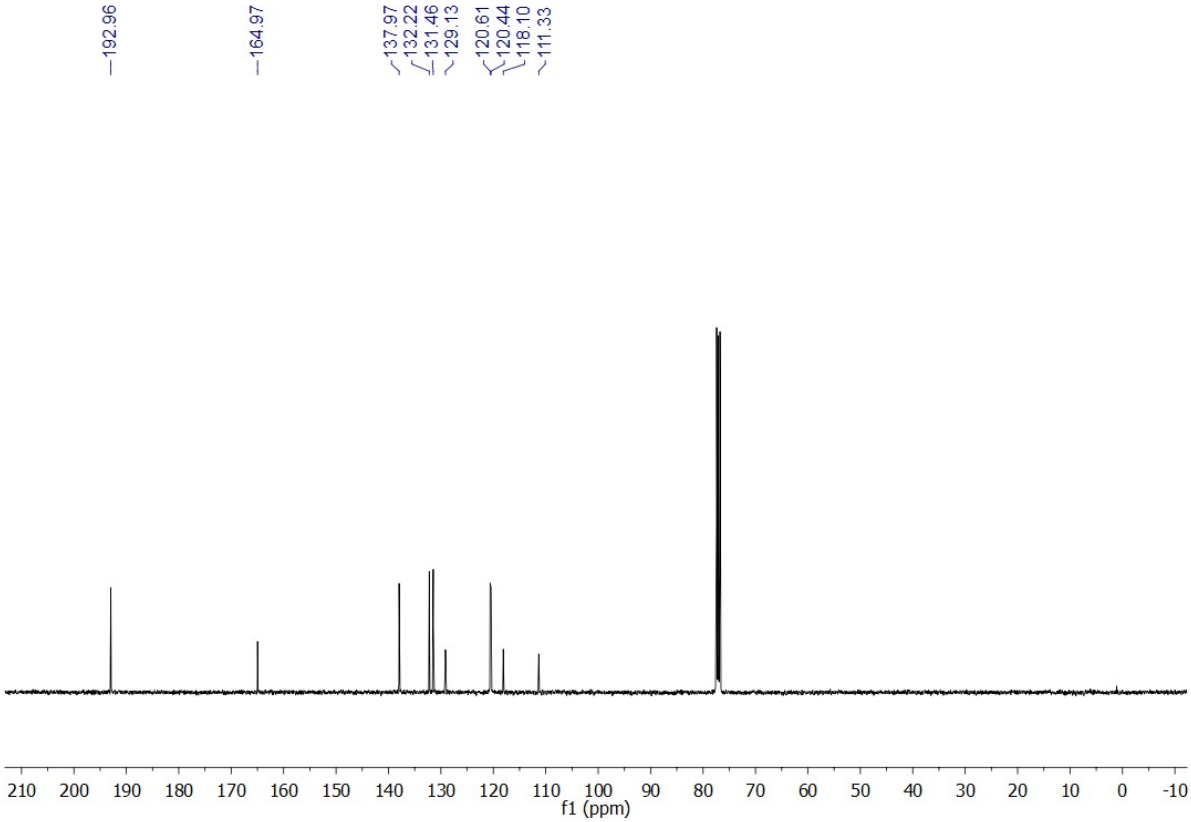
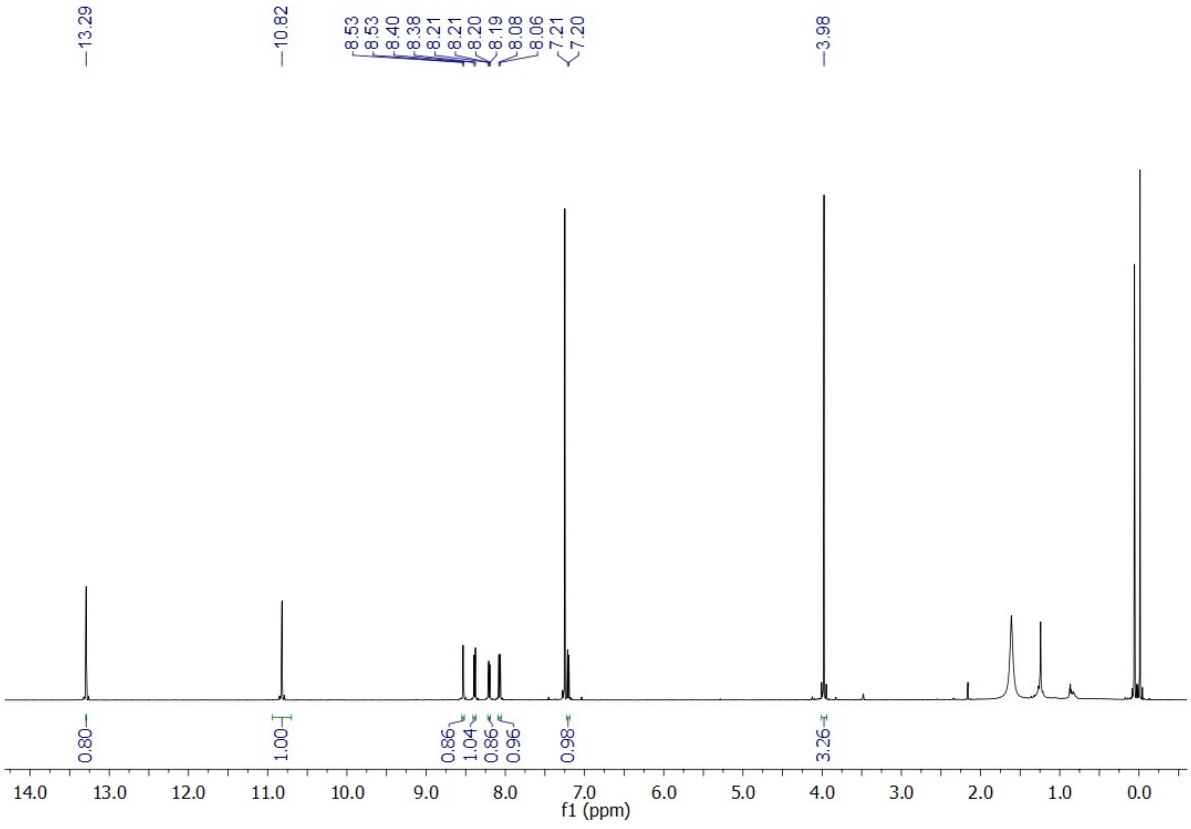


Fig S2. ^{1}H, and ^{13}C $\{^{1}H\}$ NMR for 6-bromo-2-hydroxy-1-naphthaldehyde.



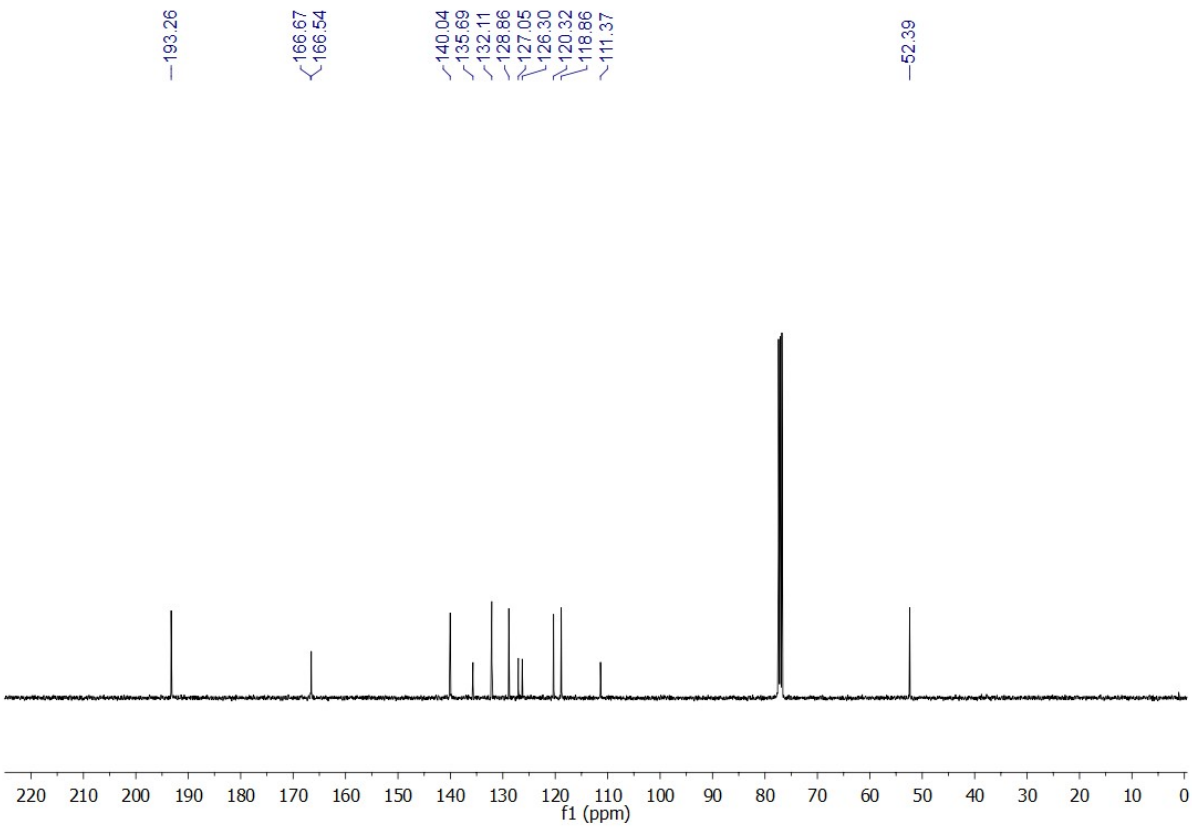


Fig S3. ^{1}H, and ^{13}C $\{^{1}H\}$ NMR for Methyl 5-formyl-6-hydroxy-2-naphthoate.

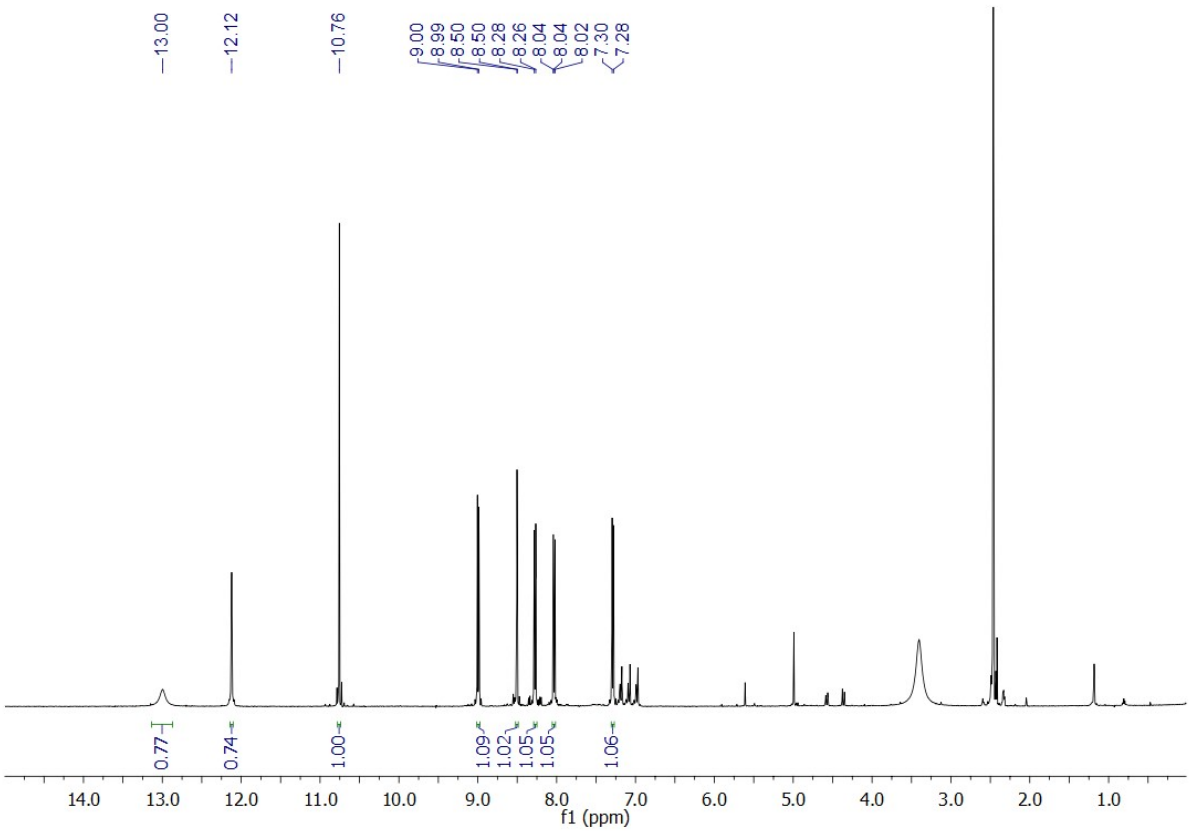


Fig S4. ^{1}H NMR for 5-formyl-6-hydroxy-2-naphthoic acid.

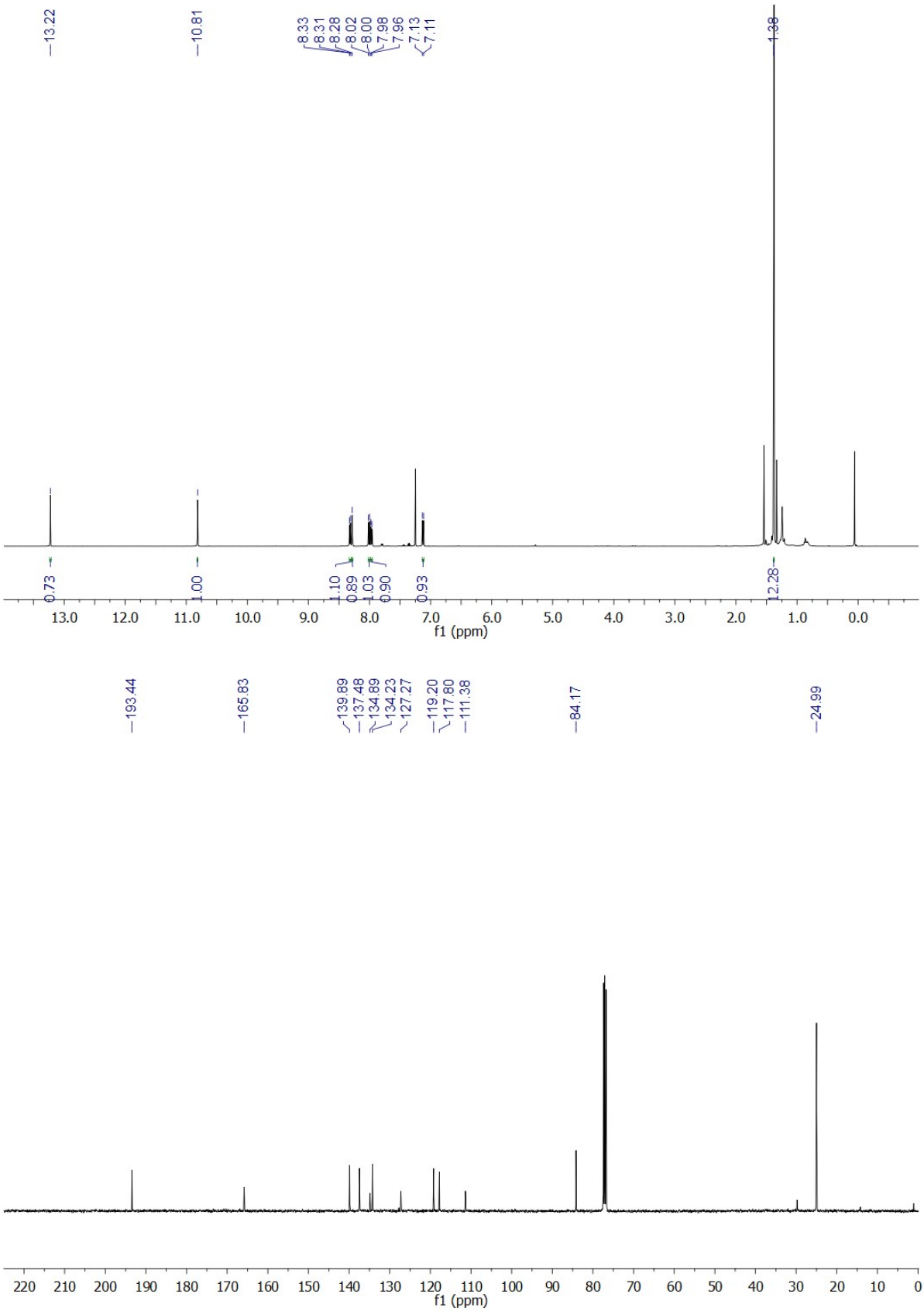
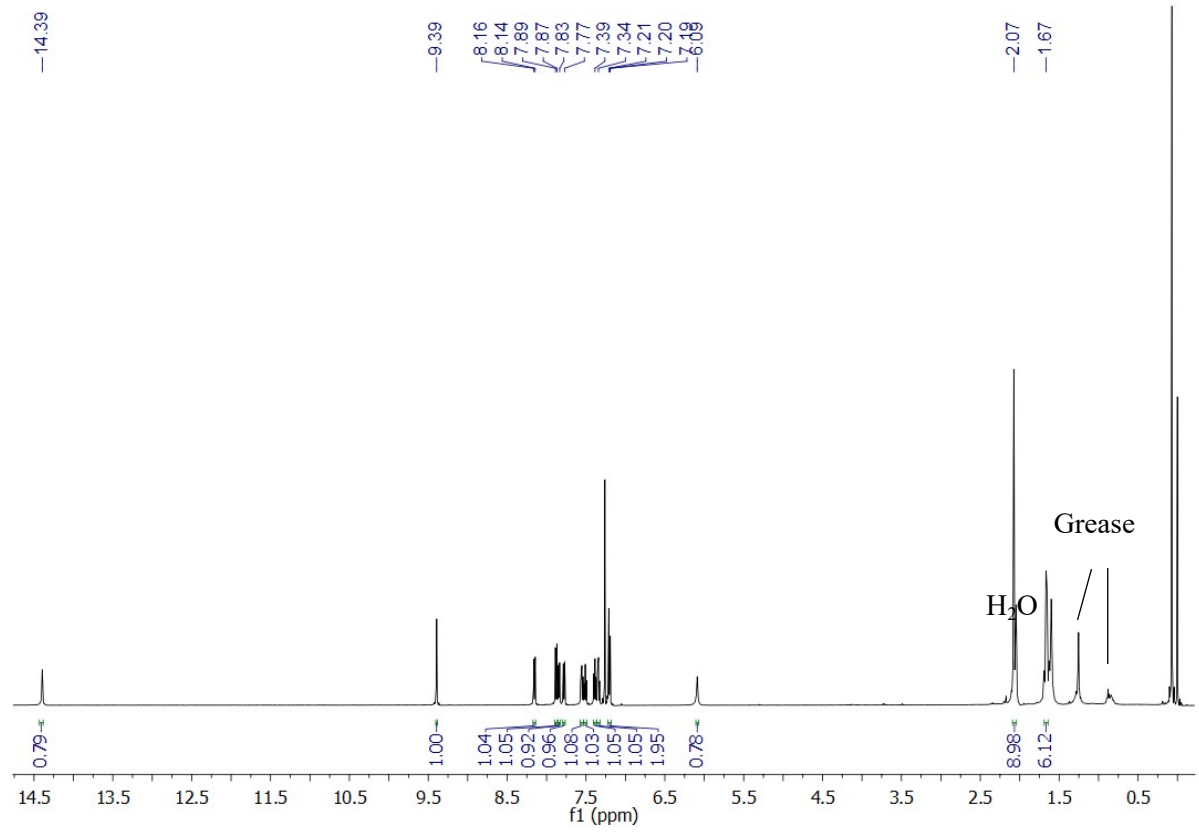
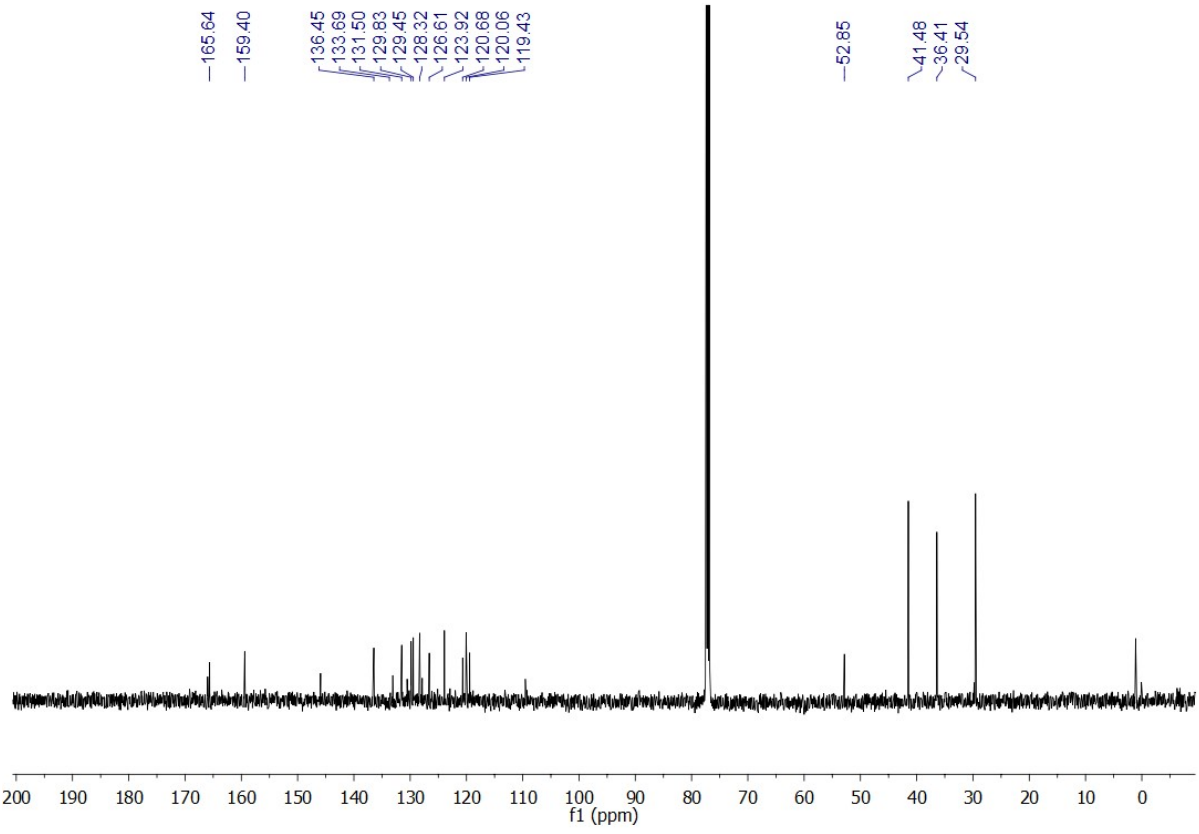


Fig S5. ^{1}H, and ^{13}C $\{^{1}H\}$ NMR for 2-hydroxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-naphthaldehyde.


14.39
9.39
8.16
8.14
7.89
7.87
7.83
7.77
7.39
7.34
7.21
7.20
7.19
6.09
2.07
1.67
Grease
H2O
0.79
1.00
1.04
1.05
0.92
0.96
1.08
1.03
1.05
1.05
1.95
0.78
8.98
6.12
14.5 13.5 12.5 11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
f1 (ppm)


165.64
159.40
136.45
133.69
131.50
129.83
129.45
128.32
126.61
123.92
120.68
120.06
119.43
52.85
41.48
36.41
29.54
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

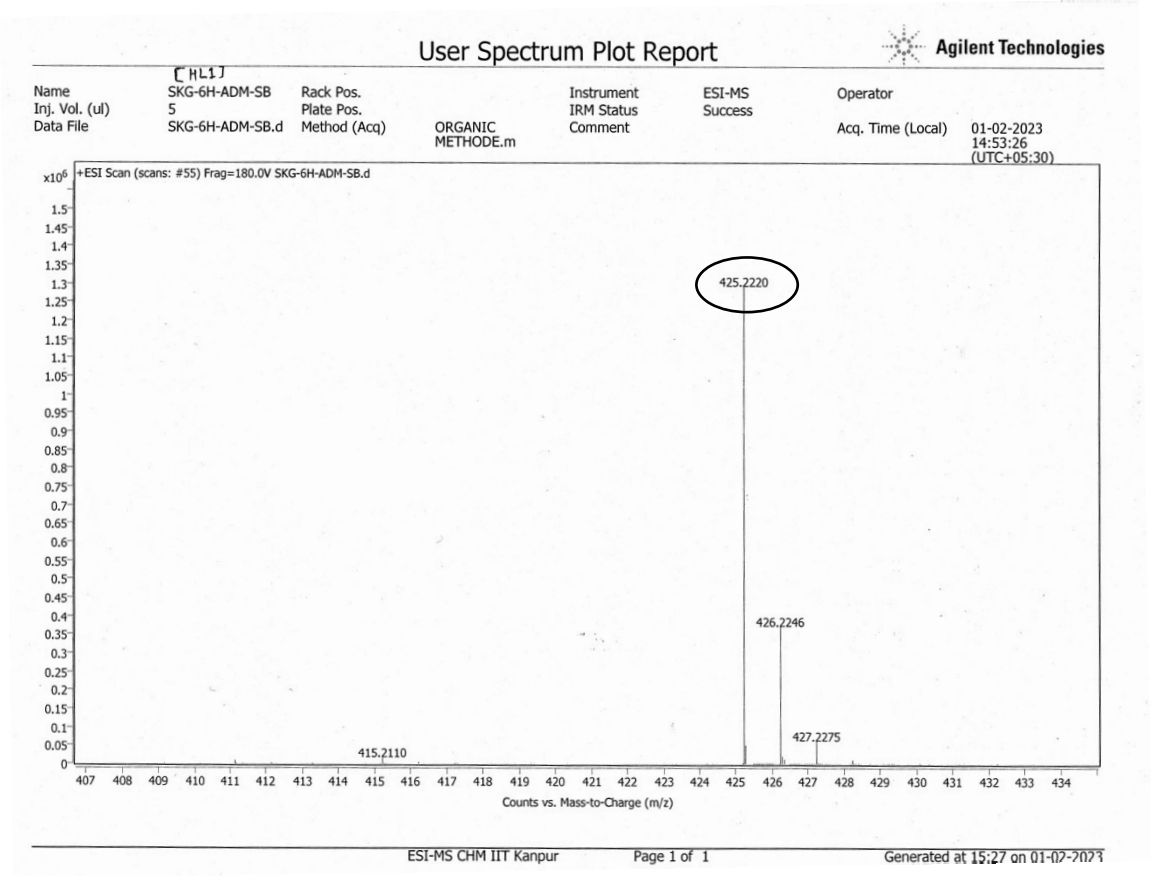
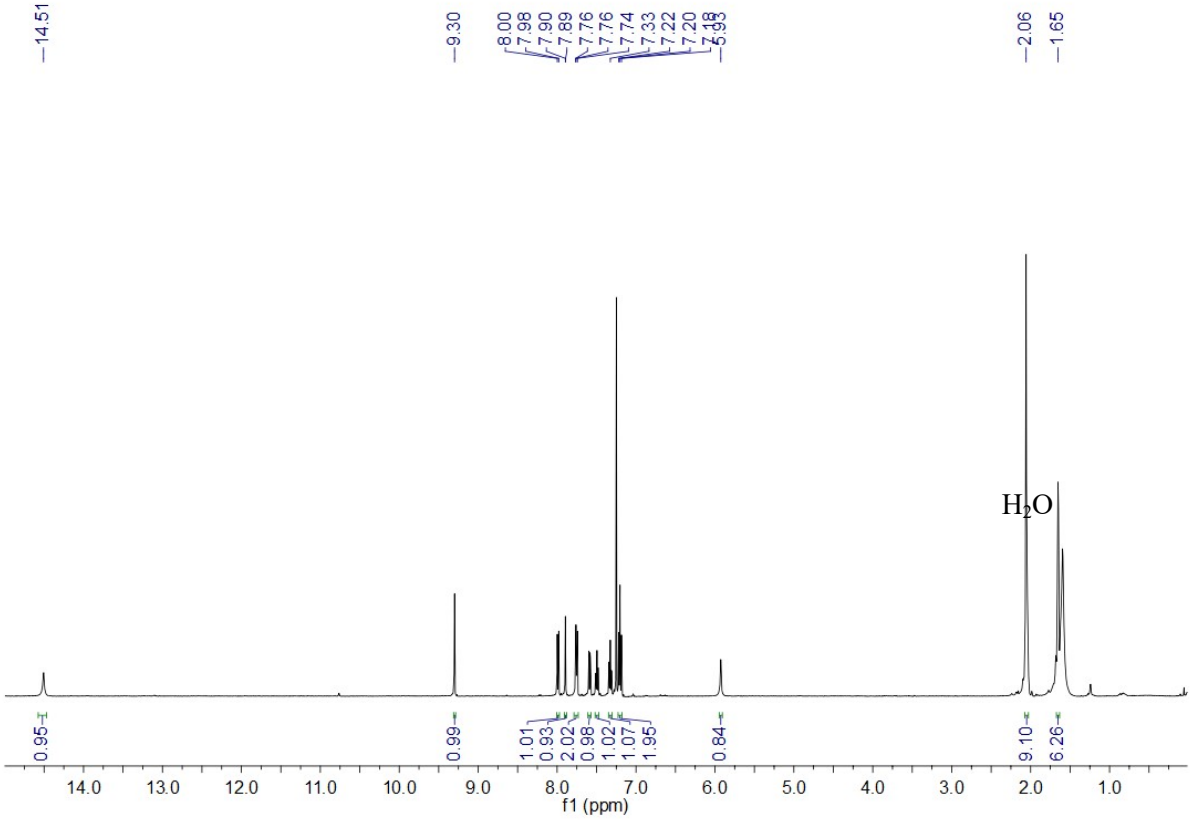
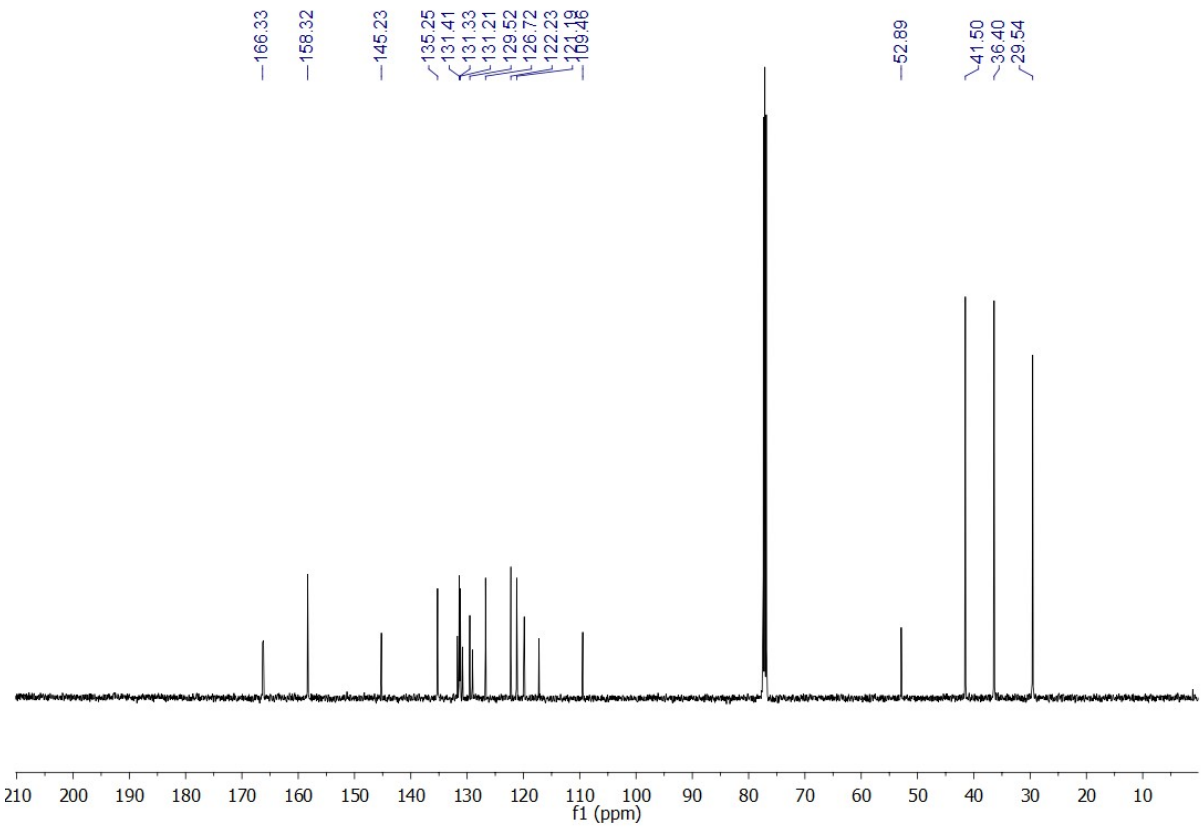


Fig S6. ^{1}H, ^{13}C $\{^{1}H\}$ NMR and ESI-MS data for compound **HL1**.





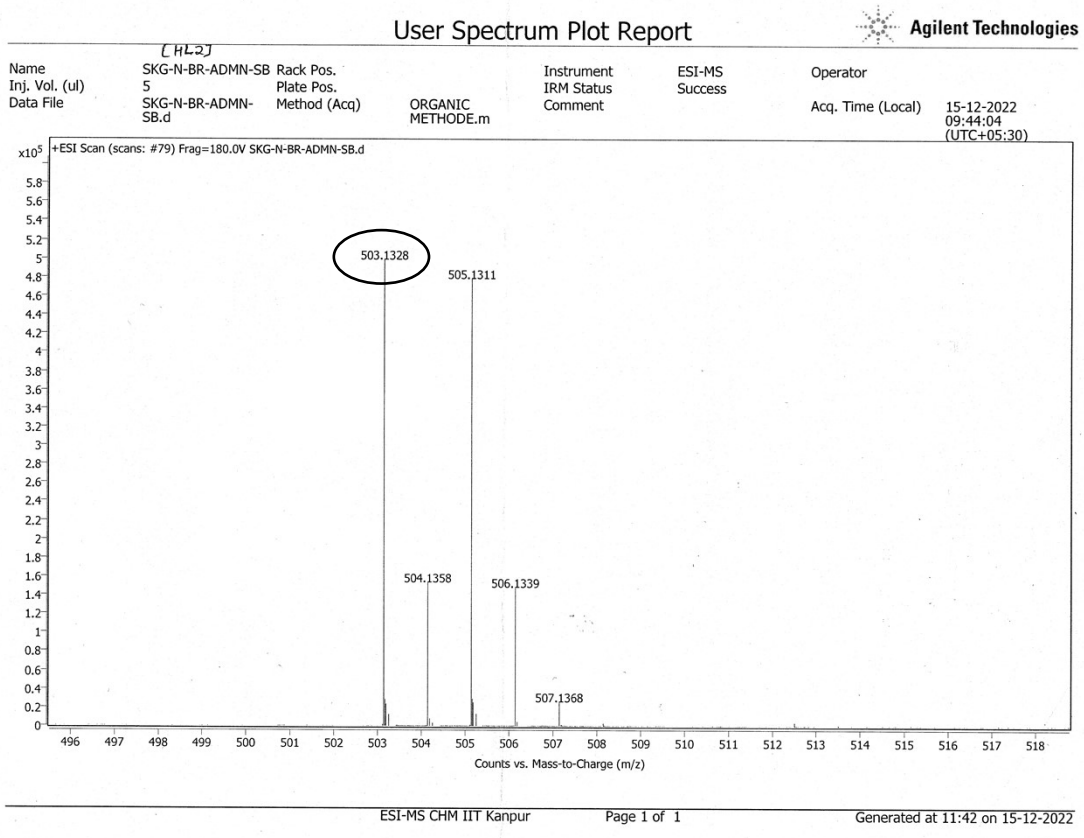
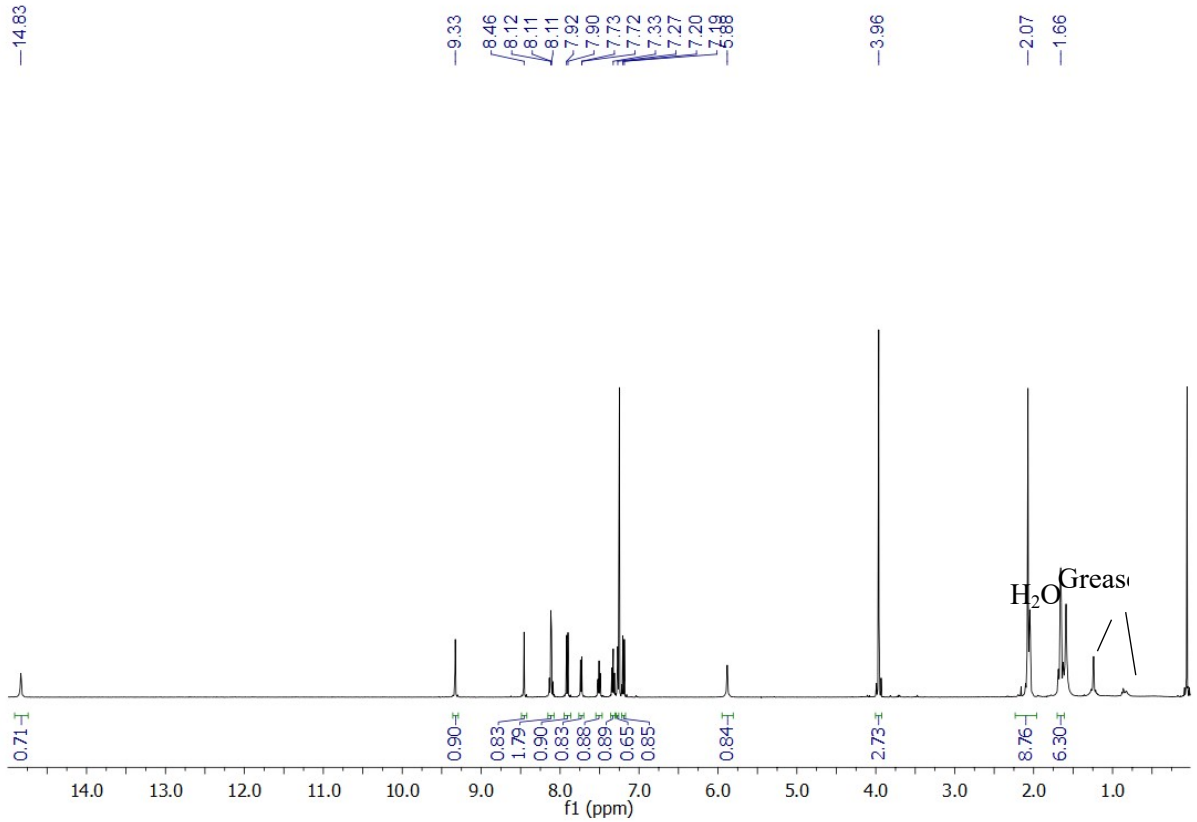
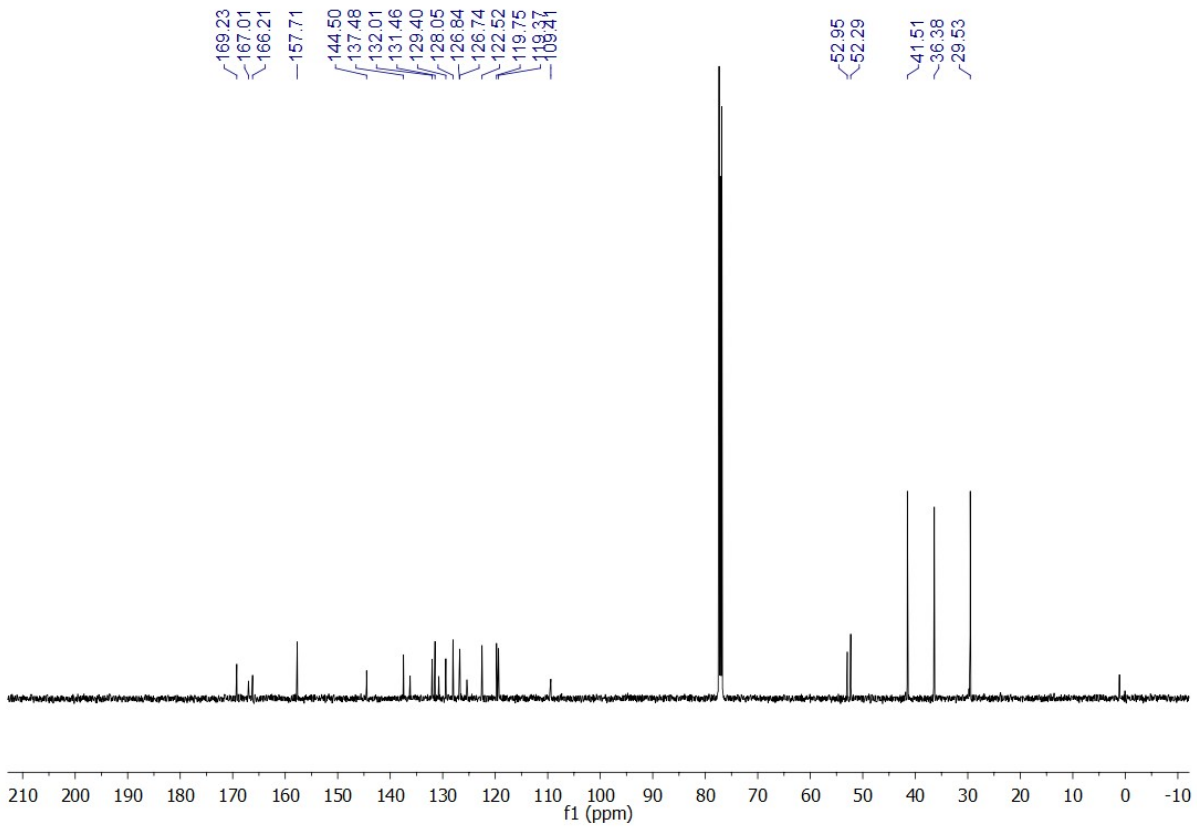


Fig S7. ^{1}H, ^{13}C $\{^{1}H\}$ NMR and ESI-MS data for compound **HL2**.


14.83
9.33
8.46
8.12
8.11
8.11
7.92
7.90
7.73
7.72
7.33
7.27
7.20
5.88
3.96
2.07
1.66
H2O
Greas
0.71
0.90
0.83
1.79
0.90
0.83
0.88
0.89
0.65
0.85
0.84
2.73
8.76
6.30
14.0 13.0 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
f1 (ppm)


169.23
167.01
166.21
157.71
144.50
137.48
132.01
131.46
129.40
128.05
126.84
126.74
122.52
119.75
52.95
52.29
41.51
36.38
29.53
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

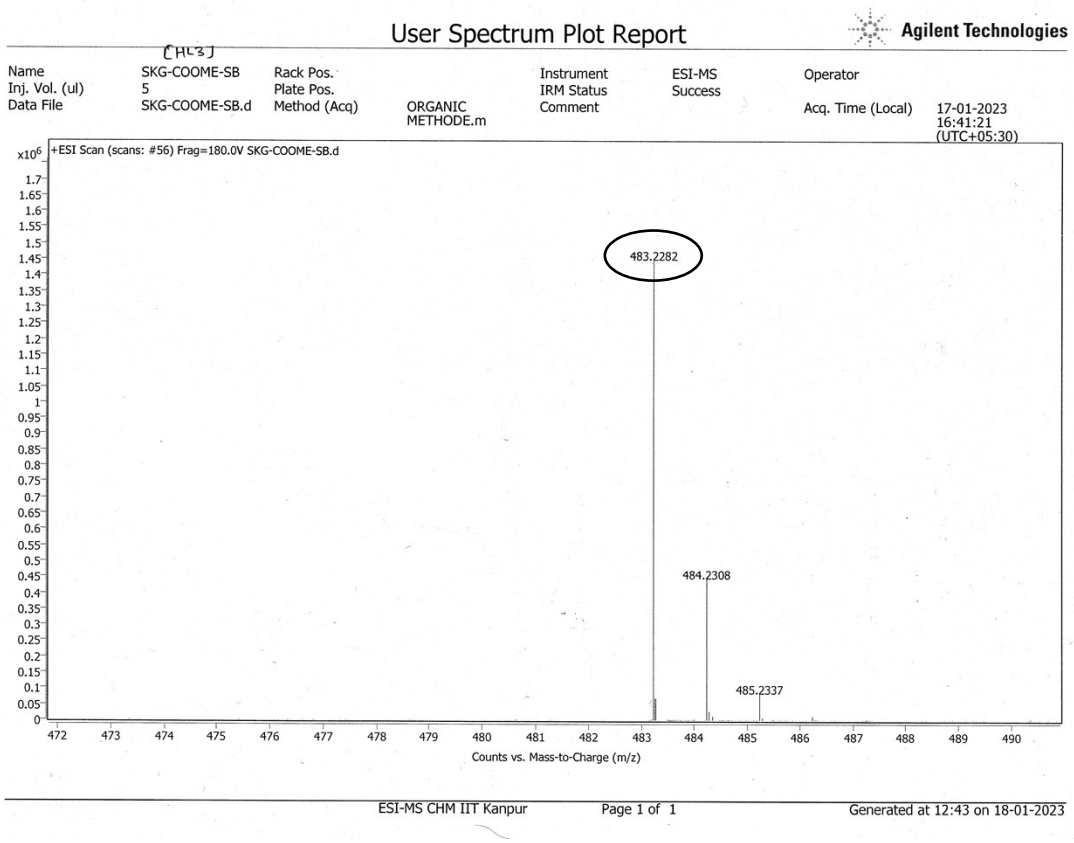
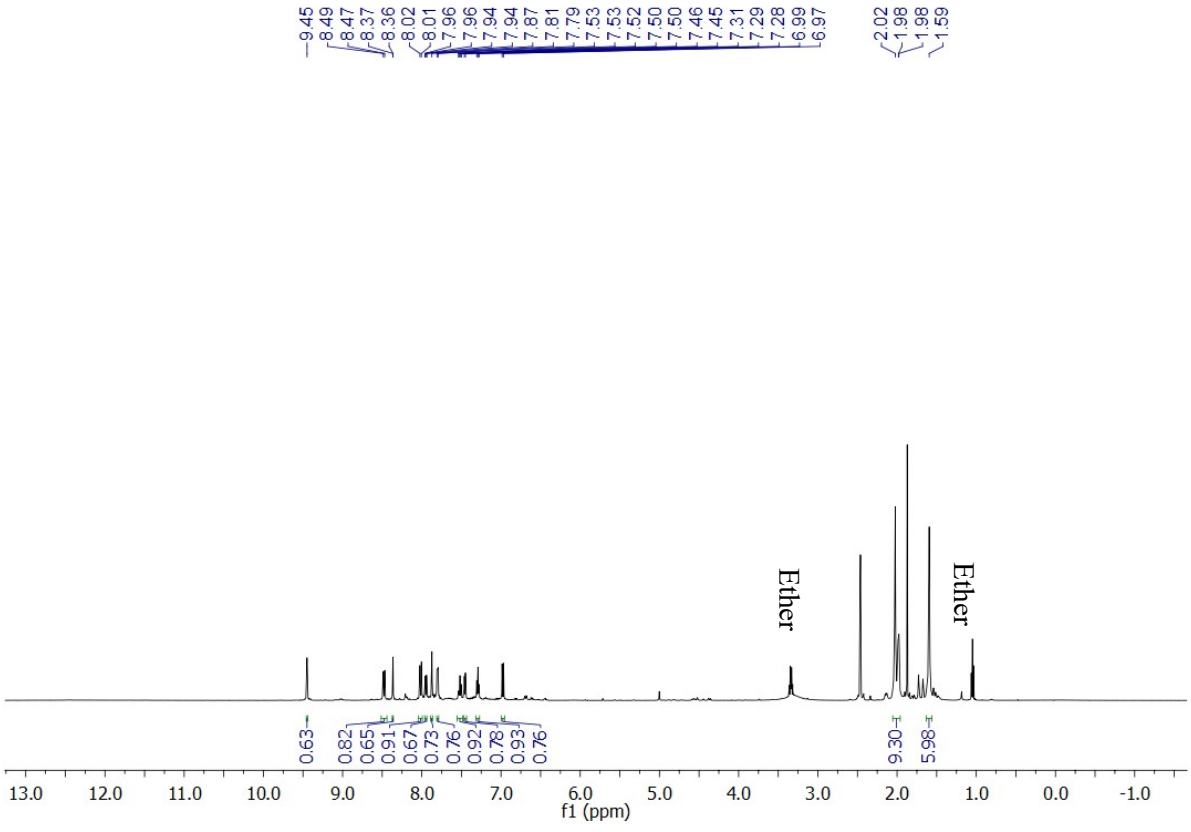
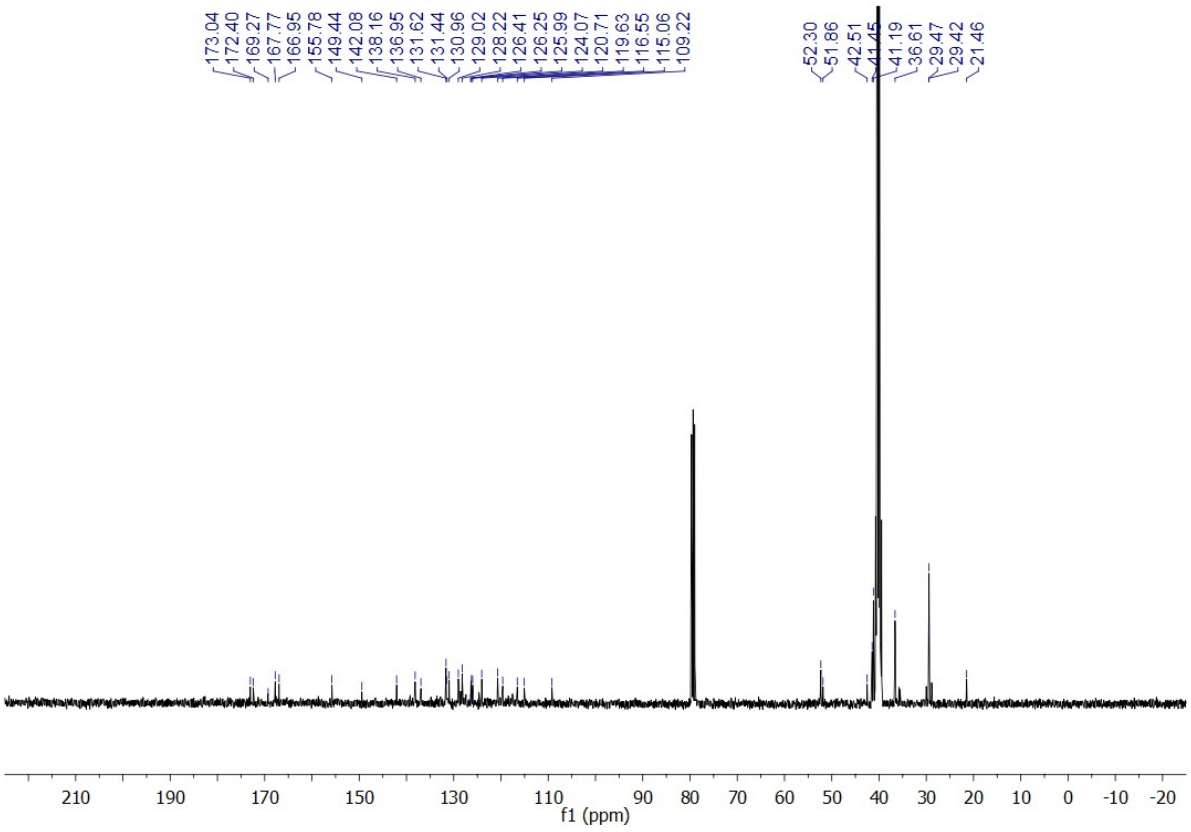


Fig S8. ^{1}H, ^{13}C $\{^{1}H\}$ NMR and ESI-MS data for compound **HL3**





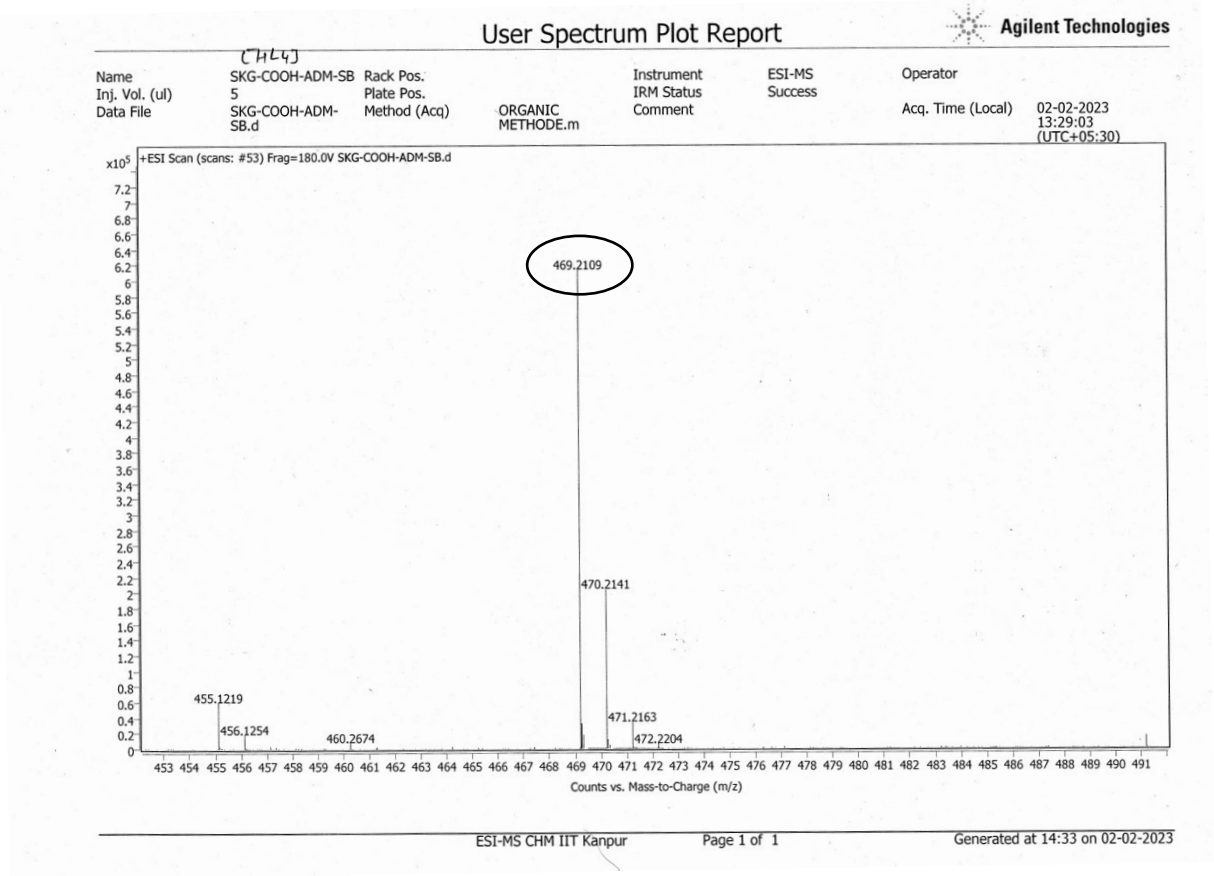
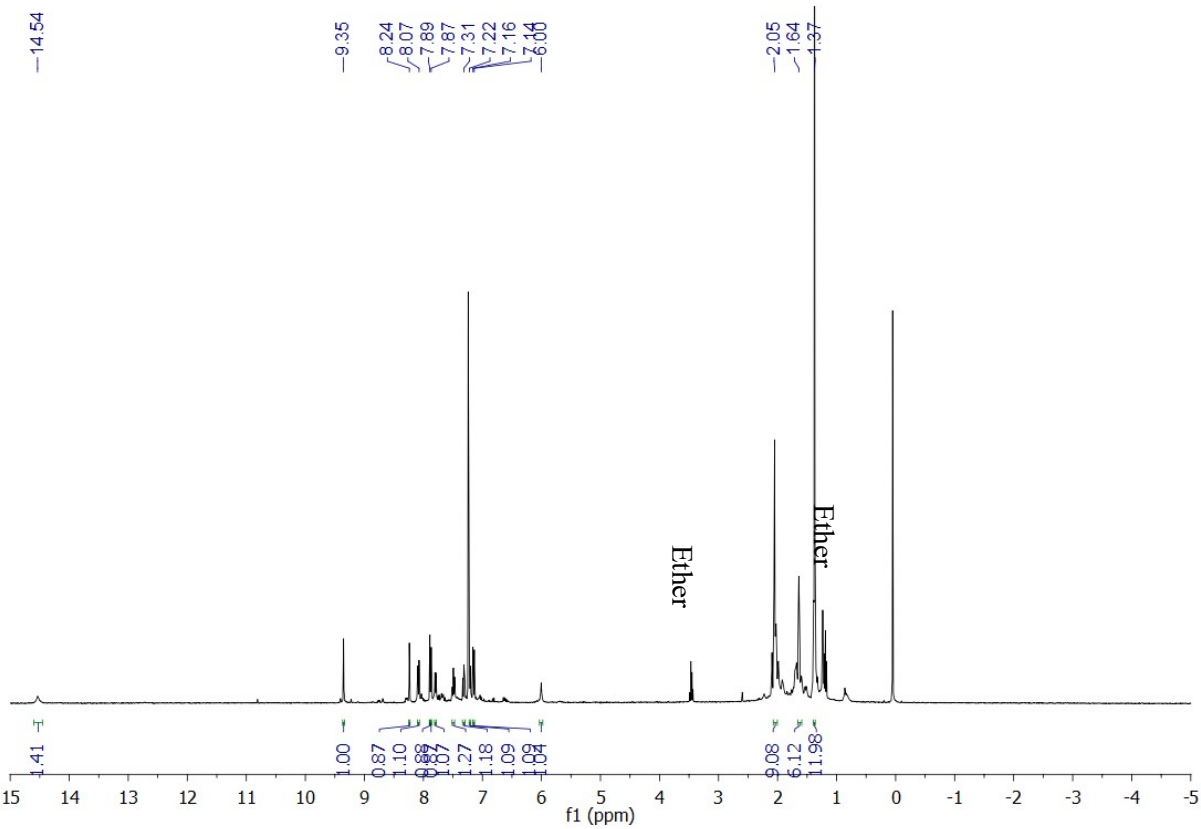
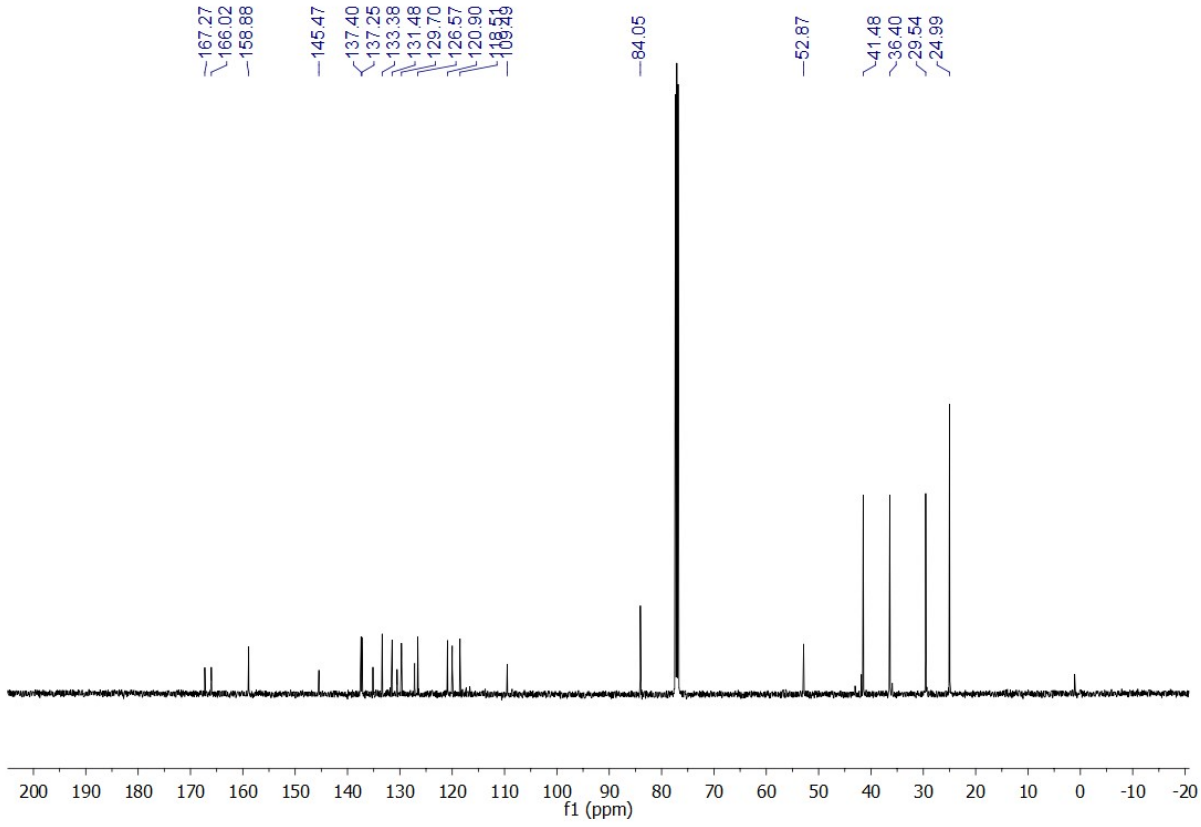


Fig S9. ^{1}H, ^{13}C $\{^{1}H\}$ NMR and ESI-MS data for compound **HL4**


14.54
9.35
8.24
8.07
7.89
7.87
7.31
7.22
7.16
7.14
7.00
2.05
1.64
1.37
Ether
Ether
1.41
1.00
0.87
1.10
1.27
1.18
1.09
1.09
1.04
9.08
6.12
11.98
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4 -5
f1 (ppm)


167.27
166.02
158.88
145.47
137.40
137.25
133.38
131.48
129.70
126.57
120.90
84.05
52.87
41.48
36.40
29.54
24.99
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

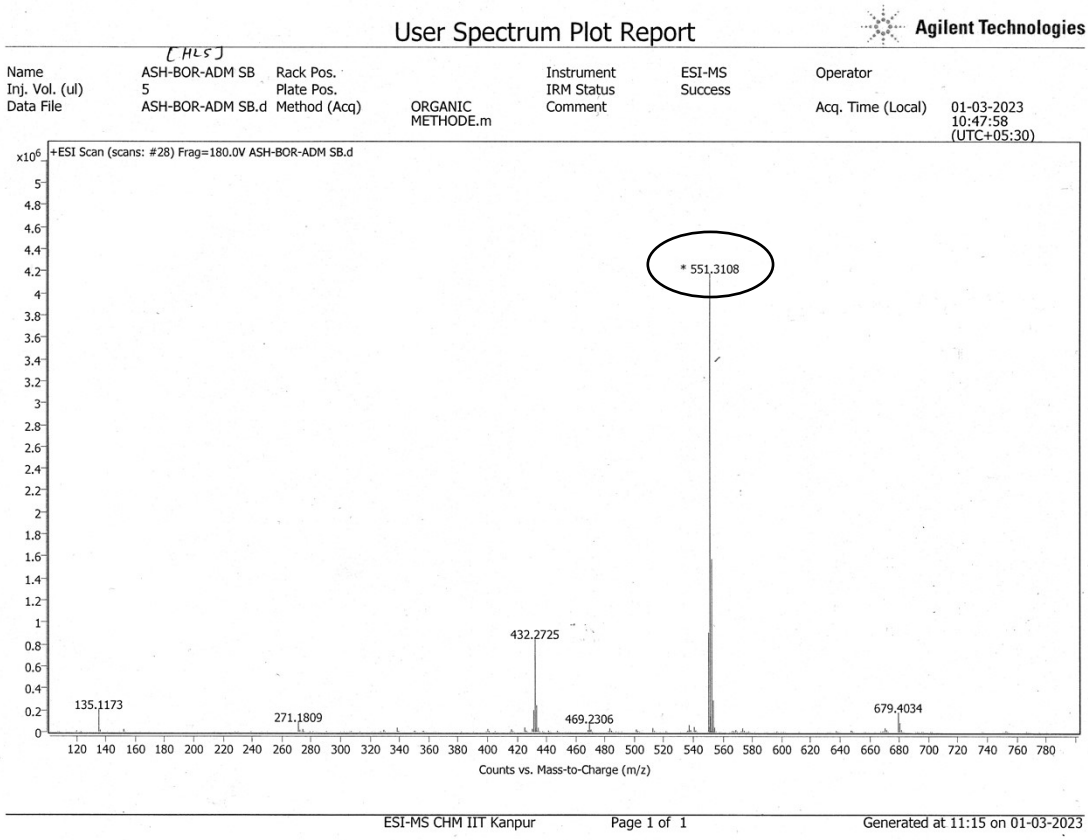
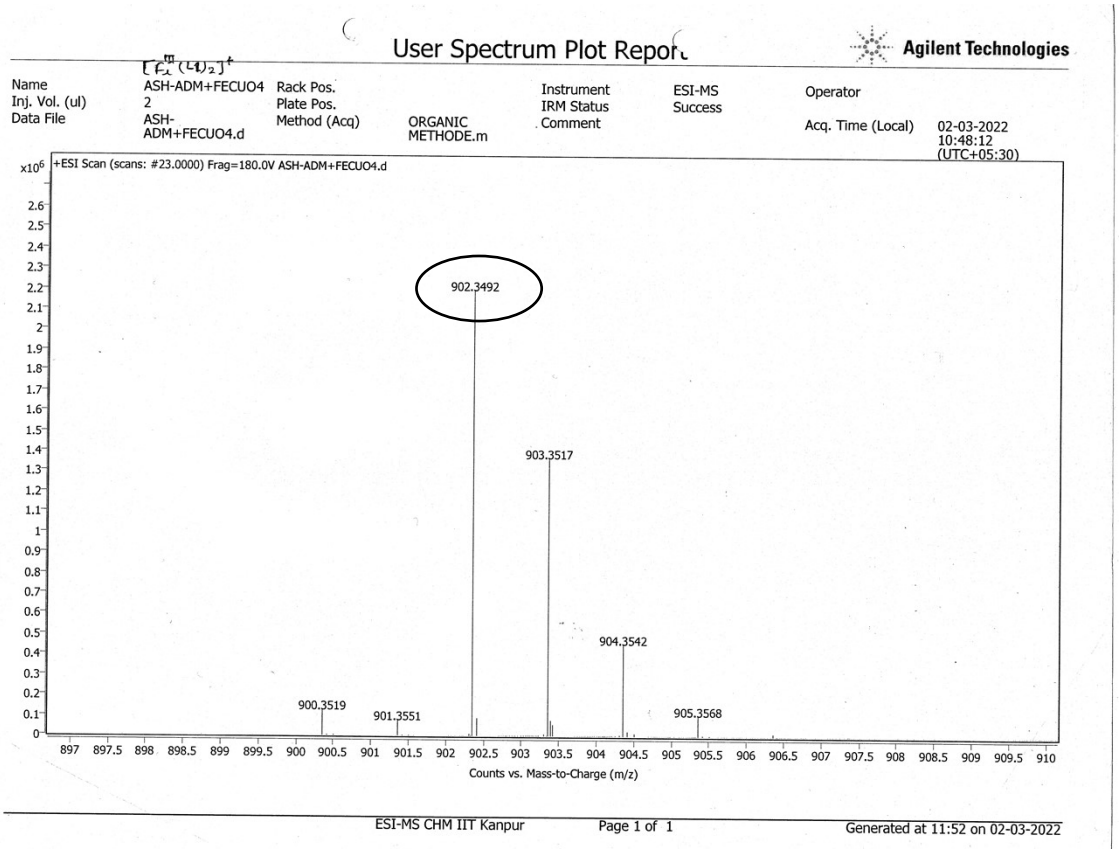


Fig S10. ^{1}H, ^{13}C $\{^{1}H\}$ NMR and ESI-MS data for compound **HL5**



Spectrum Plot Report

Agilent Technologies

[Fe^III(L2)2]+

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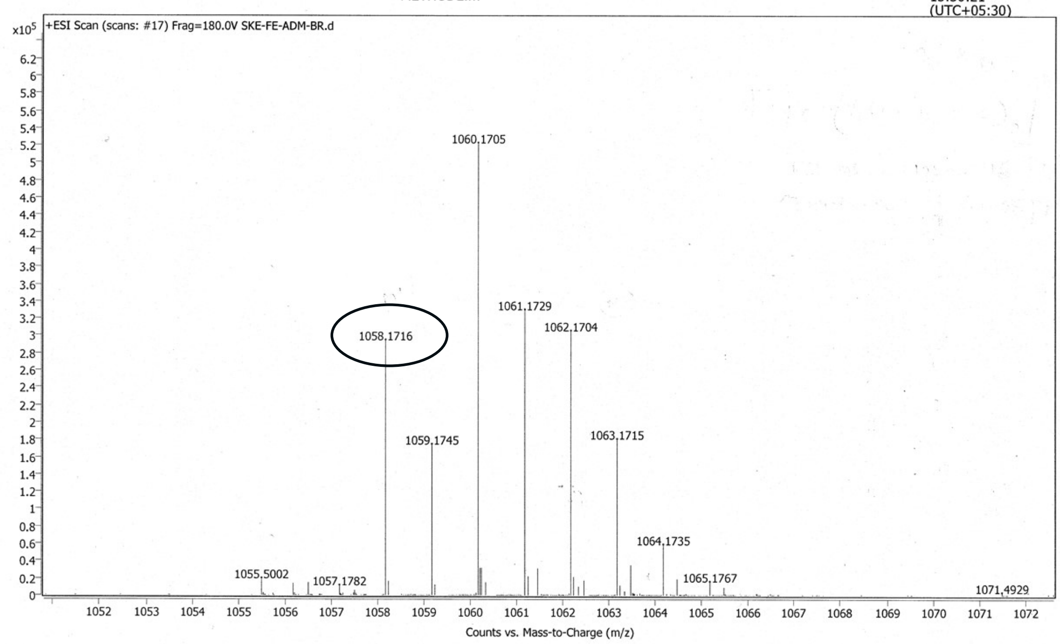
Inj. Vol. (ul)	5	Plate Pos.		IRM Status	Success		
Data File	SKE-FE-ADM-BR.d	Method (Acq)	ORGANIC METHODE.m	Comment		Acq. Time (Local)	19-12-2022 15:30:21 (UTC+05:30)



User Spectrum Plot Report

Agilent Technologies

[Fe^III(L2)2]+

Name	FECLO4-L6(ecm-ADM) SB	Rack Pos.		Instrument	ESI-MS	Operator	
Inj. Vol. (ul)	5	Plate Pos.		IRM Status	Success		
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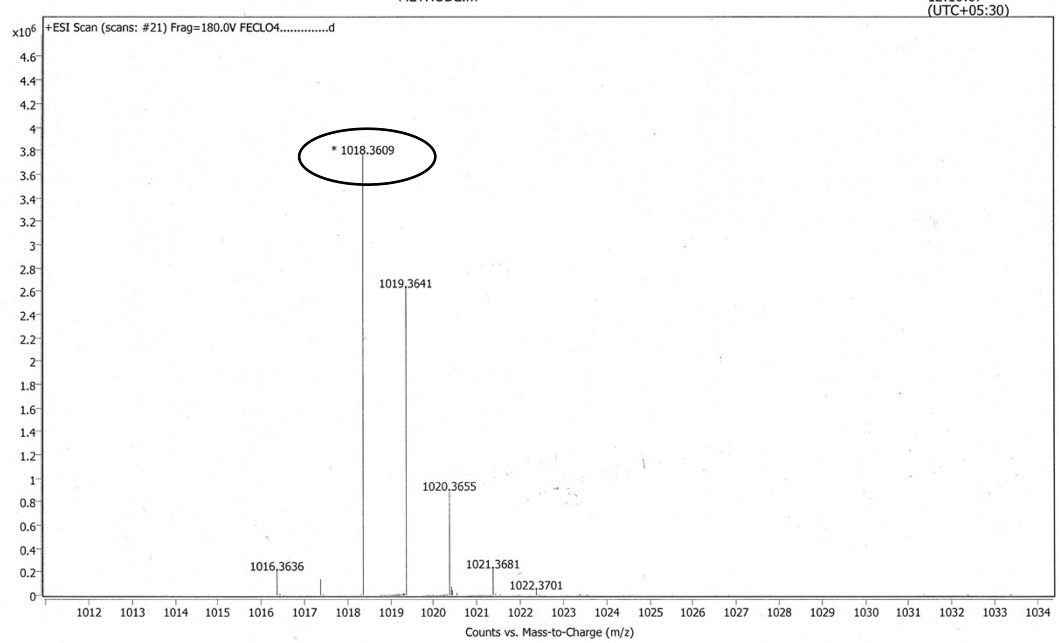
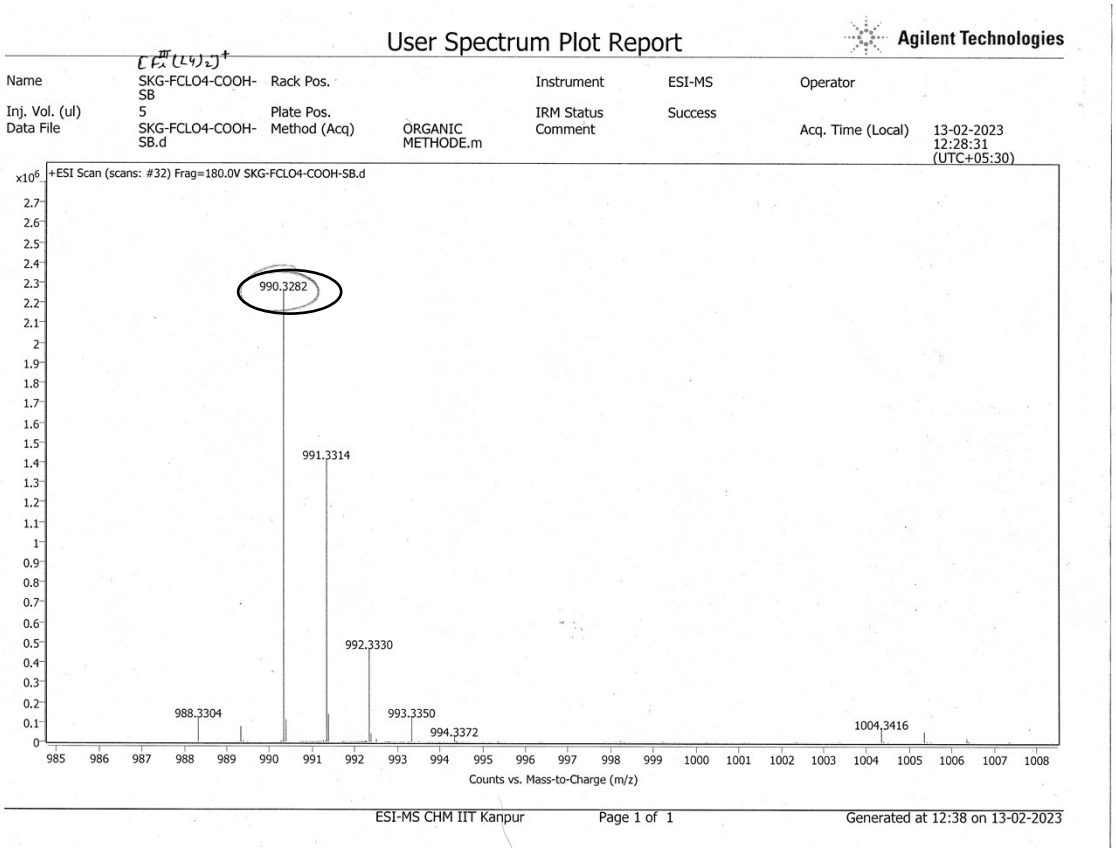




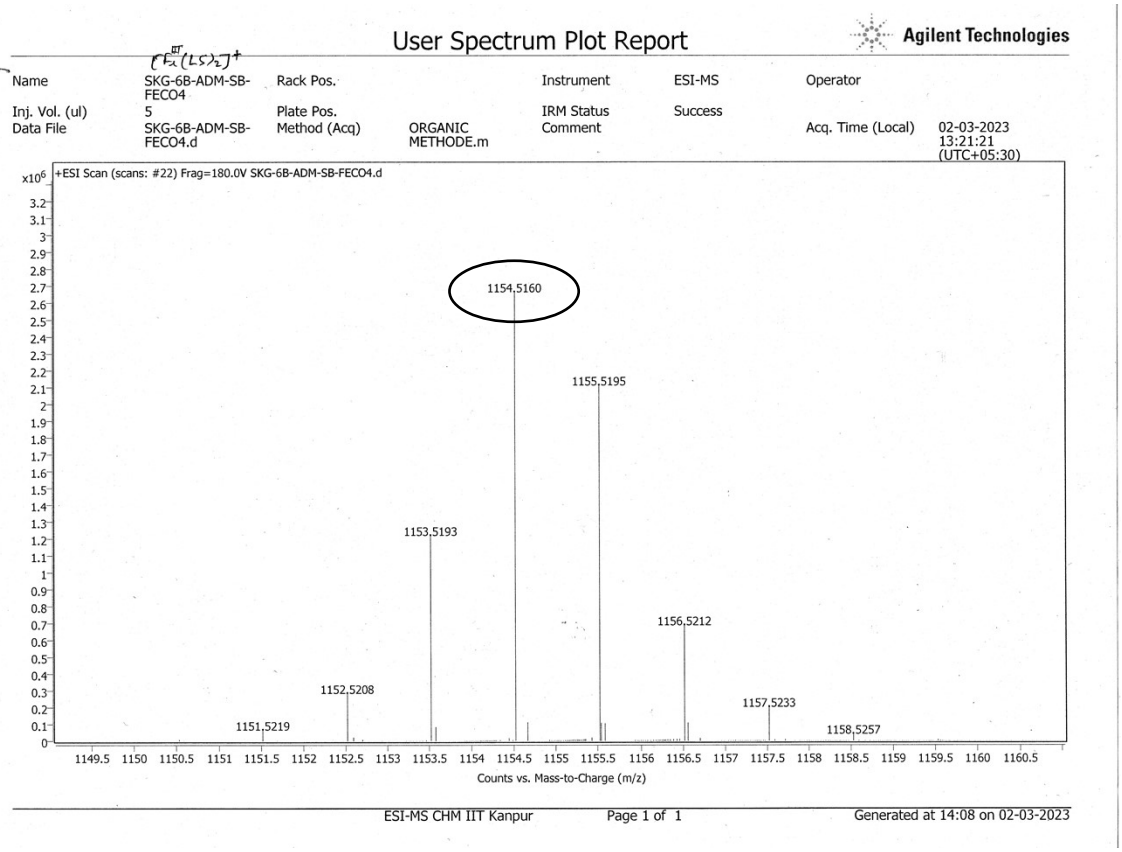


Fig S11. ESI-MS data of $Fe(L1)_2$-$Fe(L5)_2$

UV-Visible titration study

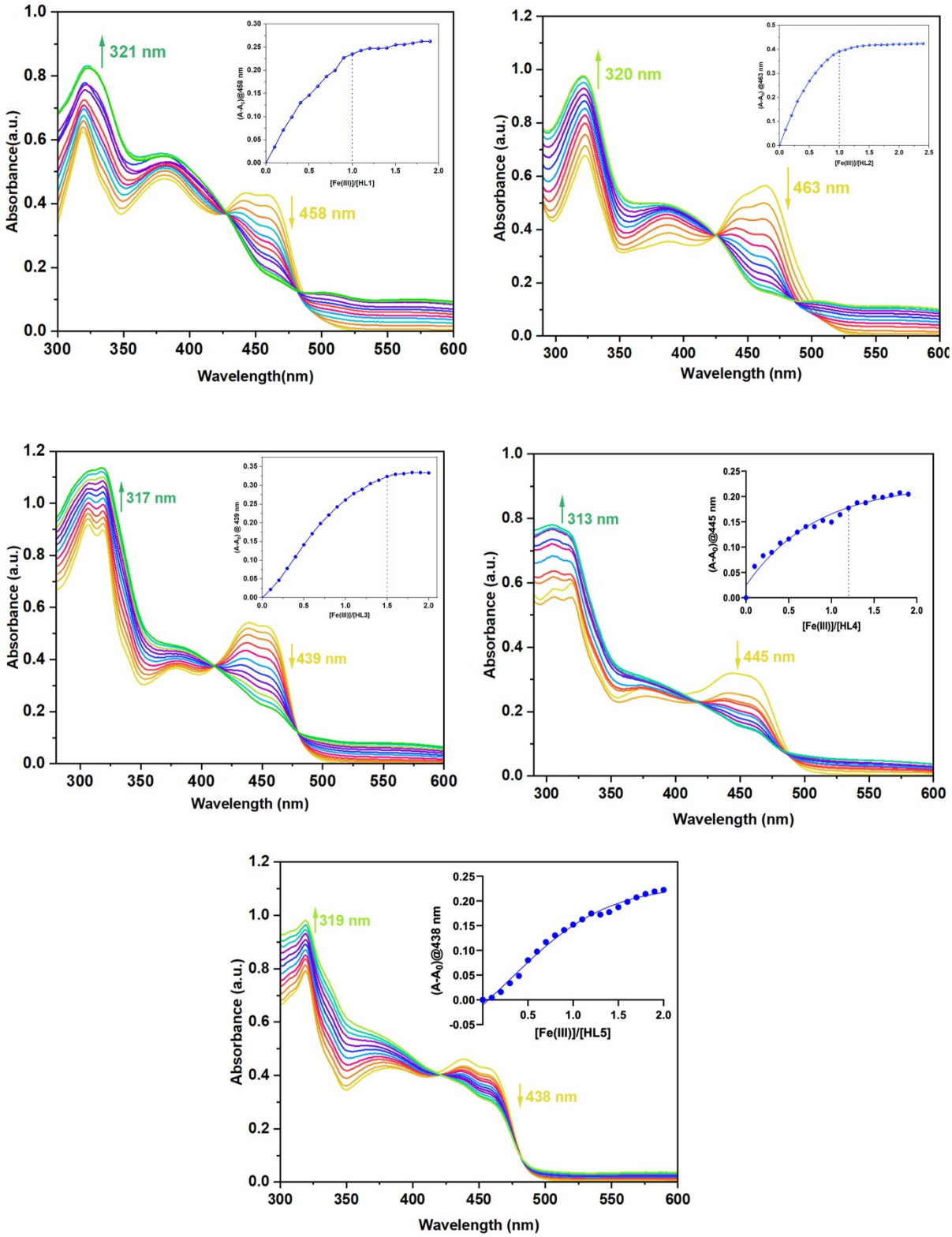
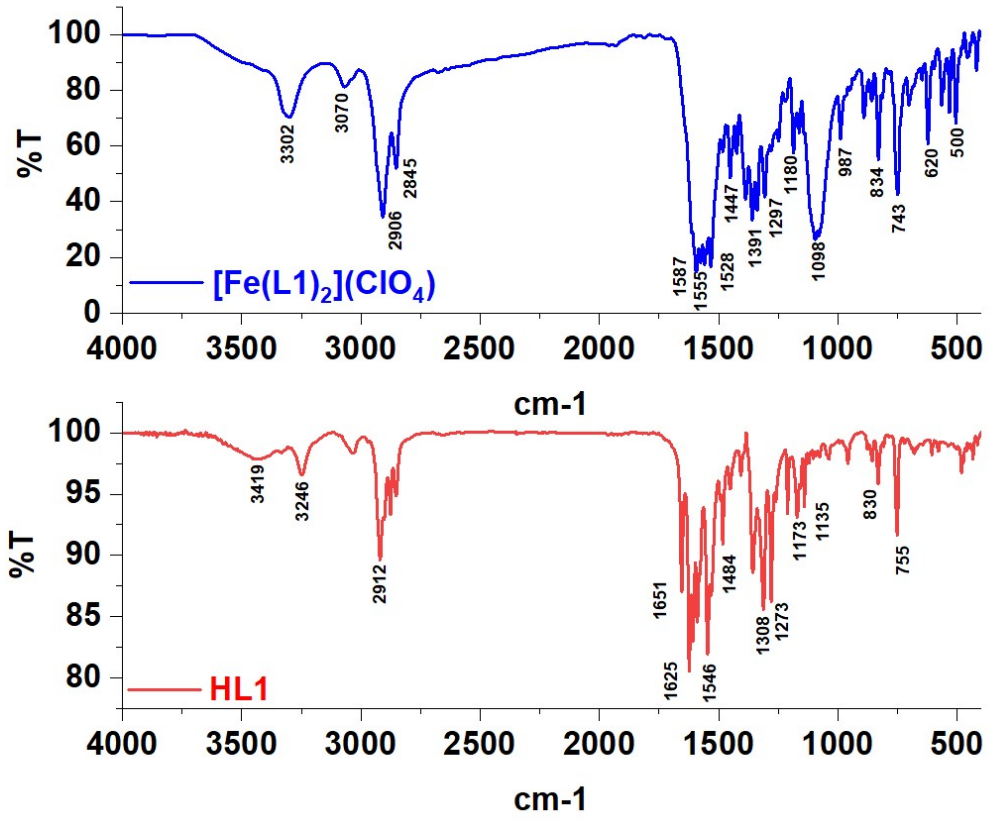
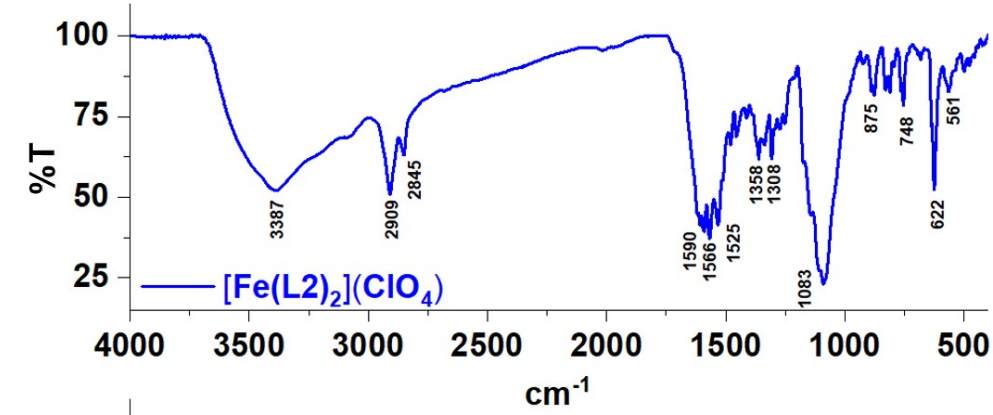
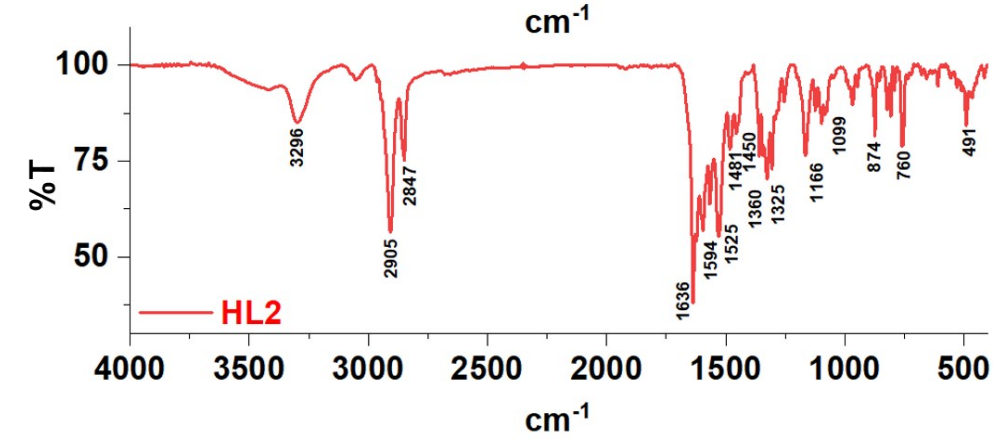


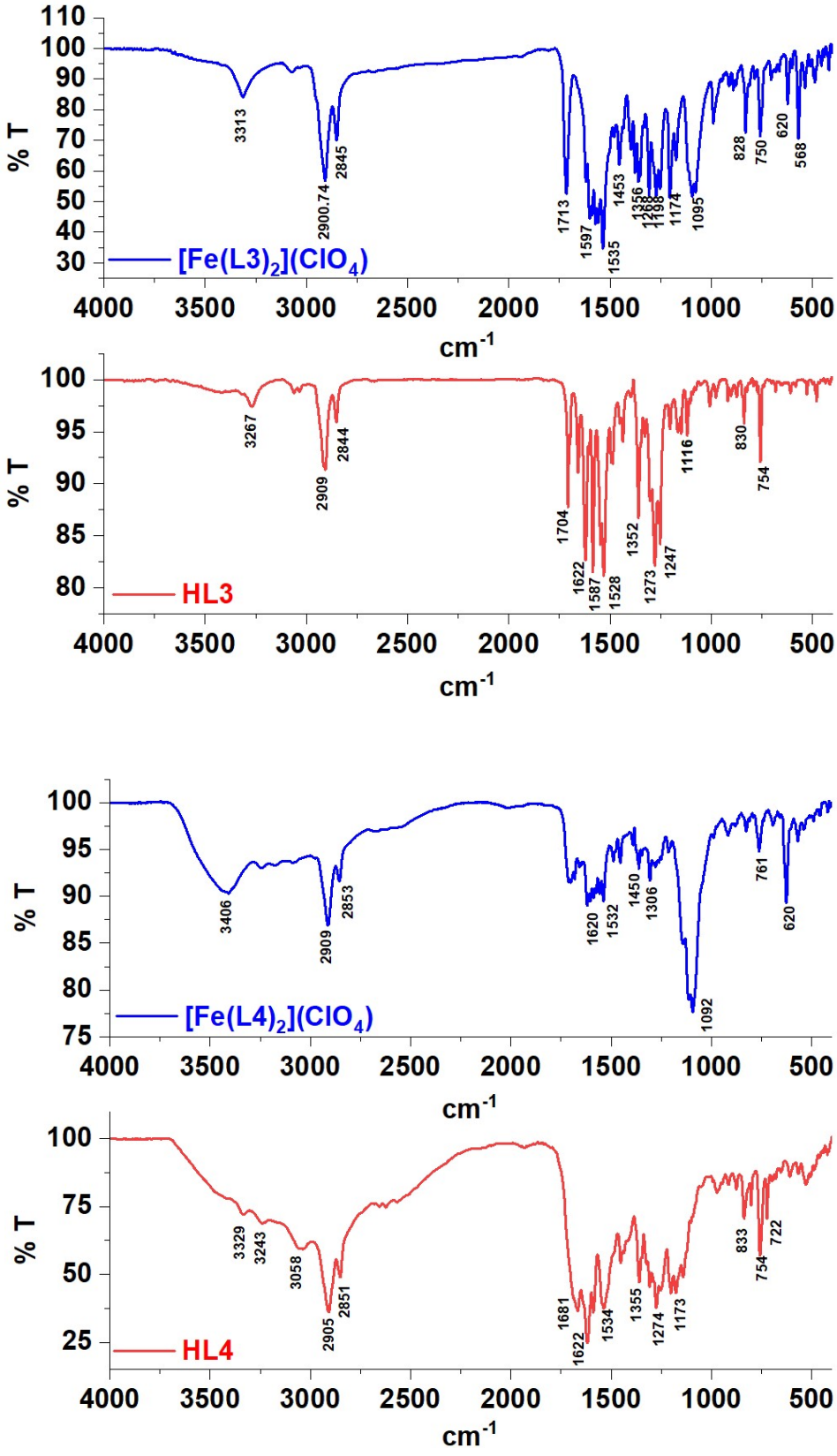
Fig S12. UV-Visible titration study of ligands HL1-HL5 with $Fe(ClO_4)_3.xH_2O$ at room temperature. Binding isotherm of Fe(III) with ligands is shown in inset images.

FT-IR data of Iron Complexes








% T
cm-1
[Fe(L3)2](ClO4)
3313
2900.74
2845
1713
1597
1535
1453
1356
1338
1198
1174
1095
828
750
620
568
HL3
3267
2909
2844
1704
1622
1587
1528
1352
1273
1247
1116
830
754
[Fe(L4)2](ClO4)
3406
2909
2853
1620
1532
1450
1306
1092
761
620
HL4
3329
3243
3058
2905
2851
1681
1622
1534
1355
1274
1173
833
754
722

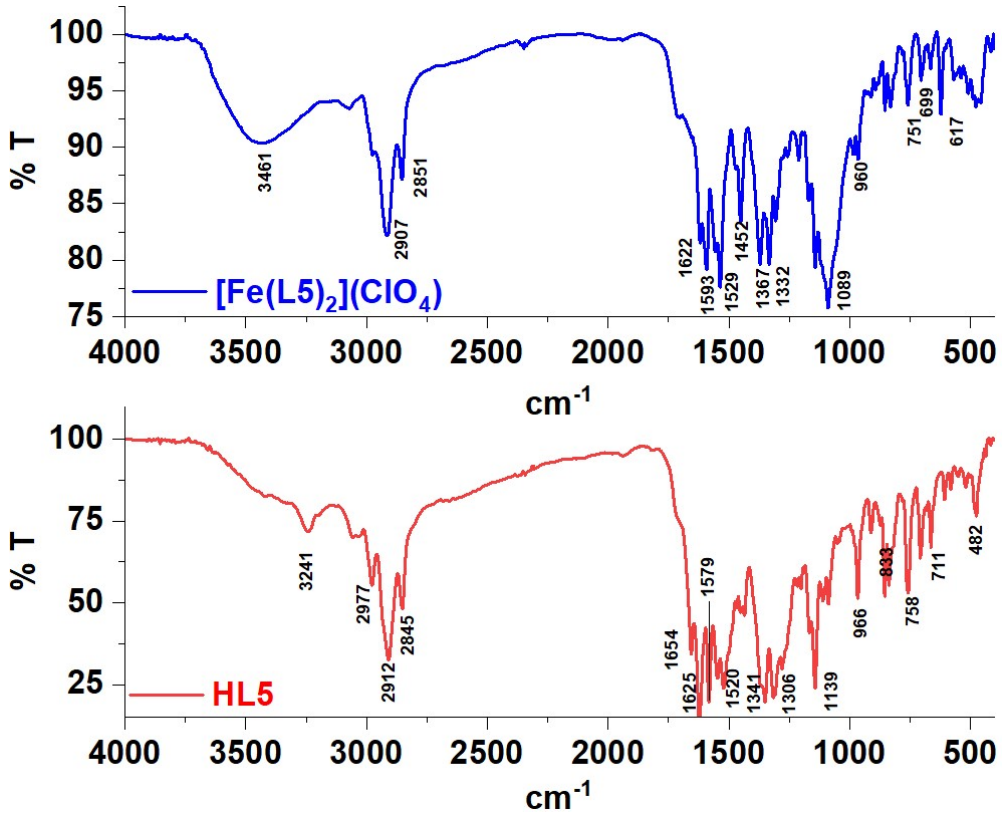
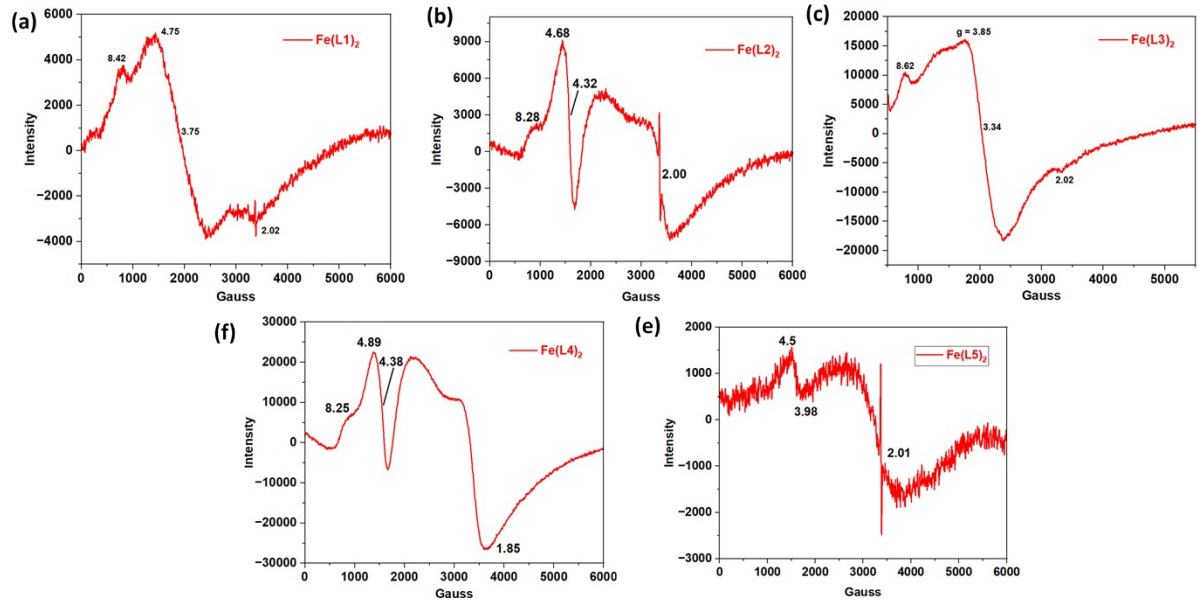


Fig S13. FT-IR spectra of ligands HL1-HL5 and its corresponding Fe(III) complexes.

EPR data



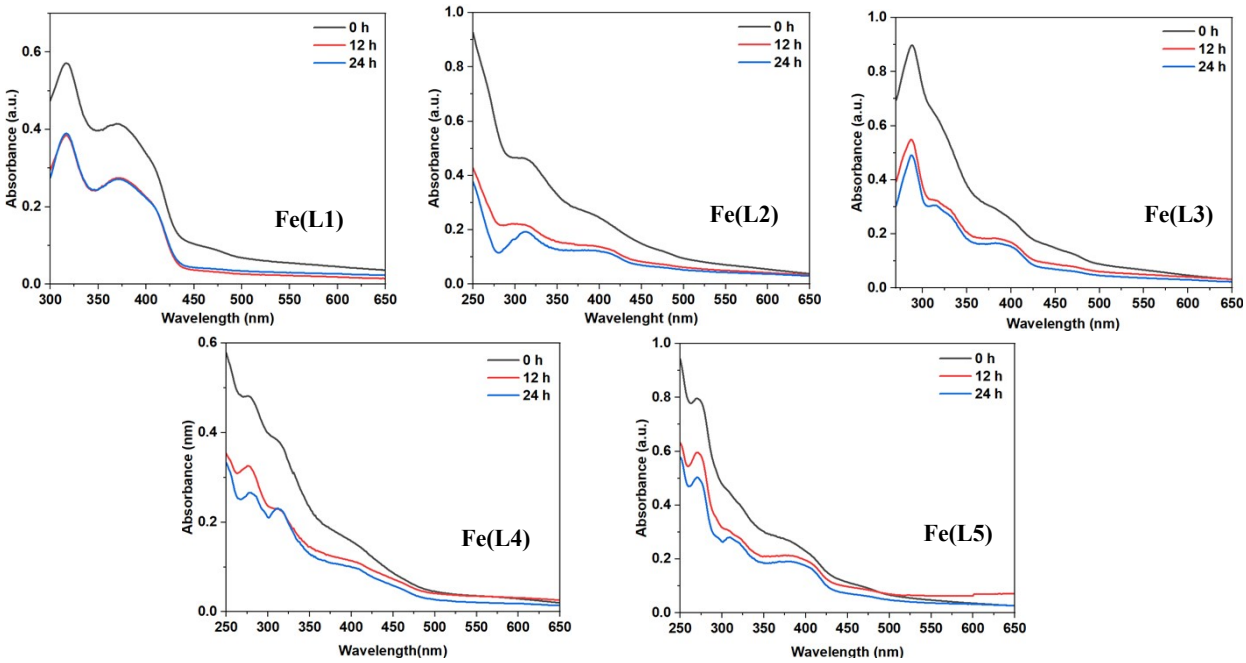
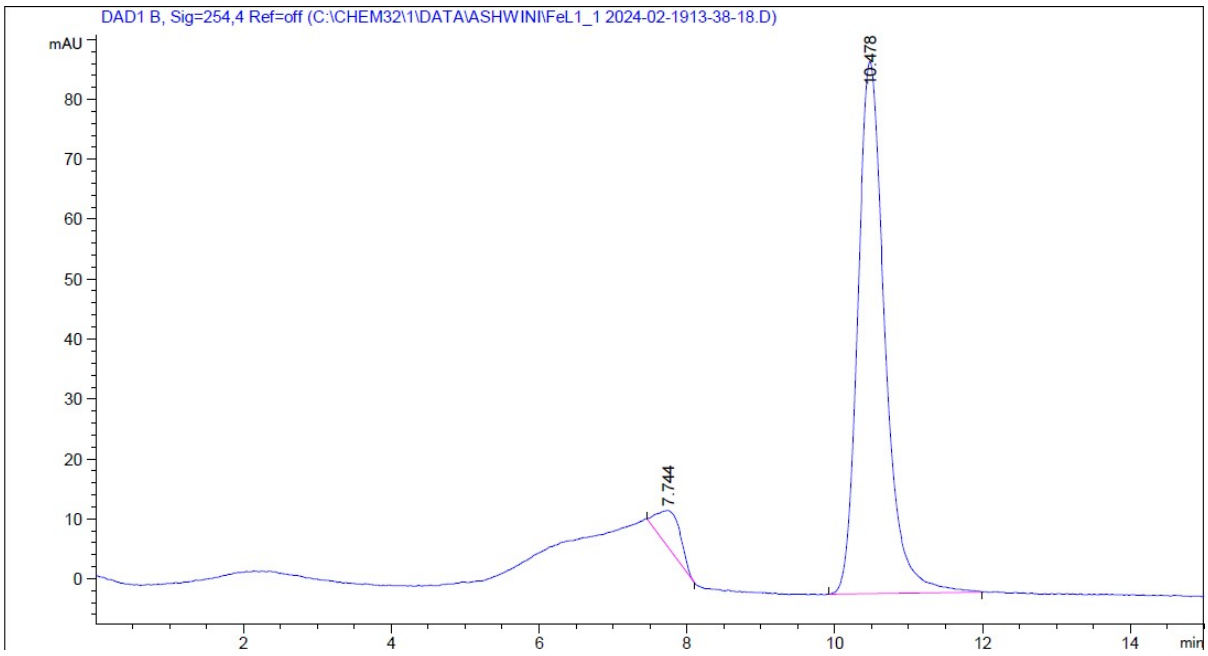


Fig S15. Stability of Iron complexes by UV-Vis absorption spectroscopy in 0.1 M HEPES buffer, recorded at 0, 12, and 24 h time duration.

HPLC analysis



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
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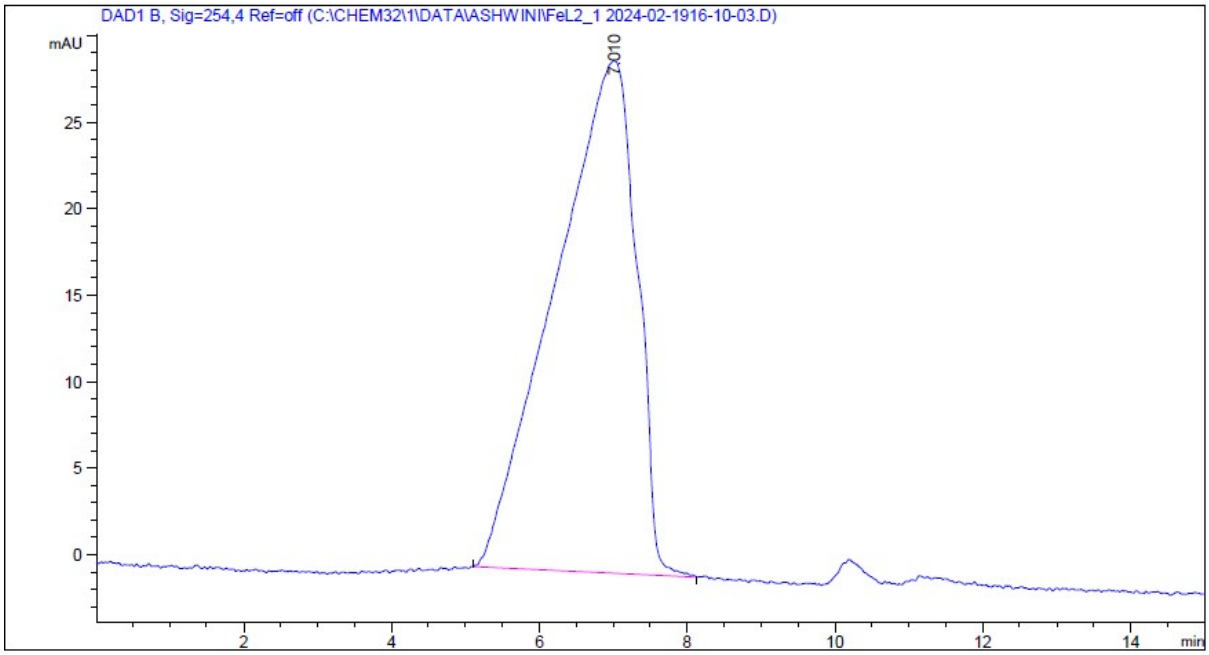
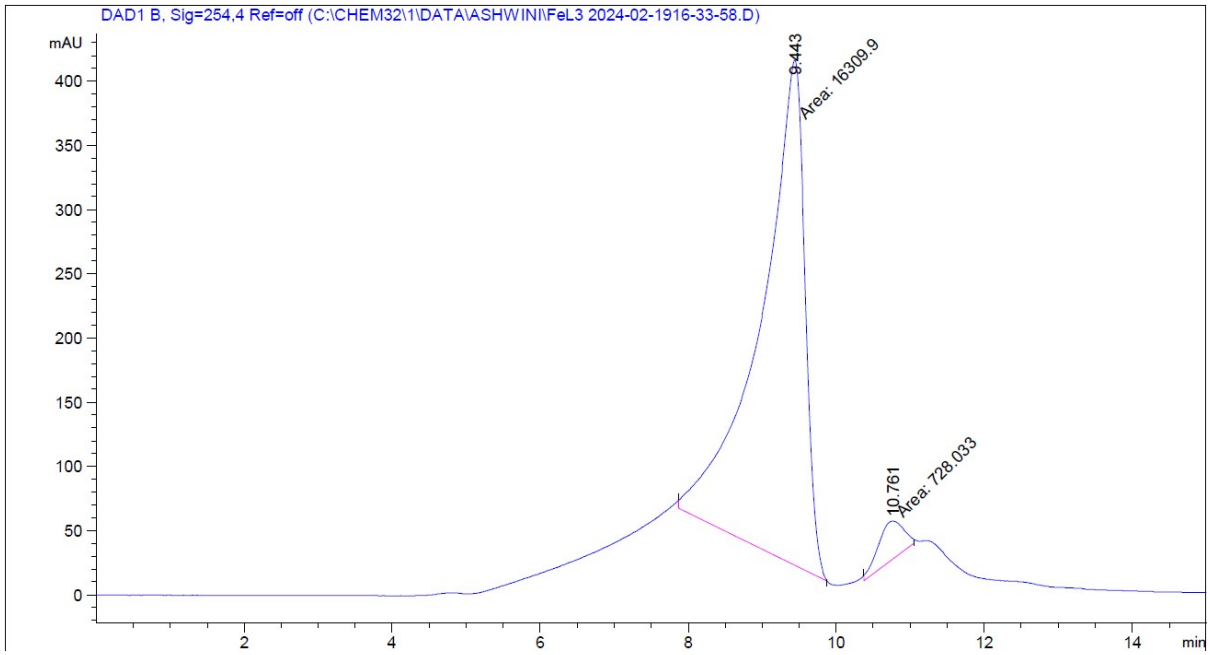
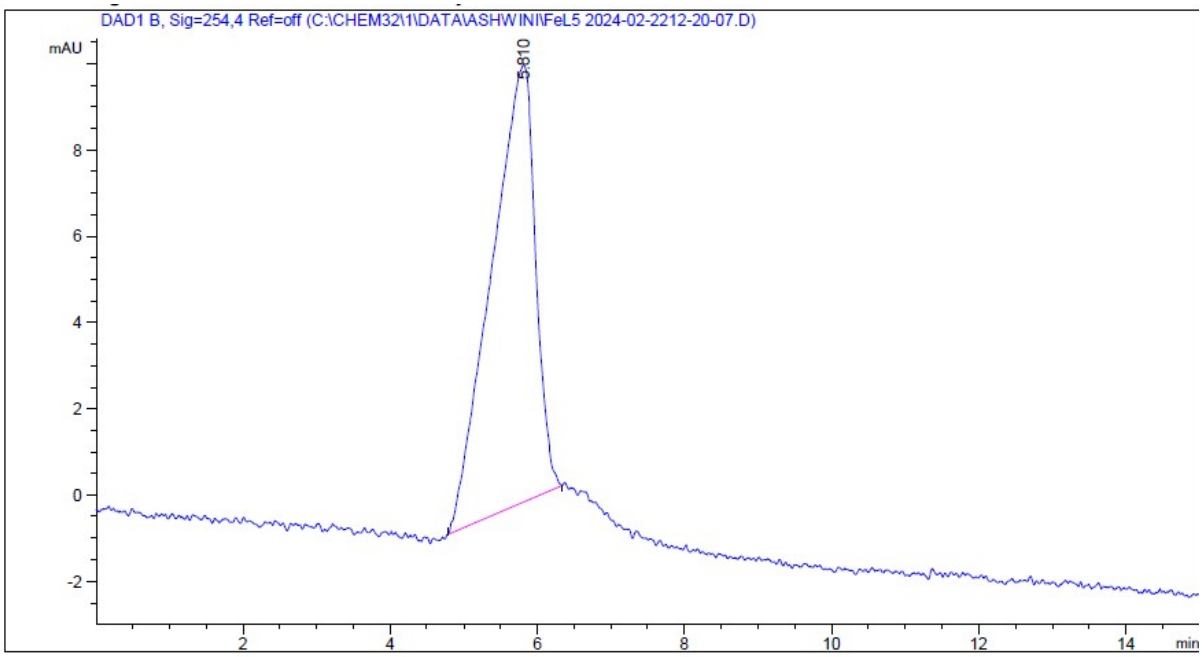
2	10.478	BB	0.3863	2255.16211	88.78318	94.2956



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.010	BB	0.9061	2269.25757	29.60982	100.0000



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.443	MM	0.6911	1.63099e4	393.34280	95.7270
2	10.761	MM	0.4046	728.03290	29.99021	4.2730



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.810	BB	0.4970	422.95221	10.13160	100.0000

Fig S16. HPLC plot of Fe(III) complexes and their retention time.

Zeta Potential.

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -30.3	Peak 1:	-30.3	100.0	4.92
Zeta Deviation (mV): 4.92	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.788	Peak 3:	0.00	0.0	0.00

Result quality : Good

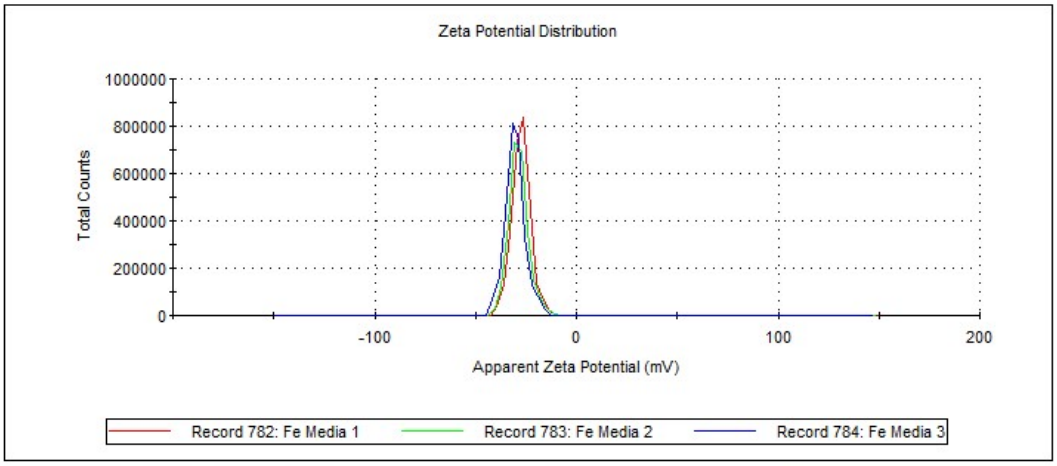


Fig S17. Zeta Potential measurement of Complex $Fe(L1)_2$.

Molecular Docking.

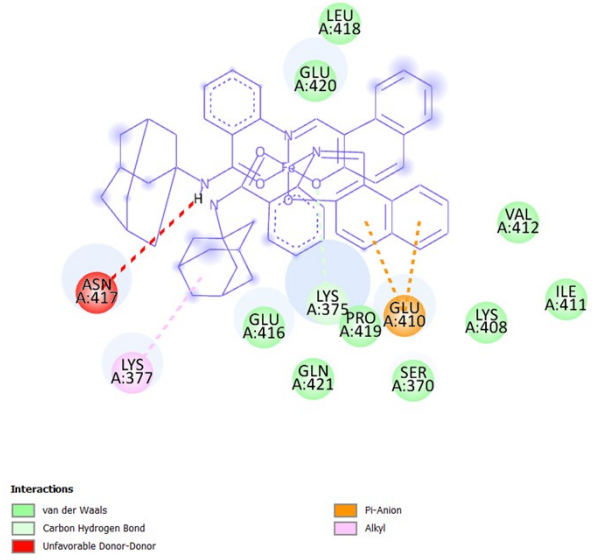


Fig S18. The 2D diagram of the SIRT1-$Fe(L1)_2$ complex interaction.

Cell Cycle Arrest.

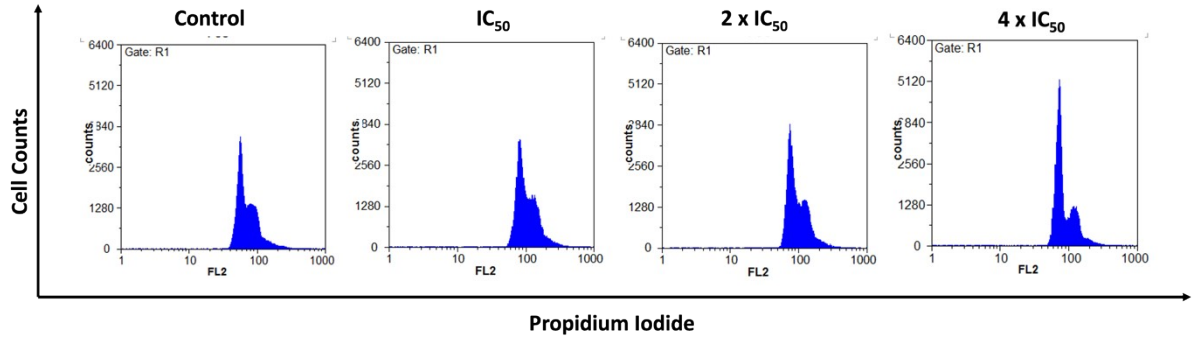


Fig S19. The cell-cycle arrest histogram, performed by flow cytometry.

Western Blotting.

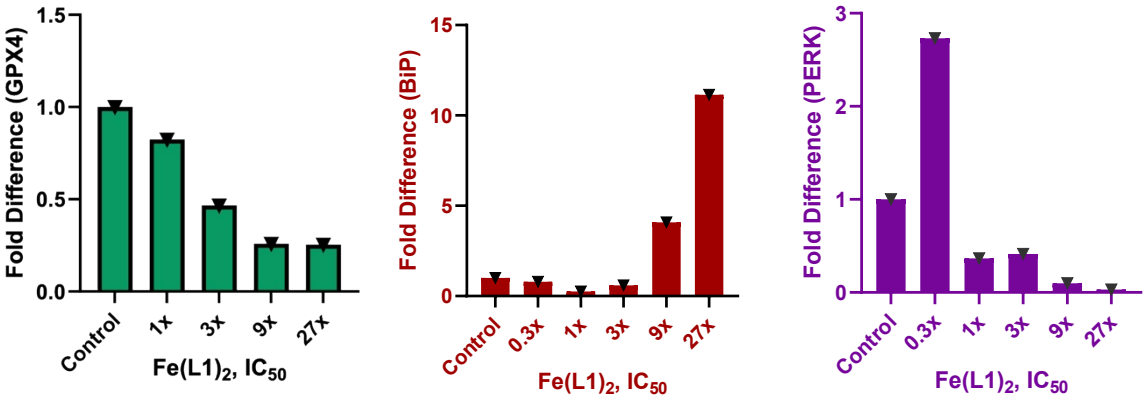


Fig S20. Fold difference of GPX4, BiP and PERK protein western blot calculated using ImageJ software.

DLS data of Fe(III)-Complexes

Fig S21. (a) and (b) are DLS data of $Fe(L1)_2$ as stability of nanoaggregation at different time such as at 0 h and 72 h. (c-f) are the DLS data of $Fe(L2\text{-}L5)_2$.

Stability of the nanoaggregates with respect to time in solution state over 72 hour

Fig S22: Dynamic Light Scattering (DLS) measurement for $Fe(L1)_2$ at 0h and 72 h

To assess the time-dependent stability of the nanoaggregates in solution, we conducted dynamic light scattering (DLS) measurements at 0 h and after 72 h. The results confirmed that the size of the synthesized iron complex nanoaggregates remained consistent over this period, indicating good stability within the nano-range.

Fig S23: Dynamic Light Scattering (DLS) measurement for $Fe(L2)_2$ $Fe(L3)_2$ $Fe(L4)_2$ and $Fe(L5)_2$ at 0h and 72 h.

Crystal Packing and Intermolecular Interactions

$Fe(L1)_2$ $Fe(L3)_2$

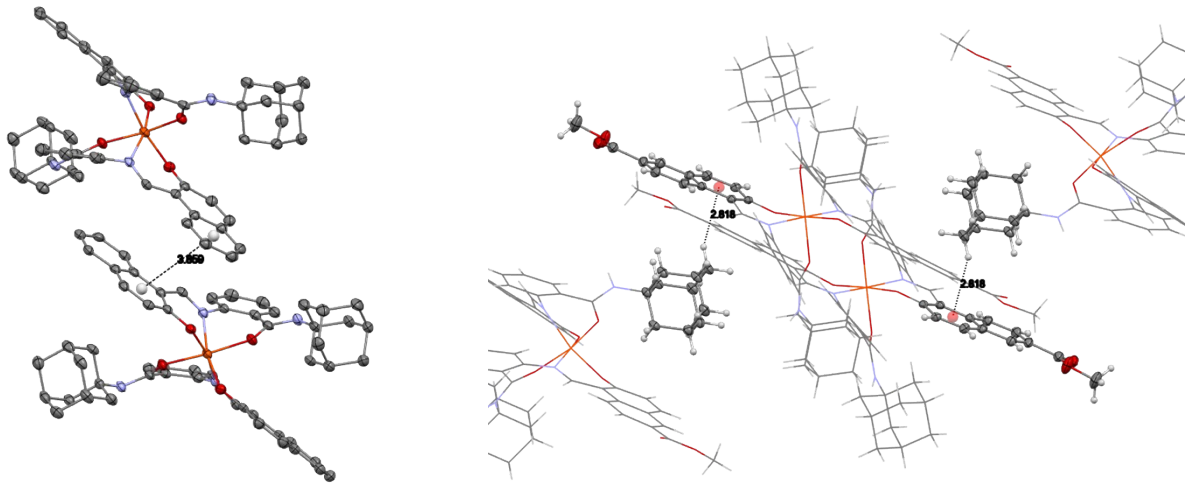


Fig S24. Crystal structure packing showing non-covalent intermolecular interactions such as π–π interaction in $Fe(L1)_2$ and σ –π interaction in $Fe(L3)_2$

References.

[1]. G. C. Senadi, V. S. Kudale, J.-J. Wang, *Green Chem.* **2019**, 21, 979.

[2]. A. Kumar, A. Chaudhary, B. Agrahari, K. Chaudhary, P. Kumar, R. G. Singh, *Chem. Commun.* **2023**, 59, 14305.

[3]. A. Kumar, A. Chaudhary, H. Sonker, S. Subhadarshini, M. K. Jolly, R. G. Singh, *ACS Org. Inorg. Au* **2024**, 4, 319.

Table S1. Crystal data and refinement parameters of Fe-Complexes.

	$Fe(L1)_2$	$Fe(L3)_2$
CCDC	**2369607**	**2369608**
Chemical formula	$C_{57}H_{55}Cl_3FeN_4O_8$	$C_{61}H_{60}Cl_3FeN_4O_{12}$
Formula weight (g/mol)	1086.25	1203.35
Temperature (K)	100	100
Wavelength (nm)	0.71073	0.71073
Crystal size	0.2 × 0.1 × 0.05	0.25 × 0.21 × 0.16
Crystal system	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/c$
Unit cell dimensions		
α (°)	90	90
β (°)	107.225(8)	103.256(6)
γ (°)	90	90
a (Å)	13.792(4)	17.160(3)
b (Å)	18.867(5)	15.966(3)
c (Å)	20.060(6)	20.287(3)
Volume	4986(2)	5410.0(18)
Z	4	4
Density (calculated)	1.447	1.476
Absorption coefficient	0.526	0.428
F(000)	2264.0	2504.0
Goodness-of-fit on F^2	1.041	1.065
Final R indices $I>2\sigma(I)$	R_1=0.0667, wR_2=0.1810	R_1=0.0690, wR_2=0.1510

Table S2: Selected bond angle and bond distance

Bond Distance (Å)	**$Fe(L1)_2$**	**$Fe(L3)_2$**
Fe1-O1	1.916(3)	1.923(2)
Fe1-O3	1.925(3)	1.901(2)
Fe1-O2	2.026(3)	2.039(2)
Fe1-O4	2.042(3)	2.038(2)
Fe1-N3	2.118(3)	2.117(3)
Fe1-N1	2.128(3)	2.105(2)
Bond Angle (˚)		
O1-Fe1-O3	99.30(12)	95.78(9)
O1-Fe1-O2	100.19(12)	103.61(9)
O1-Fe1-O4	86.59(11)	86.51(9)
O1-Fe1-N3	167.75(13)	167.22(9)
O1-Fe1-N1	85.37(12)	85.26(9)
O3-Fe1-O2	86.91(12)	86.50(9)
O3-Fe1-O4	102.48(12)	103.64(9)
O3-Fe1-N3	85.12(12)	85.53(9)
O3-Fe1-N1	166.99(12)	167.18(9)
O2-Fe1-O4	167.47(11)	165.04(9)
O2-Fe1-N3	91.41(12)	89.15(9)
O2-Fe1-N1	80.29(12)	80.84(9)
O4-Fe1-N3	81.28(12)	80.84(9)
O4-Fe1-O1	89.86(12)	86.51(9)
N3-Fe1-N1	92.79(12)	96.29(12)

Table S3. Lipophilicity of Iron complexes via octanol:water biphasic mixture. Determined by ICP-MS.

S.N.	Compounds	P = Co/Cw	LogP
1.	**$Fe(L1)_2$**	0.78	-0.11
2.	**$Fe(L2)_2$**	1.22	0.08
3.	**$Fe(L3)_2$**	2.01	0.3
4.	**$Fe(L4)_2$**	0.99	-0.004
5.	**$Fe(L5)_2$**	0.23	-0.63

Table S4. Cytotoxicity table of synthesized ligands and the corresponding iron complexes incubated for 72 h.

S.N.	Compounds	MCF-7	A549	PC-3	MDA-MB-231	4T1	HEK293
1.	HL1	17.1 ± 6.9	64.9 ±12.8	79.0 ± 9.7	60.9 ± 24.2	54.7 ± 6.7	>100
2.	HL2	18.2 ± 5.3	>100	>100	68.5 ± 10.4	64.5 ± 15.9	>100
3.	HL3	>100	>100	>100	>100	33.4 ± 1.4	>100
4.	HL4	>100	>100	81.5 ± 6.8	>100	>100	88.6 ± 19.1
5.	HL5	44.8 ± 3.8	36.4±10.9	19.3 ± 3.0	32.3 ± 2.5	31.4 ± 3.1	48.3 ± 7.1
6.	$Fe(L1)_2$	9.5 ± 5.6	9.3 ± 4.2	9.8 ± 3.9	10.6 ± 4.1	16.7 ± 5.4	24.9 ± 3.4
7.	$Fe(L2)_2$	23.0 ± 0.6	20.8 ± 5.2	30.4 ± 4.2	11.7 ± 4.8	>100	>100
8.	$Fe(L3)_2$	10.8 ± 6.5	>100	15.5 ± 3.8	14.0 ± 4.6	20.4 ± 6.2	22.0 ± 1.6
9.	$Fe(L4)_2$	73.0 ± 18.2	>100	76.1±10.0	66.8 ± 22.3	>100	58.5 ± 14.4
10.	$Fe(L5)_2$	16.4 ± 6.1	17.8 ± 4.5	18.5 ± 7.4	48.7 ± 10.0	19.90 ± 6.4	67.5 ± 8.6
11.	Cisplatin	6.4 ± 1.5	4.1 ± 0.1	3.1 ± 0.2	1.9 ± 0.1	3.3 ± 0.2	4.3 ± 0.1