Supplementary Information (SI) for Dalton Transactions.

Electronic Supporting Information

for

Photoactivated Antibacterial Activity of Fe(III) Complexes via Singlet Oxygen-Mediated Bacterial Membrane Lysis†

Ishwar Singh,[a] Rohit Rai,[b] Rajesh Kushwaha,[a] Prodyut Dhar,*[b] Samya Banerjee*[a]

[a] *Department of Chemistry, Indian Institute of Technology (BHU), Varanasi, Uttar Pradesh 221005, India. E-mail: samya.chy@itbhu.ac.in*

[b] *School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi, Varanasi, Uttar Pradesh, India 221005. E-mail: prodyut.bce@iitbhu.ac.in*

Table of contents

Tables

Schemes

Figures

1. Materials

Iron(III) chloride was purchased from SRL Chemicals, India. Dipicolyalamine (dpa), N, N-dimethylaminomethylferrocene, methylene blue, and 9,10-diphenyl anthracene were purchased from TCI (Tokyo Chemical Industry Co. Ltd.,) (Japan). Triethyl-amine was purchased from sigma chemical (USA). 2-Hydroxybenzaldehyde, and 2-aminophenol were purchased from BLD Pharma Pvt. Ltd., (China). 2-Hydroxynaphthaldehyde was purchased from Merck Specialties Pvt. Ltd. DPBF was purchased from Sigma Aldrich, USA. Methanol was purchased from Merck Life Science Private Limited. DMSO was purchased from SD-Fine Chem Limited (India). Nutrient broth and agar were purchased from Himedia, India. *E. coli* and *S. aureus* were employed as typical Gram-negative and Gram-positive bacteria, respectively, to study the antibacterial activity.

2. Instruments

Agilent Cary 60 UV-Vis. spectrophotometer was used to record the absorption spectra. The PerkinElmer spectrum-2 FT-IR instrument was used to record the IR data. For the HRMS data, maXis impact was used. For the incubation of bacterial cultures, an incubator from Amaze Instruments, India, was used.

3. Methods

3.1 UV-Vis. spectroscopy

UV-Vis. analyses of the complexes (**Fe1-Fe4)** were carried out in a 1 cm quartz cuvette and the obtained data were processed using Origin 2019b 64-bit software. The UV-Vis. spectra of **Fe1-Fe4** were recorded at ambient temperature from 800 nm-200 nm.

3.2 Single Crystal X-ray Crystallography

By slowly evaporating their solution in a 1:1 methanol-acetone ratio, the crystal of **Fe3** was produced. Crystal data for **Fe3** data was collected at 298 K temperature on a Rigaku Oxford diffractometer equipped with a CrysAlis CCD software package using a graphite mono- S7 chromated MoK$_{\alpha}$ (λ= 1.54184 Å) radiation source. X-ray diffraction intensities were collected, integrated, and scaled with APEX4 software. Empirical absorption correction was applied to the data using a multi-scan method with SADABS programming[1]. The structure was solved by intrinsic phasing with SHELXT.[2] and refined by full-matrix least-squares methods on F^2 using SHELXL using the ShelXle with the Olex2 interface.[3,4] All non-hydrogen atoms were refined with anisotropic displacement parameters.[5] The hydrogen atoms were introduced at a calculated position. The Mercury 3.8, Ortep, and Olex2 packages for Windows programs were used to generate molecular graphics.[6,7]

3.3 Photo-stability study

UV-Vis. spectral measurement in a time-dependent manner was used to conduct the photo-stability study. The experiment was conducted at ambient temperature in both Luria-Bertani

(LB) broth media (LB:DMSO; 99:1 v/v solution) and Nutrient broth (Nutrient broth: DMSO; 99:1 v/v solution). For the photo-stability experiment, the solution of **Fe1**-**Fe4** (80 µM) was kept under visible light (400-700 nm), and their corresponding UV-Vis. absorption spectra at different time intervals were recorded from 0 h to 4 h.

3.4 Lipophilicity measurement

The partition coefficient of **Fe3** was determined through the shake-flask method.[8-10] To prepare water-saturated octanol, 4 mL of octanol was mixed with 1 mL of water and left for 24 hours. Similarly, octanol-saturated water was prepared using the same procedure. **Fe3** was then solubilized in 2 mL of saturated octanol by adding a minimal volume of DMSO, and this solution was added to 2 mL of saturated water. The resulting mixture underwent 24 hours of shaking using a mechanical shaker, followed by settling and separation. The concentration of **Fe3** was subsequently assessed through UV-Vis. absorption spectroscopy. The following equation calculated the partition coefficient.

$$\text{Log } P_{o/w} = \log([\text{complex}]_{\text{octanol}}/[\text{complex}]_{\text{water}})$$

3.5 DFT calculation:

The density functional theory (DFT) calculations were carried out using the Gaussian 16 revision A. 03 quantum chemistry package. B3LYP function and the combinatorial basis set (LANL2DZ for Fe and 6-31g* for all other atoms) were employed for vacuum optimization.[11,12] A frequency calculation was employed to ensure local minima on the same level of theory. The frontier molecular orbitals (FMOs) were visualized using Gauss View 6.

3.6 Detection of singlet oxygen ($^1O_2$) generation:

The production of light-induced singlet oxygen ($^1O_2$) in solution was detected using 1,3-diphenylisobenzofuran (DPBF) as a $^1O_2$ probe. Complexes **Fe1**-**Fe4** and $[Ru(bpy)_3]Cl_2$ (10 µM) were mixed with DPBF (50 µM) in PBS-DMSO (99:1 v/v) solution. The reaction mixture was taken in a quartz cuvette, and the absorbance of DPBF was monitored by UV-Vis. spectroscopy at ambient temperature under visible light (Light source: 400-700 nm, 10 J cm^{-2}) irradiation for different time intervals.[13-15]

The $^1O_2$ generation quantum yield (Φ_Δ) was calculated by monitoring changes in UV-Vis. spectra maxima of DPBF in the presence of **Fe1**-**Fe4** and $[Ru(bpy)_3]Cl_2$. For quantum yield determination, $[Ru(bpy)_3]Cl_2$ ($\Phi_\Delta = 0.22$) was used as a standard. The singlet oxygen quantum yield values (Φ_Δ) were obtained by using the following equation:

$$\Phi_{\Delta X} = \Phi_{\Delta S} \times (S_X/S_S) \times (F_S/F_X) \text{ .... (2)}$$

where Φ_Δ represents $^1O_2$ generation quantum yield; subscripts x and s designate the sample and $[Ru(bpy)_3]Cl_2$, respectively; S stands for the slope of plot matching difference value of absorbance against the irradiation time (s); F stands for the absorption correction factor, which is calculated by $F = 1\text{-}10^{-OD}$ (OD represents the optical density of samples).[14]

This experiment was similarly conducted with another $^1O_2$ probe, i.e., 9,10-diphenyl anthracene (DPA), to validate the $^1O_2$ generation capacity of **Fe3**. Briefly, **Fe3** (10 µM) was mixed with DPA (50 µM) in PBS-DMSO (99:1 v/v) solution. The reaction mixture was taken in a quartz cuvette, and the absorbance of DPA was monitored by UV-Vis. spectroscopy at ambient temperature under visible light (Light source: 400-700 nm, 10 J cm^{-2}) irradiation for different time intervals.[13-15]

3.7 Detection of $^•$OH generation

The production of hydroxyl radical ($^•$OH) in solution was detected using Methylene Blue (MB) as an $^•$OH probe. The reaction between **Fe1-Fe4** (2 µM) methylene blue (MB) (10 µM) in H_2O-DMSO (99:1 v/v) solution was monitored by UV-vis spectroscopy after different durations of visible light (Light source: 400-700 nm, 10 J cm^{-2}) irradiation.

3.8 Antibacterial study

To determine the antibacterial activity of complexes **Fe1-Fe4** against gram-positive and gram-negative bacteria, a fresh culture of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) was inoculated in the nutrient broth and incubated for 24 hours (Amaze Instruments, India) for the assessment of antibacterial activity. The growth in the culture tubes can be observed by a turbidity change (0.6 O. D.), which is then plated to nutrient agar (NA), (100 µL). After 20 minutes of incubation of agar plates at 37 °C, three concentrations (2, 1, and 0.5 µg/mL) of Fe(III) complexes (**Fe1**-**Fe4**) were added to NA plates (5 µL), and exposed to the visible light source (400-700 nm, 10 J cm^{-2}) for 15 minutes. The plates were incubated at 37 °C for the next 16 hours. Thereafter, the inhibition of bacterial growth was determined to confirm the antibacterial activity of **Fe1**-**Fe4** on nutrient agar plates.

3.9 Minimum inhibitory concentration (MIC) determination

For the determination of minimum inhibitory concentration (MIC) of complexes (**Fe1**-**Fe4**) against gram-positive and gram-negative bacteria, overnight grown cultures of *S. aureus* (gram positive) and *E. coli* (gram negative) (0.6 optical density (O.D), 100 µL) were suspended aseptically in a 10 mL fresh nutrient broth cultures. The bacteria were allowed to grow for 30 minutes at 37 °C, after which, the synthesized complexes (**Fe1**-**Fe4**) were added aseptically to the tubes with inoculated bacteria, at varying concentrations.[12,13] The concentration range of the complexes, **Fe1**-**Fe4**, was varied from 0.05 µg/mL - 2 µg/mL, and 1% of each concentration was added to the tubes. For light-treated groups, a visible light (400-700 nm, 10 J cm^{-2}) was exposed for 15 minutes. The bacteria with the inoculated compound were further incubated for 16 hours at 37 °C for growth. The grown bacteria were then plated in nutrient agar (NA) by taking 100 µL volume from the culture tubes and incubated for 24 hours at 37 °C. The plates showing no growth after 24 hours of incubation were considered for the MIC of the respective complexes.

3.10 Membrane lysis studies

The complexes' effect on the cell membrane integrity was measured with the A_{260} value of the intracellular material that gets released upon interaction with the complexes.[16,17] An inoculum of gram-negative (*E. coli*) and gram-positive (*S. aureus*) was produced by transferring a loopful of culture from the nutrient agar plate to the nutrient broth and incubated at 37 °C for 12 hours. The culture was pelleted by centrifugation at 8000 rpm for 8 minutes, and subsequently washed and resuspended in 0.01 M phosphate buffer solution. The absorbance of the solution was adjusted to 0.6 at 420 nm. **Fe1-Fe4** of 1.5 mL of different concentrations (5, 15, 25 µg/mL) were added to 1.5 mL of bacterial culture, and kept in the presence of visible light for 20 minutes. The release of intracellular material at different time intervals was recorded by measuring the absorbance at 260 nm using an Agilent Cary spectrophotometer.

3.11 Synthesis and Characterization

A previously reported approach was followed in the synthesis of the dipicolylamine derivatives (dpa and Fc-dpa) and schiff bases (L1 and L2).[18-20] 1 mmol of $FeCl_3$ (1 mmol) was dissolved in 10 mL MeOH. A methanolic solution of 1 mmol dipicolylamine (1 mmol, for **Fe1**/**Fe3**), and ferrocenyl dipicolylamine (1 mmol for **Fe2**/**Fe4**) was added slowly to the above solution of $FeCl_3$ (**Scheme S1**). The solution mixture was allowed to stir for 30 min at room temperature to get a precipitate of the precursor complex [Fe(dpa/Fc-dpa)Cl_3] that was filtered and air-dried (**Scheme S1**). In a separate RB, the Schiff based ligands (L1 for **Fe1**/**Fe4** or L2 for **Fe2**/**Fe3**) were dissolved in MeOH (10 mL) and deprotonated with triethylamine (2 mmol). The methanolic solution (10 mL) of the precursor complex ([Fe(dpa)Cl_3] for **Fe1**/**Fe3** or [Fe(Fc-dpa)Cl_3] for **Fe2/Fe4**) was added into the above solution and stirred for 3 h (**Scheme S1**). After 3 h, the reaction mixture was filtered and concentrated with the rotary evaporator up to 4 mL. An excess amount of diethyl ether was added to precipitate out the desired complexes. The complexes were recrystallized in MeOH:Toluene mixture.[19,20] For crystallization, the ClO_4 analogs of complexes were prepared. For this, the complexes dissolved in a minimum amount of EtOH, and an excess amount of aqueous $NaClO_4$ was added to replace the Cl counter anion with ClO_4 ion.

Fe1: Brown powder. 72% yield. $C_{25}H_{22}ClFeN_4O_2$ (M. W. = 501.8 g/mol) calcd: C = 59.84, H = 4.42, N = 11.17; found: C = 59.51, H = 4.12, N = 10.98, HR-MS **(**m/z for $[M]^+$**)**: calcd. 466.1092, found 466.1259. FT-IR data (cm^{-1}): 3400 (m, br), 2926 (m, sh), 2941 (m, sh), 2738 (m, sh), 2679 (s, sh), 2491 (m. sh), 1600 (s, sh), 1533 (m, sh), 1476 (s, sh), 1436 (w, br), 1382 (w, br), 1292 (w, br), 1171 (m, sh), 1147 (w, br), 1036 (m, sh), 837 (s, sh), 754 (s, sh), 541 (w br). [w, week; m, medium; s, strong; br, broad; sh, sharp]. UV-Vis. in LB media-DMSO (99:1 v/v), where λ_{max} was observed at 425 nm (ε = 7375 M^{-1} cm^{-1}).

Fe2: Brown powder. 69% yield. $C_{36}H_{32}ClFe_2N_4O_2$ (M. W. = 699.8 g/mol) calcd. C = 61.79, H = 4.61, N = 8.01; found C = 61.56, H = 4.43, N = 7.67. HR-MS (m/z for $[M]^+$: calcd. 664.1224, found 664.1185. FT-IR data (cm^{-1}): 3400 (m, br), 2975, (w, br), 2940 (m, br), 2739 (w, br), 2679 (s, sh), 2491 (m, sh), 1600 (s, sh), 1533 (m, sh), 1464 (m, br), 1434 (m ,br), 1382 (m, w), 1293 (m, w), 1149 (m, w), 1103 (w, sh), 1024 (m, sh), 837 (w, sh), 764 (s, sh), 650 (w, sh), 613 (m, sh), 542 (s, sh). [w, week; m, medium; s, strong; br, broad; sh, sharp]. UV-Vis. in LB media-DMSO (99:1 v/v), where λ_{max} was observed at 426 nm (ε = 10867 M^{-1} cm^{-1}).

Fe3: Brown powder. 86% yield. $C_{29}H_{24}ClFeN_4O_2$ (M. W. = 551.8 g/mol) calcd. C = 63.12, H = 4.38, N = 10.15 found C = 63.38, H =4.09, N = 9.92. HR-MS (m/z for $[M]^+$): calcd. 516.1249 found. 516.1238. FT-IR data (cm^{-1}): 3415 (m, br), 2936 (m, sh), 2976 (w, sh), 2601 (w, sh), 2496 (w, sh), 1595 (m, sh), 1527 (w, sh), 1473 (w, br), 1396 (w, br), 1361 (w, sh), 1283 (w, br), 1260 (w, br), 1169 (w, sh), 1104 (w, sh), 1025 (w, sh), 833 (w, sh), 754 (w, sh), 648 (w, br), 600 (w, br), 556 (w, br). [w, week; m, medium; s, strong; br, broad; sh, sharp]. UV-Vis in LB media-DMSO (99:1 v/v), where λ_{max} was observed at 400 nm (ε= 7790 M^{-1} cm^{-1}).

Fe4: Dark brown powder. 72% yield. $C_{40}H_{34}ClFe_2N_4O_2$ (M. W. = 749.9 g/mol) calcd. C = 64.07, H = 4.57, N = 7.47 found. C = 63.58, H = 4.31, N = 7.26. HR-MS (m/z for $[M]^+$) caccd. 714.1381, found 714.1375. FT-IR data (cm^{-1}): 3412 (m, br), 2976 (w, br), 2734(w, br), 2680 (w, br), 2601 (w, br), 2492 (w, br), 1595 (m, sh), 1528 (m, sh), 1472 (m, sh), 1444 (w, br), 1398 (w, br), 1362 (m, sh), 1283 (s, br), 1171 (m, br), 1105 (m, br), 1025 (m, sh), 832 (m, sh), 755 (m, sh), 649 (w, sh), 550 (w, sh), 500 (w, sh). [w, week; m, medium; s, strong; br, broad; sh, sharp]. UV-Vis in LB media-DMSO (99:1 v/v), where λ_{max} was observed at 425 nm (ε = 6390 M^{-1} cm^{-1}).

Tables

Table S1. Crystallographic Data for **Fe3**.

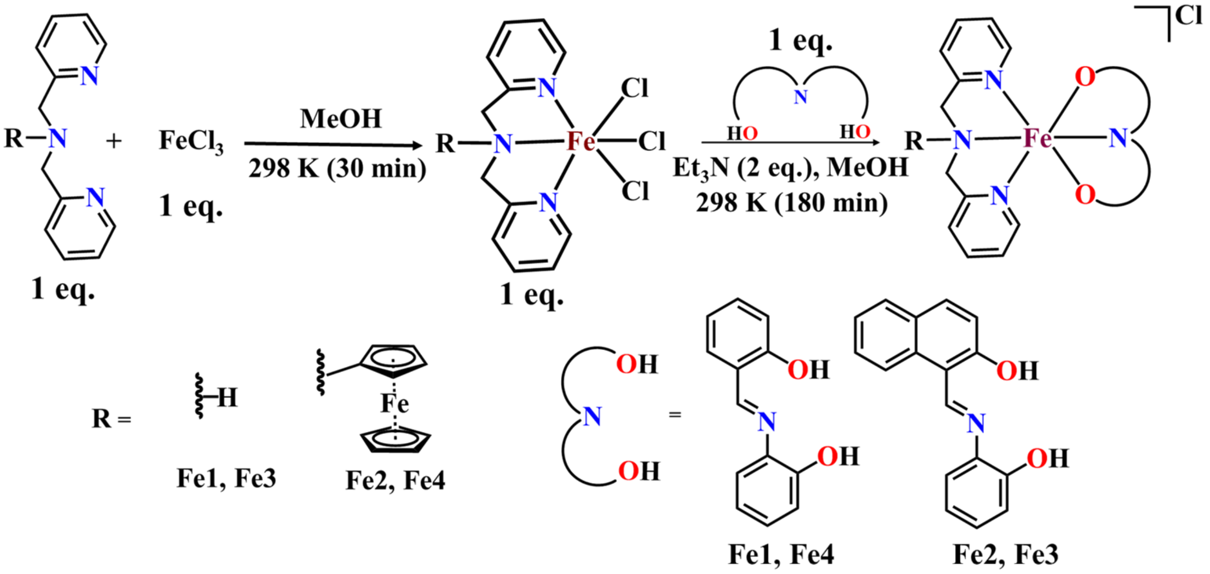
Entry	Fe3
Empirical formula	$C_{29}H_{24}ClFeN_4O_6$
Formula weight	615.82
Temperature/K	298
Crystal system	monoclinic
Space group	P1n1
a/Å	10.6073(2)
b/Å	11.5933(2)
c/Å	11.0071(2)
α/°	90
β/°	99.432(2)
γ/°	90
Volume/Å^3	1335.28(4)
Z	2
ρ_{calc}g/cm^3	1.532
μ/mm^{-1}	5.898
F(000)	634.0
Crystal size/mm^3	0.03 × 0.02 × 0.01
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	7.626 to 136.328
Index ranges	$-12 \leq h \leq 12$, $-13 \leq k \leq 13$, $-12 \leq l \leq 13$

Reflections collected	12851
Independent reflections	3455 [R_{int} = 0.0379, R_{sigma} = 0.0348]
Data/restraints/parameters	3455/2/370
Goodness-of-fit on F^2	1.056
Final R indexes [I>=2σ (I)]	R_1 = 0.0407, wR_2 = 0.1088
Final R indexes [all data]	R_1 = 0.0458, wR_2 = 0.1138
Largest diff. peak/hole / e $Å^{-3}$	0.30/-0.27
CCDC	2455812

Table S2 Selected bond distances (Å) and bond angles (°) of **Fe3**.

	Fe3
Fe-N1	2.130 (5)
Fe-N2	2.136 (4)
Fe-N3	2.120 (5)
Fe-N4	2.089 (5)
Fe-O5	1.916 (4)
Fe-O6	1.930 (5)
N1-Fe-N2	85.2 (2)
N1-Fe-N3	153.5 (2)
N2-Fe-N3	77.3 (2)
N2-Fe-N4	176.8 (2)
N4-Fe-O5	85.5 (2)
N4-Fe-O6	81.3 (2)
O5-Fe-O6	166.6 (2)
O6-Fe-N3	92.2 (2)
O6-Fe-N2	97.1 (2)
O6-Fe-N1	90.6 (2)
O5-Fe-N3	92.1 (2)
O5-Fe-N2	95.9 (2)
O5-Fe-N1	91.2 (2)

Schemes



Scheme S1. Synthetic route of **Fe1-Fe4**.

Figures

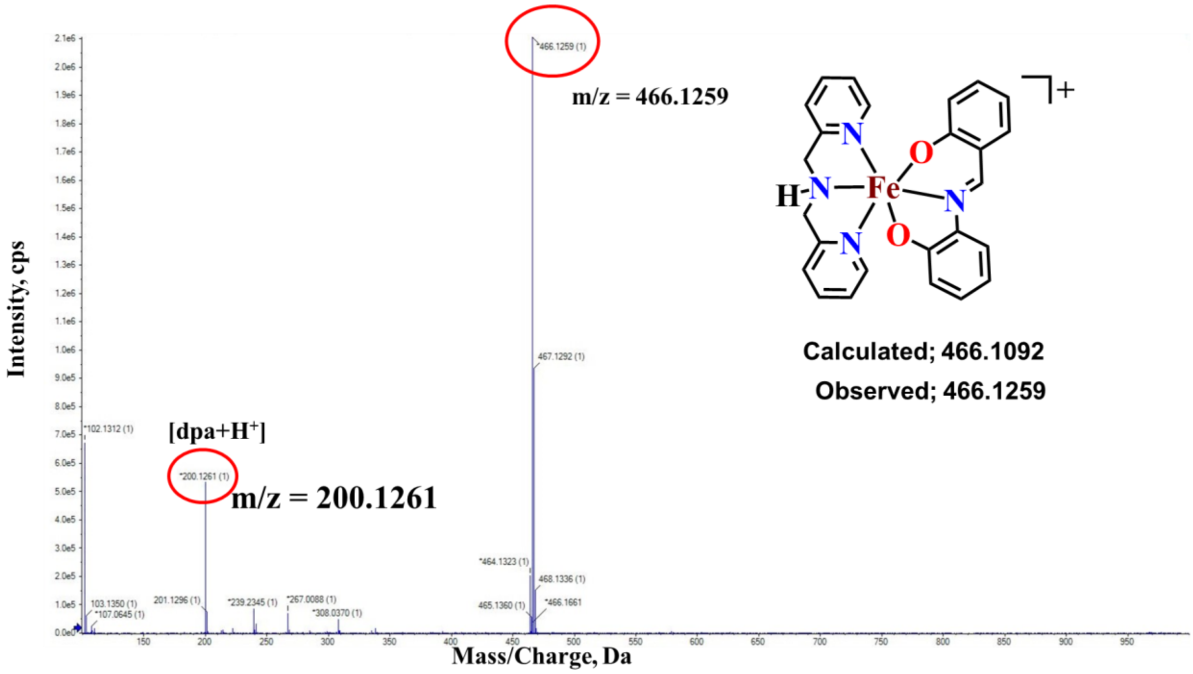


Figure S1. The HR-MS spectrum of complex **Fe1** in MeOH.

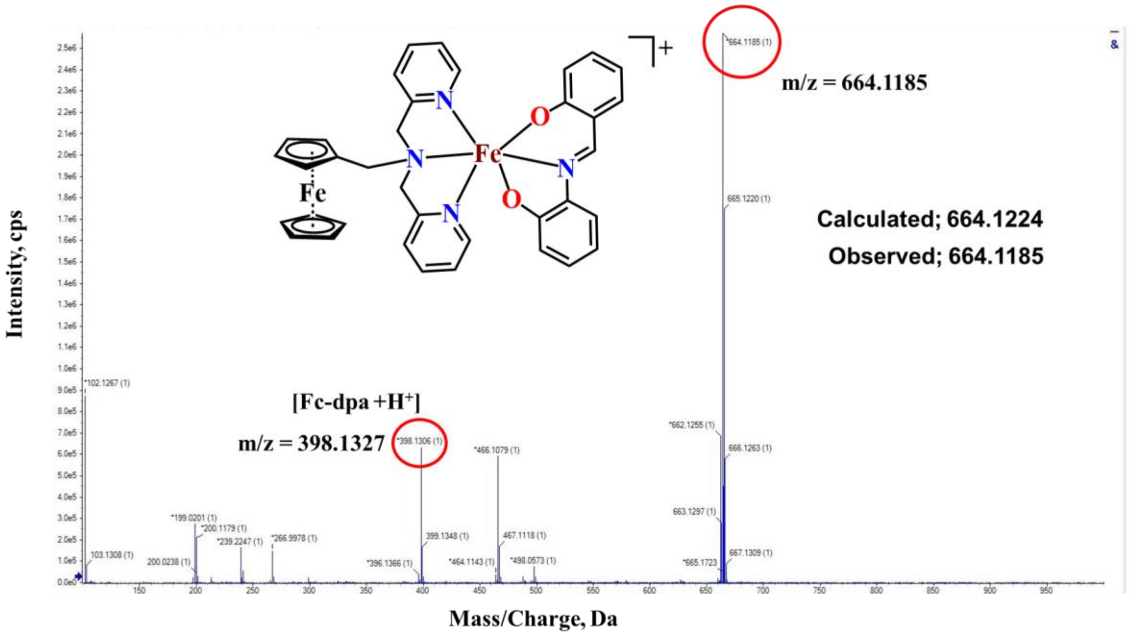


Figure S2. The HR-MS spectrum of complex **Fe2** in MeOH.

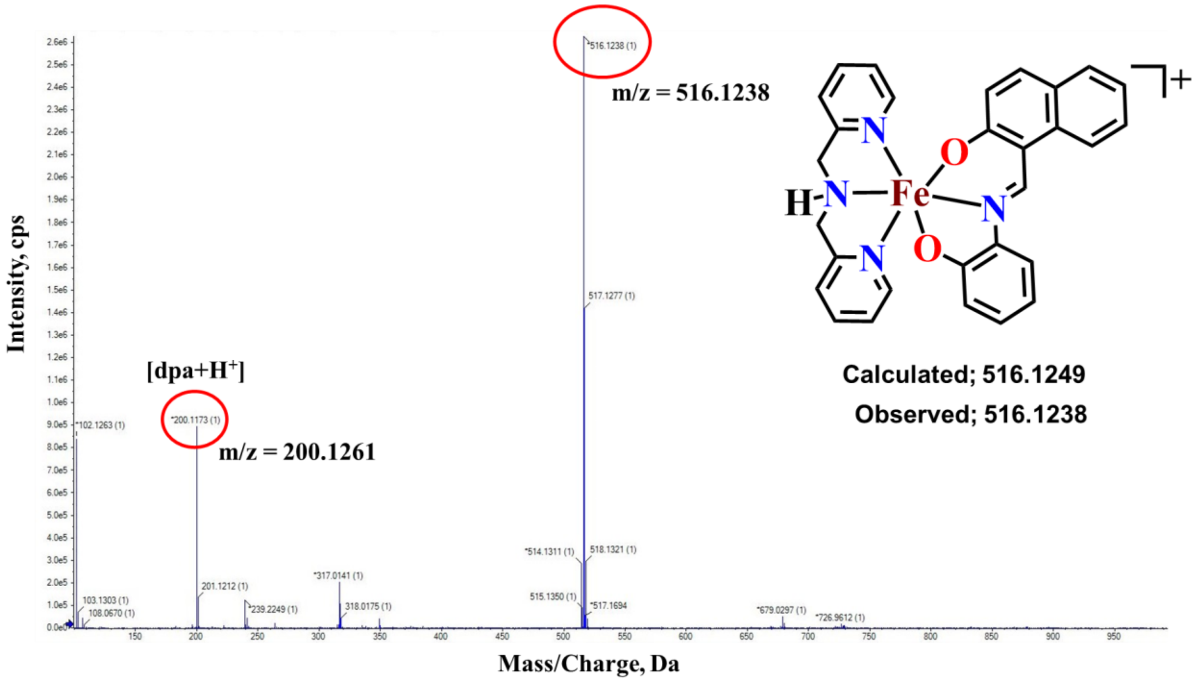


Figure S3. The HR-MS spectrum of complex **Fe3** in MeOH.

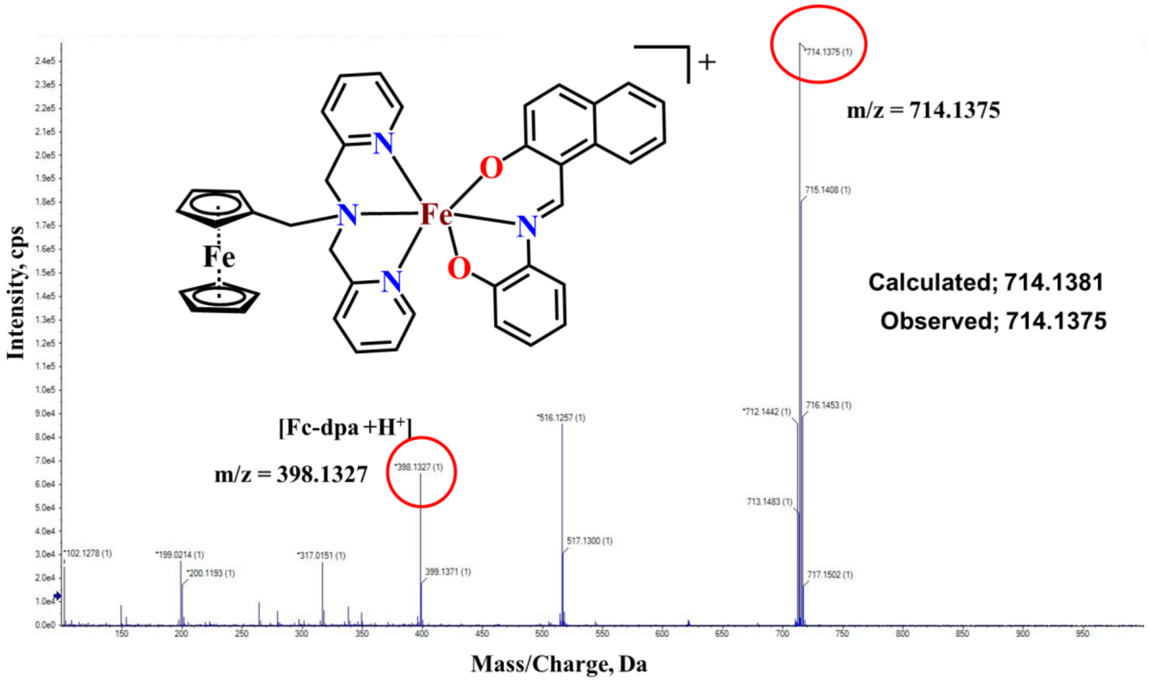


Figure S4. The HR-MS spectrum of complex **Fe4** in MeOH.

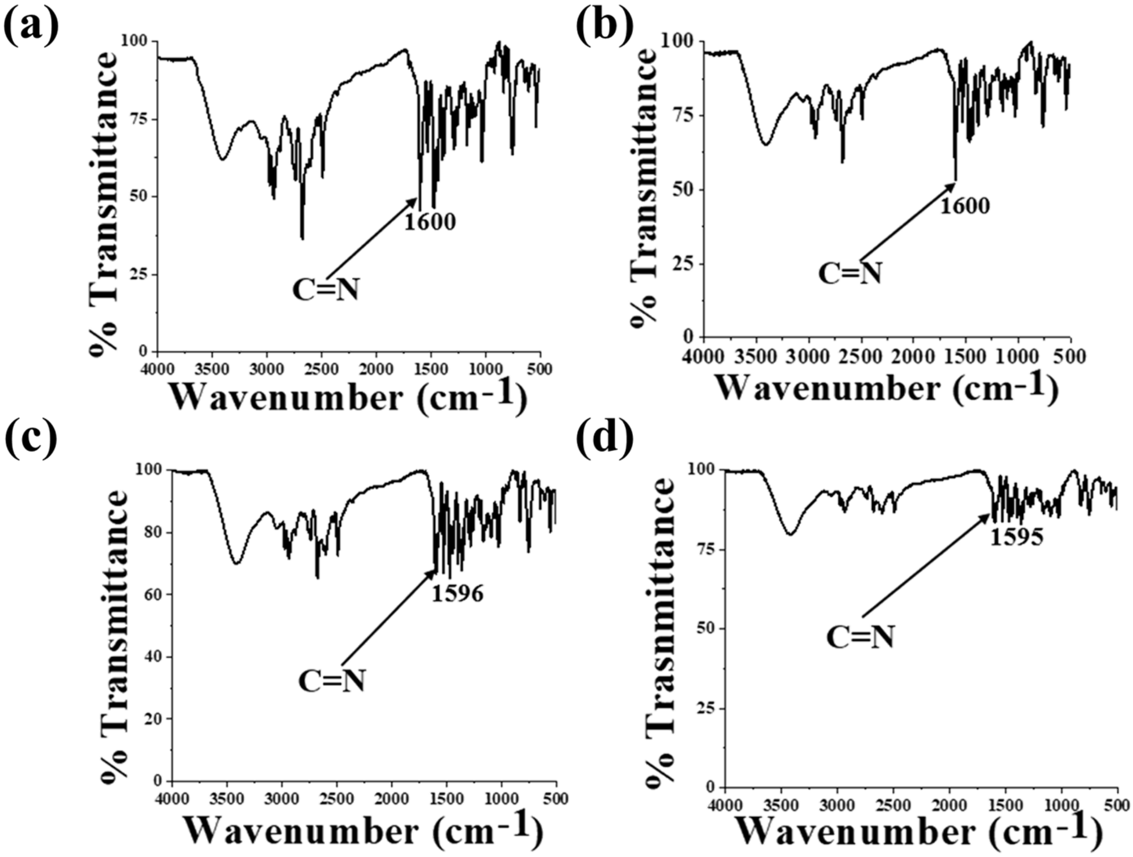


Figure S5. IR spectra of **Fe1** (a**), Fe2** (b), **Fe3** (c), and **Fe4** (d).

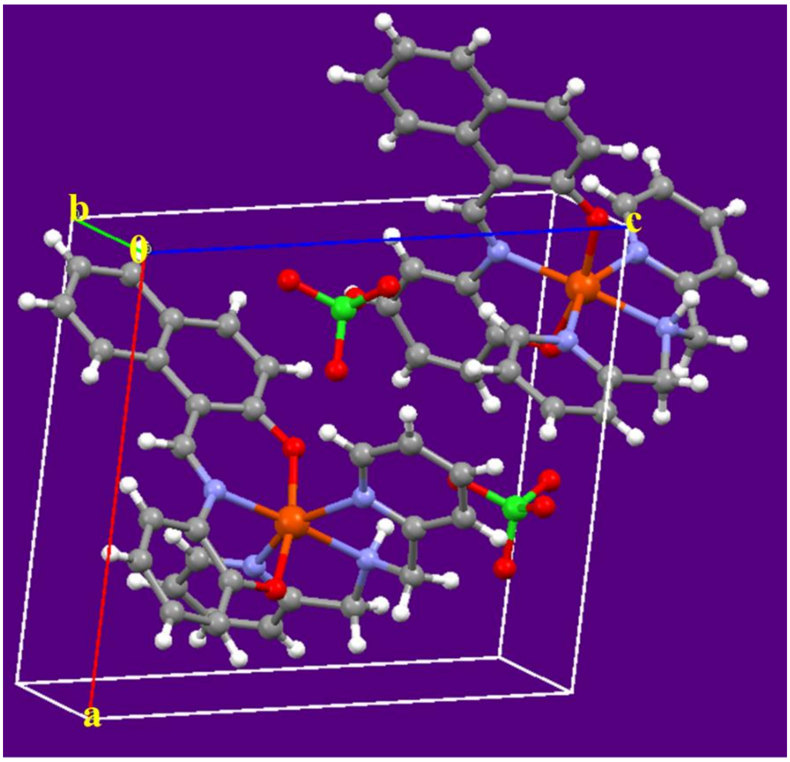


Figure S6. Unit cell packing of **Fe3**, the plot is drawn using mercury 3.8 software.

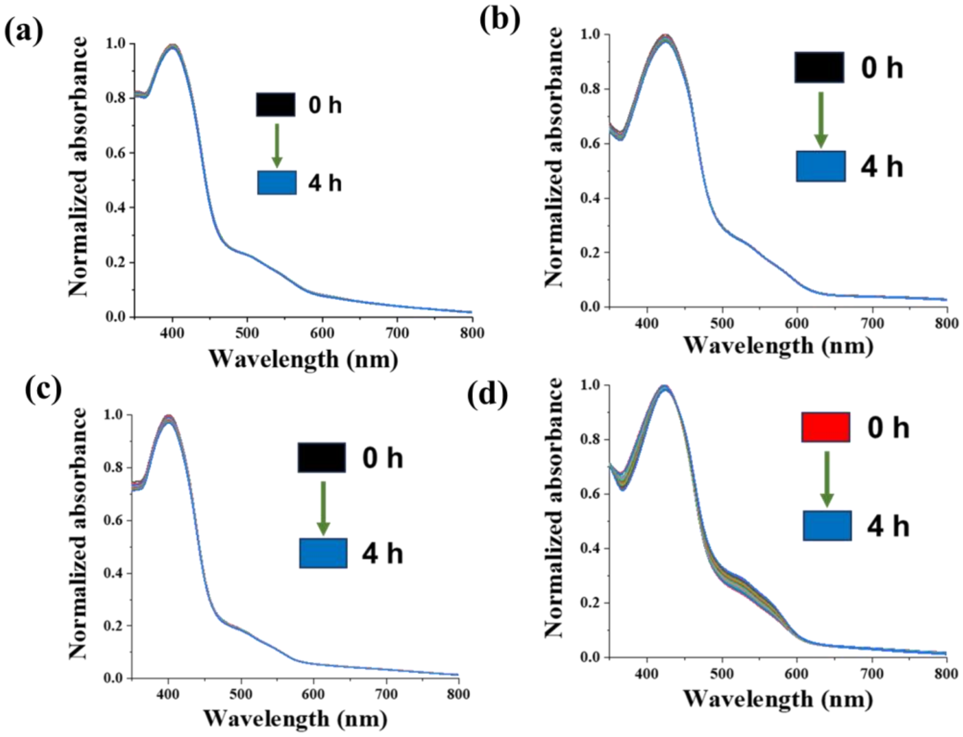


Figure S7. Photo-stability of **Fe1** (a), **Fe2** (b), **Fe3** (c), and **Fe4** (d), in LB broth-DMSO (99:1 v/v) solution, under visible light (400-700 nm, 10 J cm^{-2}) irradiation up to 4 h.

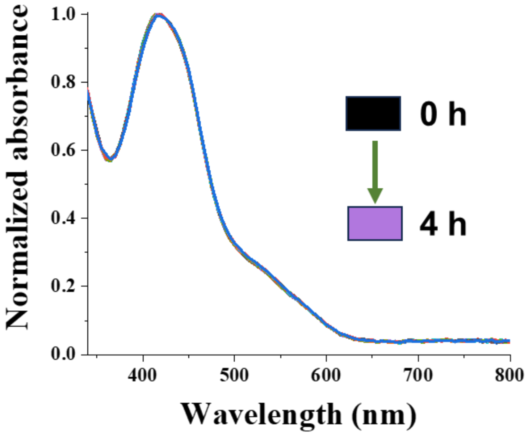


Figure S8. Photo-stability of **Fe3** in Nutrient broth-DMSO (99:1 v/v) solution, under visible light (400-700 nm, 10 J cm^{-2}) irradiation up to 4 h.

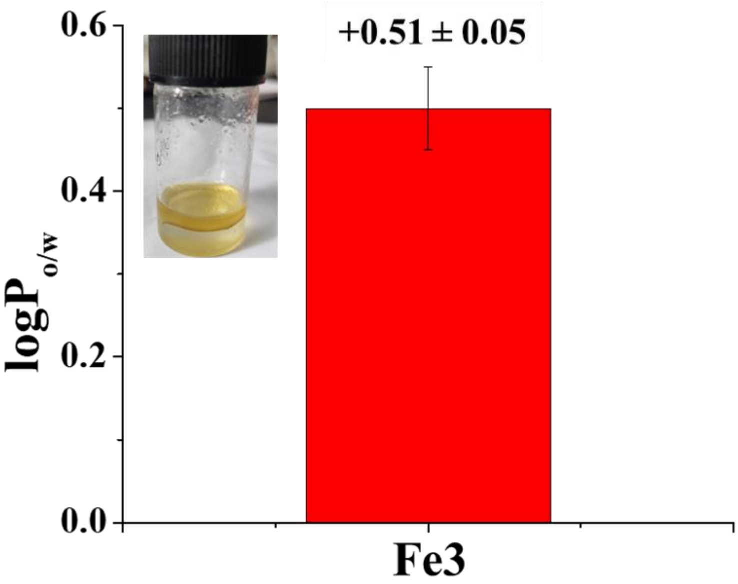


Figure S9. The distribution of **Fe3** in water and n-octanol and the corresponding Log $P_{o/w}$ value.

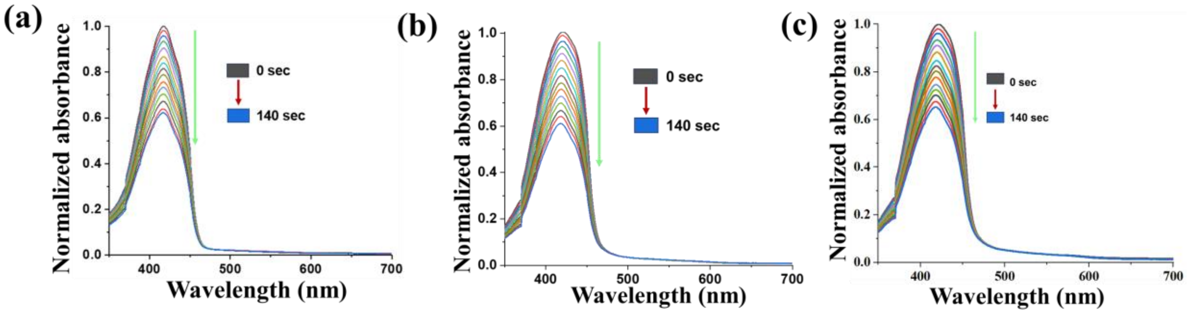


Figure S10. Absorption spectral plots showing change in the absorbance of (a) DPBF + **Fe2** (10 µM), (b) DPBF + **Fe3** (10 µM), (c) DPBF + **Fe4** (10 µM), in LB broth-DMSO (99:1 v/v) solution, (Light source: 400-700 nm, 10 J cm^{-2}).

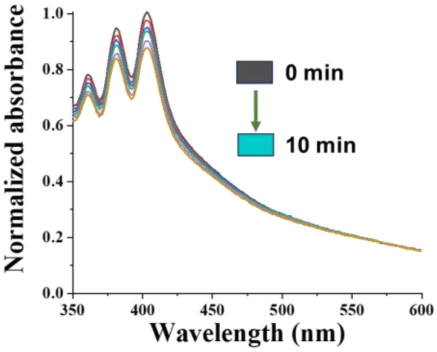


Figure S11. Time-dependent UV–vis. spectra of **Fe3** (10 µM) + DPA (diphenyl anthracene, the $^1O_2$ probe, 50 µM) under visible light (400–700 nm) exposure in PBS-DMSO (99:1 v/v) solution, indicating its $^1O_2$ generation ability.

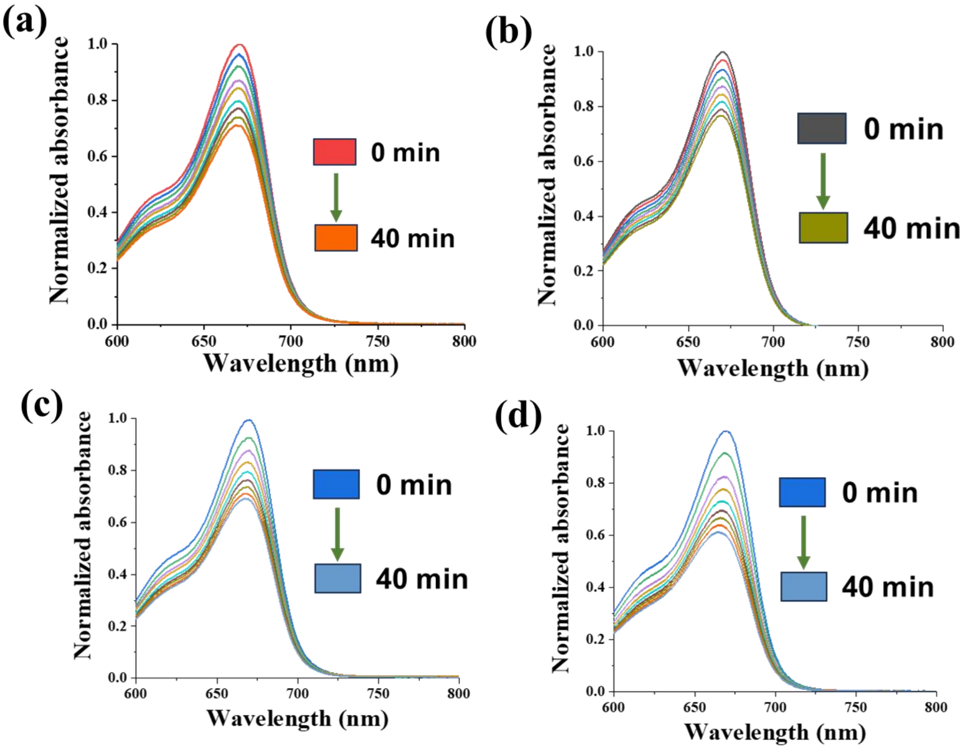


Figure S12. Absorption spectral plots showing the changes in the absorbance of, (a) MB + **Fe1** (2 µM), (b) MB+ **Fe2** (2 µM), (c) MB + **Fe3** (2 µM) (d) MB + **Fe4** (2 µM) in H_2O-DMSO (99:1 v/v) solution, under visible light irradiation (Light source: 400-700 nm, 10 Jcm^{-2}).

DFT-optimized coordinates of **Fe1**-**Fe4**

Fe1

Fe	1.65886	0.49121	4.06868
N	2.69265	1.41541	5.30389
N	1.33567	-0.68959	5.48872
N	0.48423	-0.31671	2.8206
N	2.36124	1.73453	2.85071
O	0.19827	1.59128	4.42526
O	3.08325	-0.62834	3.7274
C	2.73912	2.82754	4.92675
H	3.51977	3.34237	5.44623
H	1.80674	3.30502	5.14552
C	2.92055	2.80992	3.41345
C	3.56778	3.79053	2.66221
H	4.03767	4.62734	3.1354
C	3.57874	3.64846	1.2700
H	4.06439	4.37564	0.65342
C	2.9389	2.53684	0.69374
H	2.91963	2.42006	-0.36954
C	2.32592	1.5768	1.52132
H	1.83951	0.72592	1.09187
C	2.10091	1.2502	6.63473
H	1.22502	1.8575	6.7296
H	2.79456	1.52162	7.40283
C	1.66495	-0.21066	6.69179
C	1.58285	-0.98702	7.84752
H	1.88582	-0.59693	8.79655
C	1.08601	-2.28995	7.72815

H	1.00445	-2.92099	8.58815
C	0.69264	-2.75592	6.46111
H	0.29214	-3.74218	6.35221
C	0.83065	-1.92075	5.33627
H	0.53889	-2.26839	4.36707
C	-0.86906	1.43687	3.49295
C	-0.76342	0.3773	2.66818
C	-1.77467	0.03637	1.76754
H	-1.68782	-0.83466	1.15243
C	-2.90507	0.86476	1.68223
H	-3.69464	0.62856	0.99963
C	-2.99371	2.0103	2.49734
H	-3.84263	2.65708	2.42221
C	-1.96603	2.30059	3.41183
H	-2.02442	3.16571	4.03908
C	3.13537	-1.58339	2.65941
C	4.4001	-2.11014	2.34769
C	3.40368	-3.63329	0.7559
H	3.49531	-4.43658	0.05477
C	0.67217	-1.36833	2.10592
C	2.02842	-2.02247	1.94538
C	2.15054	-3.07878	1.02976
H	1.28115	-3.4563	0.53293
H	5.26814	-1.73283	2.84677
H	3.60924	1.01722	5.27249
C	4.5354	-3.12949	1.39856
H	5.50319	-3.52372	1.16891
H	-0.16122	-1.78444	1.57916

Fe2

Fe	1.47677	-0.23704	0.00427
N	-0.28567	-1.26427	-0.18456
N	1.27726	-0.69425	1.92973
N	3.27636	0.54038	0.11268
N	1.33297	-0.03223	-1.97737
O	2.35221	-1.8903	-0.22517
O	0.61793	1.39788	0.32483
C	-0.18888	-1.87947	-1.55549
H	-1.16547	-2.23136	-1.90038
H	0.48931	-2.73377	-1.47922
C	0.41509	-0.88634	-2.51356
C	0.12253	-0.85443	-3.87829
H	-0.61253	-1.53808	-4.28682
C	0.78832	0.06037	-4.70114
H	0.57449	0.09604	-5.76327
C	1.7271	0.92994	-4.13664
H	2.25811	1.65349	-4.74235
C	1.97345	0.85967	-2.7662
H	2.68105	1.51475	-2.27929
C	-0.22436	-2.29716	0.90895
H	0.45469	-3.08516	0.57319
H	-1.2097	-2.73302	1.09718
C	0.35913	-1.67462	2.14893
C	0.04913	-2.08351	3.44721
H	-0.68808	-2.86209	3.60519

C	0.70149	-1.48104	4.52858
H	0.47534	-1.78892	5.54307
C	1.6423	-0.47484	4.28586
H	2.16266	0.01571	5.09887
C	1.90355	-0.09949	2.96856
H	2.61163	0.67965	2.7244
C	3.71048	-1.7649	-0.24858
C	4.26529	-0.47831	-0.05041
C	5.65748	-0.30112	-0.04057
H	6.09804	0.67761	0.11788
C	6.49316	-1.40272	-0.23443
H	7.56893	-1.27003	-0.22648
C	5.94156	-2.67921	-0.43799
H	6.59712	-3.53031	-0.58819
C	4.55812	-2.86549	-0.4458
H	4.1196	-3.84496	-0.59697
C	1.16558	2.62601	0.41994
C	0.29507	3.73013	0.55801
C	2.17904	5.26632	0.67598
C	3.54745	1.81353	0.26996
C	2.57675	2.86498	0.40967
C	3.05083	4.19685	0.54298
H	-0.77084	3.53409	0.56763
C	0.79083	5.02329	0.68161
H	0.09953	5.8532	0.78568
H	4.59126	2.1275	0.28933
C	-2.83202	-1.07508	-0.17566
C	-3.5945	-1.66588	0.90158

C	-3.57469	-1.30549	-1.39473
C	-4.78031	-2.25036	0.34883
H	-3.33505	-1.63396	1.94983
C	-4.7682	-2.02793	-1.06762
H	-3.29858	-0.95429	-2.3784
H	-5.56141	-2.74588	0.90433
H	-5.5386	-2.32724	-1.76127
C	-1.52008	-0.3631	-0.05171
H	-1.42321	0.1391	0.91096
H	-1.41057	0.4102	-0.81282
C	-6.43971	0.9344	-0.61211
C	-5.25488	1.67383	-0.94127
C	-6.4463	0.71425	0.80554
H	-7.18724	0.59037	-1.31024
C	-4.52897	1.91057	0.27436
H	-4.96048	1.99145	-1.92981
C	-5.26556	1.31733	1.35424
H	-7.19958	0.17567	1.35966
H	-3.59337	2.4419	0.36139
H	-4.98039	1.31952	2.39511
Fe	-4.72557	-0.16912	-0.04945
C	4.57138	4.44078	0.53705
C	5.10442	5.64587	0.68426
H	6.18881	5.81984	0.68002
C	2.71928	6.70153	0.81713
C	4.25314	6.91494	0.87505
H	2.04952	7.53405	0.87403
H	4.69069	7.87795	1.03651

H	5.2247	3.60315	0.40884

Fe3

Fe	1.88803	0.43665	4.18092
N	2.91628	1.58415	5.21601
N	1.56283	-0.47534	5.78543
N	0.74992	-0.49161	3.01219
N	2.42878	1.53355	2.75742
O	0.37264	1.39233	4.67555
O	3.32077	-0.69911	4.03025
C	2.89652	2.92432	4.62427
H	3.6884	3.53064	5.01284
H	1.96216	3.40528	4.82711
C	2.98136	2.69321	3.12207
C	3.5384	3.57502	2.19654
H	4.01413	4.47886	2.51407
C	3.44971	3.24358	0.84004
H	3.86164	3.89109	0.09519
C	2.80776	2.04834	0.47089
H	2.7104	1.78756	-0.56203
C	2.29704	1.19492	1.46721
H	1.81202	0.27997	1.19649
C	2.35382	1.63066	6.57233
H	1.4763	2.2428	6.58487
H	3.06276	2.02561	7.26995

C	1.91684	0.2011	6.88192
C	1.85452	-0.36425	8.15457
H	2.1825	0.17953	9.01582
C	0.916	-2.3326	7.11241
C	1.03865	-1.70632	5.85718
H	0.72179	-2.21131	4.96875
C	-0.77707	1.01851	3.90552
C	-0.61034	-0.03168	3.05991
C	-1.66694	-0.55504	2.31297
H	-1.52016	-1.40554	1.68067
C	-2.92394	0.06318	2.40229
H	-3.75036	-0.31809	1.84086
C	-3.09194	1.1898	3.23183
H	-4.04288	1.67703	3.28579
C	-2.0107	1.67192	3.99089
H	-2.13176	2.52349	4.62791
C	3.55134	-1.42334	2.81378
C	4.87994	-1.73604	2.48113
C	1.07756	-1.41694	2.18333
C	2.52275	-1.84698	1.9728
C	2.80141	-2.68288	0.88753
H	5.67968	-1.37034	3.09056
H	3.84361	1.21064	5.22099
C	5.16499	-2.5272	1.36033
H	6.17936	-2.75674	1.108
H	0.30966	-1.8861	1.60307
C	1.34383	-1.66139	8.27201
H	1.27688	-2.13422	9.22948

H	0.50131	-3.31671	7.18386
C	4.12035	-3.02115	0.57472
C	4.38846	-3.84818	-0.52564
C	1.74581	-3.17441	0.11037
C	3.33116	-4.33704	-1.30702
H	5.39752	-4.10723	-0.76923
C	2.00923	-4.00234	-0.98581
H	0.73676	-2.9158	0.35555
H	3.53276	-4.96511	-2.14834
H	1.20093	-4.37874	-1.57736

Fe4

Fe	1.40548	0.06331	-0.29583
N	-0.03252	0.65455	-1.66157
N	1.68912	-1.43975	-1.58901
N	2.90604	-0.32595	0.89578
N	0.86353	1.73195	0.6656
O	2.63771	1.06021	-1.29038
O	0.21434	-1.04151	0.62938
C	-0.0108	2.1441	-1.56216
H	-0.90401	2.59438	-2.00521
H	0.86638	2.49572	-2.11079
C	0.14876	2.56837	-0.12153
C	-0.34129	3.77623	0.36958
H	-0.9204	4.42524	-0.27859
C	-0.08888	4.12366	1.69539
H	-0.46342	5.06057	2.09631
C	0.64156	3.24923	2.50017

H	0.85438	3.47806	3.53858
C	1.09842	2.05862	1.94681
H	1.66455	1.34111	2.52776
C	0.53182	0.17231	-2.95863
H	1.34986	0.84104	-3.23608
H	-0.22128	0.19182	-3.75554
C	1.11341	-1.20894	-2.78847
C	1.13004	-2.17211	-3.79386
H	0.65284	-1.96785	-4.74694
C	1.76769	-3.39015	-3.55493
H	1.79501	-4.15391	-4.32609
C	2.36116	-3.61473	-2.31332
H	2.86263	-4.54895	-2.08583
C	2.29366	-2.61268	-1.34994
H	2.72305	-2.73856	-0.36309
C	3.84958	1.09893	-0.72679
C	4.07058	0.35563	0.45599
C	5.33066	0.35167	1.06822
H	5.51209	-0.21987	1.9737
C	6.36982	1.09039	0.50782
H	7.34708	1.08886	0.97975
C	6.1525	1.83178	-0.662
H	6.96699	2.40577	-1.09441
C	4.90476	1.84079	-1.27995
H	4.72693	2.40859	-2.18801
C	0.42815	-1.7175	1.74581
C	-0.64511	-2.46399	2.28418
C	0.72775	-3.232	4.13635

H	0.83587	-3.80946	5.04839
C	2.84922	-1.06478	1.96562
C	1.68181	-1.75823	2.43568
C	1.79737	-2.52087	3.62345
H	2.75782	-2.54163	4.13385
H	-1.58933	-2.44612	1.74879
C	-0.49927	-3.19897	3.45216
H	-1.34694	-3.75879	3.83806
H	3.75136	-1.18115	2.56859
C	-2.46897	0.51323	-2.47074
C	-2.81466	-0.19875	-3.67328
C	-3.32255	1.66553	-2.40108
C	-3.86109	0.51074	-4.33088
H	-2.38578	-1.13966	-3.99648
C	-4.17331	1.66064	-3.54398
H	-3.32384	2.39398	-1.60481
H	-4.36287	0.20542	-5.23948
H	-4.95335	2.37992	-3.75596
C	-1.43282	0.06911	-1.48266
H	-1.32879	-1.01965	-1.56638
H	-1.73489	0.31555	-0.46574
C	-6.46184	-0.13713	-1.86721
C	-5.63344	-0.14842	-0.70491
C	-6.13601	-1.28305	-2.65266
H	-7.18821	0.62278	-2.1232
C	-4.79429	-1.30146	-0.7737
H	-5.631	0.59593	0.08029
C	-5.10527	-2.0033	-1.97764

H	-6.57159	-1.5429	-3.60837
H	-4.0453	-1.58668	-0.04621
H	-4.62977	-2.90976	-2.3285
Fe	-4.45647	-0.07462	-2.42303

References

1 G. M. Sheldrick, SADABS, a software for empirical absorption correction, Ver. 2.05, University of Göttingen, Göttingen, Germany, 2002.

2 G. M. Sheldrick, *Acta Cryst*., 2015, **71**, 3-8.

3 O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann*, J. Appl. Cryst.* 2009, **42**, 339-341.

4 G. M. Sheldrick, *Acta Cryst*., 2008, **64**, 112-122.

5 L. J. Farrugia, *J. Appl. Crystallogr*., 1997, **30**, 565.

6 C. F. Macrae, I. Sovago, S. J. Cottrell, P. T. A. Galek, P. McCabe, E. Pidcock, M. Platings, G. P. Shields, J. S. Stevens, M. Towler and P. A. Wood, J. *Appl. Cryst*., 2020, **53**, 226-235.

7 A. A. Mandal, V. Singh, S. Saha, S. Peters, T. Sadhukhan, R. Kushwaha, A. K. Yadav, A. Mandal, A. Upadhyay, A. Bera, A. Dutta, B. Koch, and S. Banerjee, *Inorg. Chem*., 2024, **63**, 7493-7503.

8 U. Das, S. Shanavas, A. H. Nagendra, B. Kar, N. Roy, S. Vardhan, S. K. Sahoo, D. Panda, B. Bose and P. Paira, *ACS Appl. Bio Mater*., 2023, **6**, 410-424.

9 M. Kubanik, H. Holtkamp, T. Söhnel, S. M. F. Jamieson and C. G. Hartinger, *Organometallics* 2015, **34**, 5658-5668.

10 S. P. Westrip, *J. Appl. Cryst*., 2010, **43**, 920-925.

11 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman and D. J. Fox*, Gaussian 16, Revision A.03, Gaussian, Inc., Wallingford CT*, 2016.

12 T. Yanai, D. P. Tew and N. C. Handy, *Chem. Phys. Lett*., 2004, **393**, 51-57.

13 A. K. Yadav, A. Upadhyay, A. Bera, R. Kushwaha, A. A. Mandal, S. Acharjee, A. Kunwar and S. Banerjee, *Inorg. Chem. Front*., 2024, **11**, 5435-5448.

14 A. A. Mandal, A. Upadhyay, A. Mandal, M. Nayak, M. S. K, S. Mukherjee and S. Banerjee, *ACS Appl. Mater. Interfaces,* 2024, **16**, 28118-28133.

15 F. D. Abreu, T. d. F. Paulo, M. H. Gehlen, R. m. A. Ando, L. G. Lopes, A. C. S. Gondim, M. A. Vasconcelos, E. H. Teixeira, E. H. S. Sousa and I. M. M. de Carvalho, *Inorg. Chem.,* 2017, **56**, 9084-9096.

16 S. Rajalakshmi, A. Fathima, J. R. Rao and B. U. Nair, *RSC advances,* 2014, **4**, 32004-32012.

17 A. Mandal, R. Rai, A. A. Mandal, P. Dhar and S. Banerjee, *Chem. Asian J.,* 2024, **19**, e202400943.

18 F. Zapata, A. Caballero, A. Espinosa, A. Ta´rraga and P. Molina, J. *Org. Chem*., 2008, **73**, 4034.

19 U. Basu, I. Khan, A. Hussain, B. Gole, P. Kondaiah and A. R. Chakravarty, *Inorg. Chem.,* 2014, **53**, 2152-2162.

20 S. Sahoo, S. Podder, A. Garai, S. Majumdar, N. Mukherjee, U. Basu, D. Nandi and A. R. Chakravarty, *Eur. J. Inorg. Chem*., 2018, **2018**, 1522-1532.