Supporting Information

A Ratiometric Fluorescent Chemosensor for Iron: Discrimination of Fe^{2+} and Fe^{3+} and Living Cell Application

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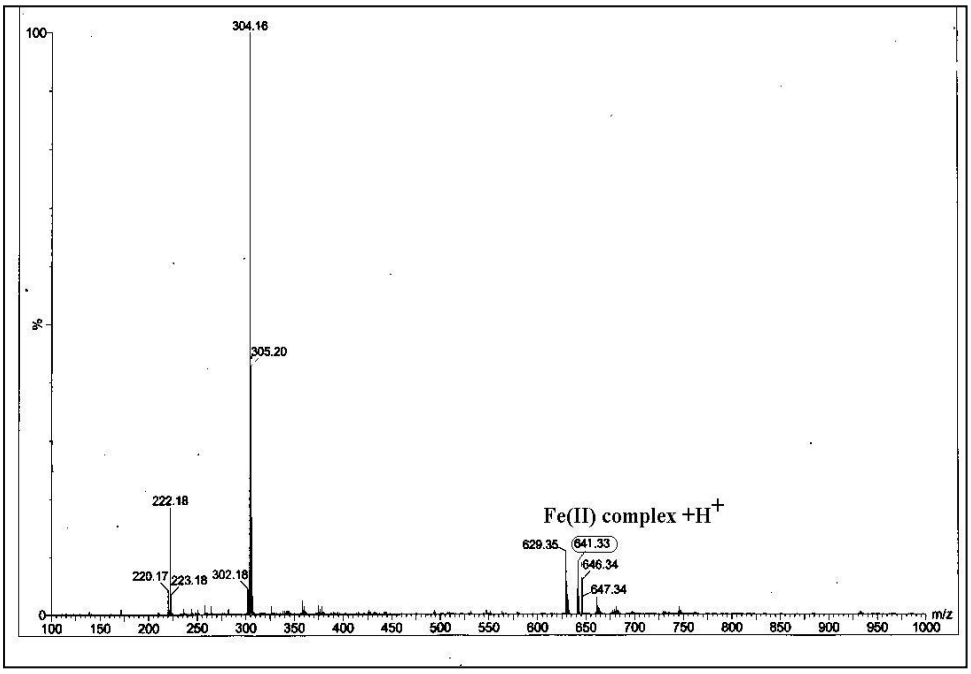
Characterization of all compounds



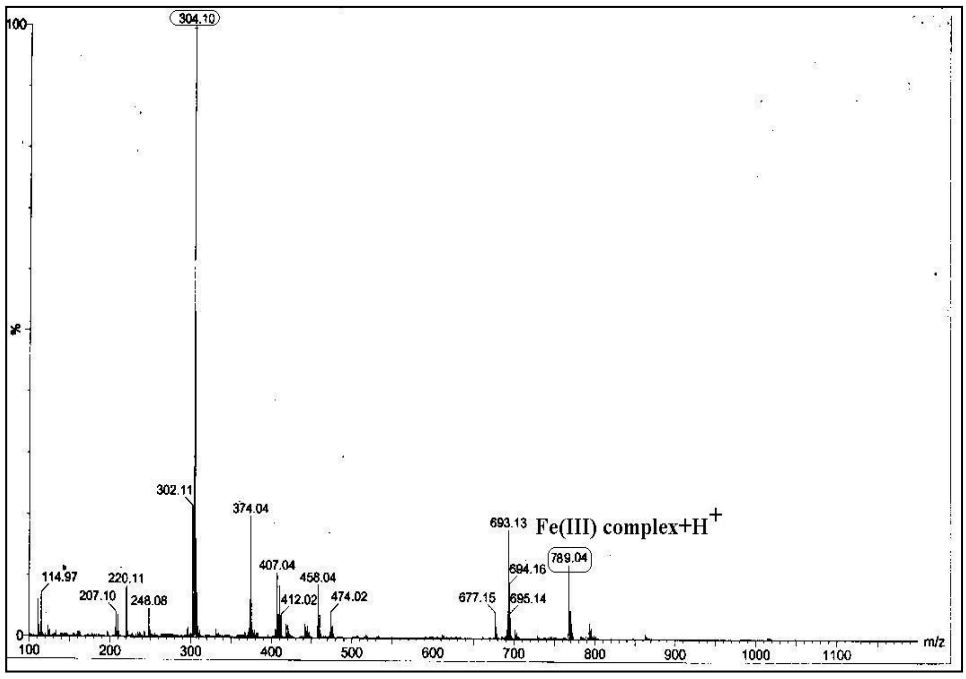


Fig. S1A ESI-MS of $[Fe(HL)(ClO_4)_2(CH_3CN)_2]$ (**1**) and $[Fe(L)_2(H_2O)(ClO_4)]$ (**2**)

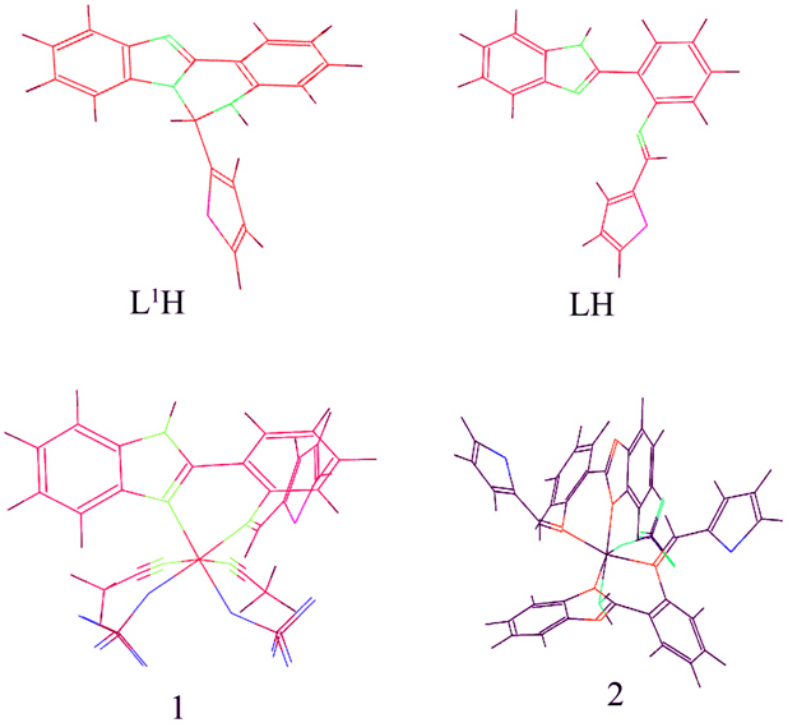


Fig. S1B DFT optimized structures of (a) **HL**[1] and (b) **HL** (c) Complex **1** and (d) Complex **2**.

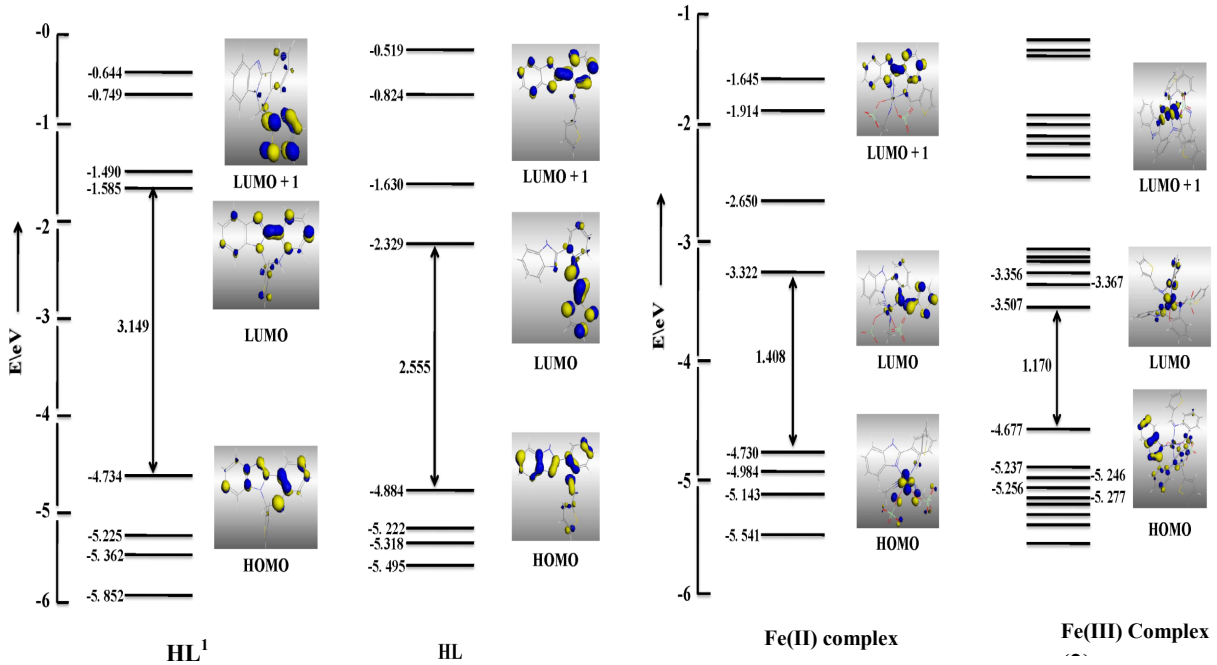


Fig. S1C Molecular Orbital diagram of **HL**[1], **HL**, Complex **1** and Complex **2**

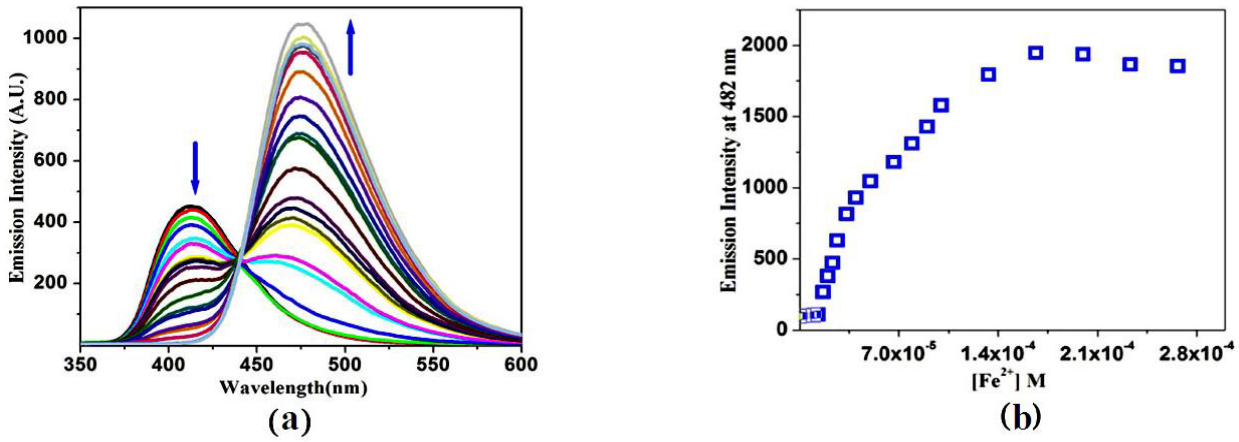


Fig. S2 (a) Emission spectra of **HL** (1.66×10^{-5}) in presence of Fe^{2+} (1.66×10^{-6} - 8.33×10^{-5}) in a HEPES buffer (100 mM, acetonitrile:water = 1:4 (v/v), pH 4.5) at 25°C (b) Fluorescence intensity as a function of Fe^{2+} concentration.

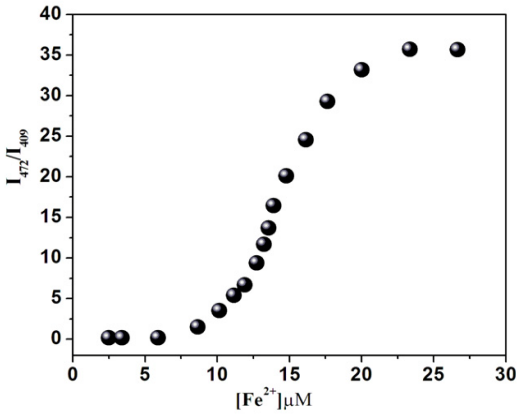


Fig. S3 Ratiometric signaling of fluorescence output at two different wavelengths is plotted as a function of concentration of Fe^{2+}.

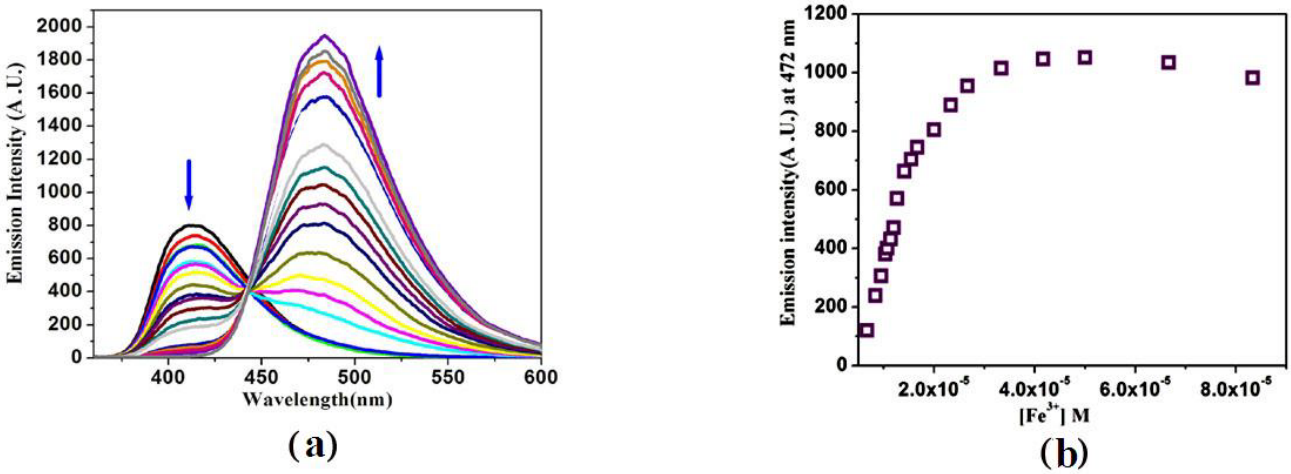


Fig. S4 (a) Emission spectra of **HL** (1.66×10^{-5}) in presence of Fe^{3+} (3.33×10^{-6}-3.33×10^{-4}) in a HEPES buffer (100 mM, acetonitrile:water = 1:4 (v/v), pH = 7.4) at 25 ^{o}C; (b) Fluorescence intensity as a function of Fe^{3+} concentration.

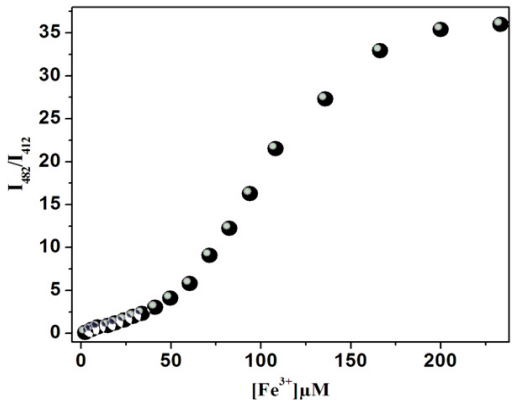


Fig. S5 Ratiometric calibration curve I_{482} /I_{412} as a function of Fe^{3+}concentration.

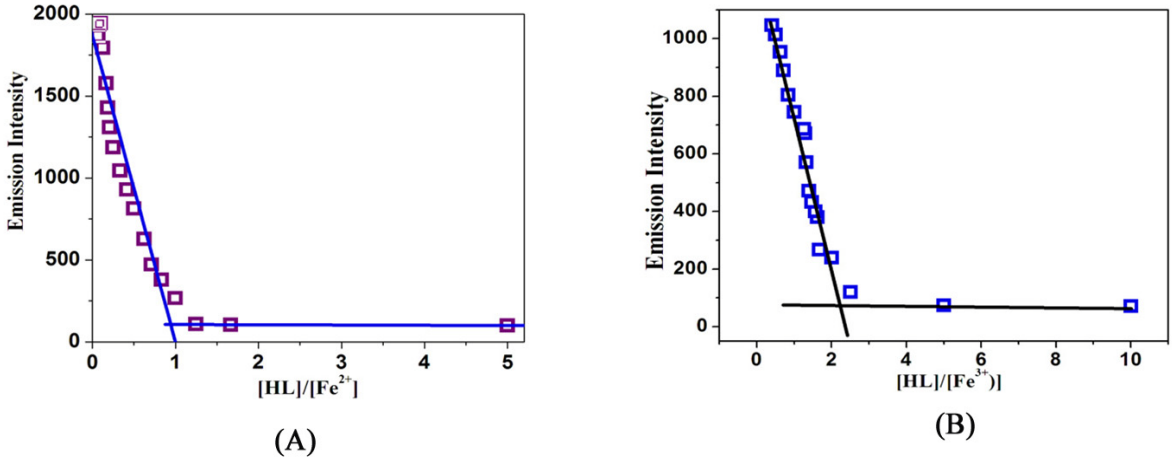


Fig.S6 (a) Job's plot analysis showing maximum emission at 1:1 ratio [**HL**: Fe^{2+}] and (b) 2:1 ratio [**HL**: Fe^{3+}] in a HEPES buffer (100 mM, acetonitrile:water = 1:4 (v/v)).

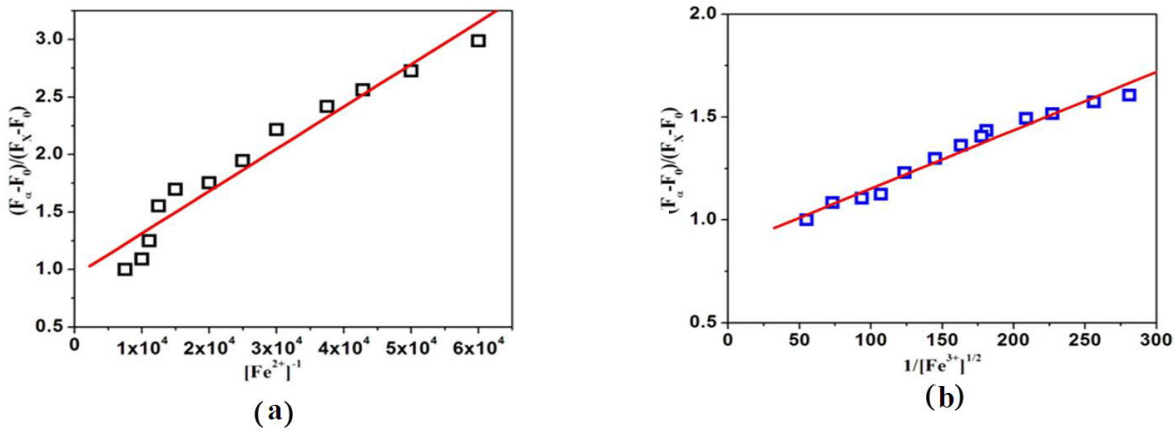


Fig. S7 (a) Binding constant (K_a) value 3.88×10^5 M^{-1}determined from the interaction of **HL** with Fe^{2+} (b) Binding constant (K_a) value 0.205×10^3 $M^{-1/2}$ determined from the interactions of **HL** with Fe^{3+}.

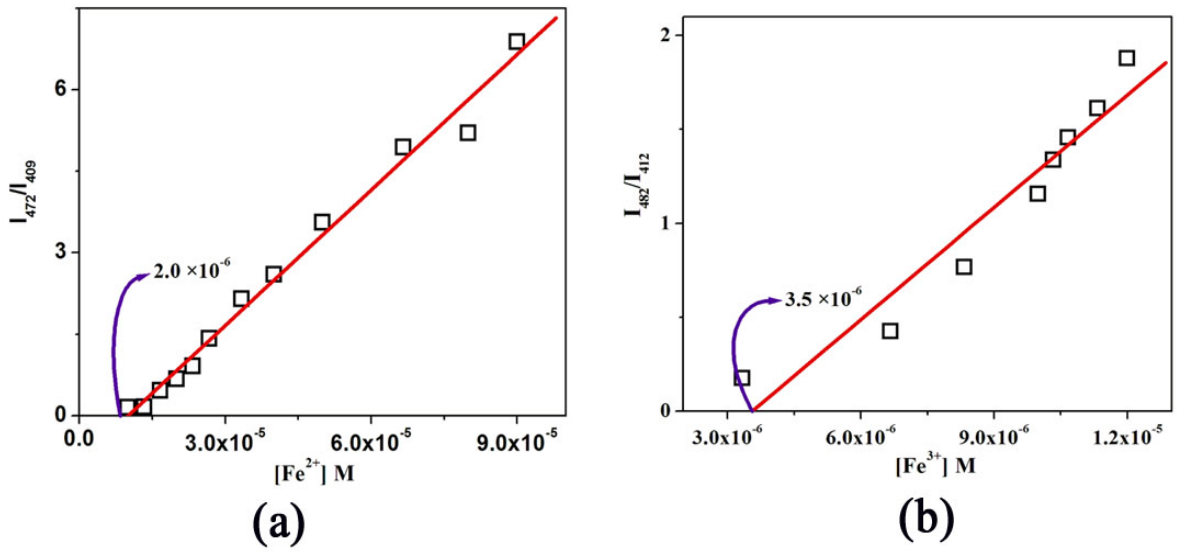


Fig.S8. Ratiometric detection limit of (a) Fe^{2+} (*ca*. 2.0 μM) and (b) Fe^{3+}(*ca*. 3.5 μM) in a HEPES buffer (100 mM, acetonitrile:water = 1:4 (v/v), at respective adjusted pH).

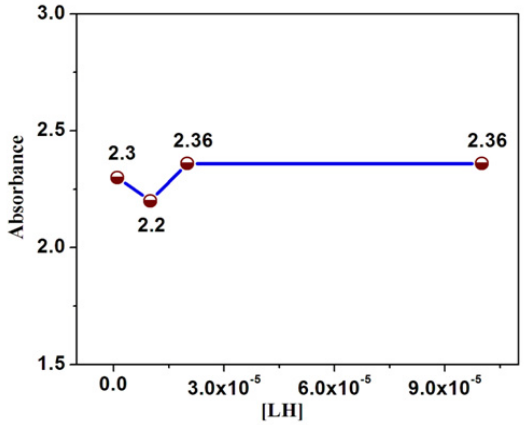


Fig. S9 MTT assay for the determining cytotoxic effect of **HL**[1] which was incubated with HeLa cell for 18 hours in 24 wells plate. MTT was added and after 3 hours, absorbency was measured in 590 nm

Table S1. Selectivity coefficients (k)[a] for Fe^{2+} and Fe^{3+} over competent ions

Ions	Selectivity coefficient (k)	
	for Fe^{2+}	for Fe^{3+}
Na^{+}	1392	1220
K^{+}	1273	1255
Mg^{2+}	1312	1277
Ca^{2+}	1244	1227
Cr^{3+}	541	534
Mn^{2+}	1142	1094
Ni^{2+}	1094	1079
Cu^{2+}	1546	1525
Zn^{2+}	618	667
Cd^{2+}	970	1051
Hg^{2+}	1098	940

[a]Selectivity coefficient (k) was calculated as $k_{B,A} = m_B/m_A$; where m_B = d/dc(signal of B) and m_A = d/dc(signal of A); dc = change of concentration of species; B = Fe^{3+} or Fe^{2+} and A = other interfering metal ion