Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Idiosyncratic recognition of Zn^{2+} and CN^- in environment and live cell imaging using Pyrazolyl-Hydroxy-Coumarin scaffold: Depiction of Luminescent Zn(II)-Metallocryptand

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Experimental Section

Materials and Methods

All the reagents of analytical grade (A.R) were collected from the commercial suppliers and used without further purification. 3-Amino-5phenyl pyrazole, Resorcinol, Ethylacetoacetate, Hexamine were purchased from Sigma-Aldrich. Inorganic salts and other organic chemicals ($ZnCl_2$, $CdCl_2$, $HgCl_2$, $CuCl_2.2H_2O$, $CoCl_2.6H_2O$, $PbCl_2$, $AlCl_3$, $CaCl_2.6H_2O$, $FeCl_3.6H_2O$, $BaCl_2.2H_2O$,$NiCl_2.6H_2O$, NaCl,$MnCl_2.4H_2O$, $PdCl_2$, $CrCl_3.6H_2O$, KCl, $MgCl_2$,$Na_2S_2O_3.5H_2O$, K_3PO_4, NaCl, NaF, NH_4HF_2, KNO_3, KBr, $NaNO_2$, NaN_3, , CH_3COONa, , KIO_3, $Na_4P_2O_7$,KI,Na_2SO_4, Na_2S, $Na_2S_2O_5$, $C_4H_9N(CN)$) were bought from TCI chemicals and Merck. For spectroscopic measurement UV graded solvents spectroscopic were used. The solvents were dried by standard procedure for spectroscopic studies.[1]Milli-Q water (Millipore) has been used for preparation of aqueous solutions of metal salts. Perkin-Elmer (2400 Series-II, Perkin Elmer, USA) CHN analyzer has been used for elemental analysis purpose. The spectra were recorded by Lambda 25 spectrophotometer: UV-Vis; LS55: fluorescence and LX-1FTIR spectrophotometer: FT-IR spectra (KBr disk, 4000-400 cm^{-1}) on Perkin Elmer instruments. ^1H and ^{13}C NMR spectra were taken by Bruker 300 MHz FT-NMR spectrometer. The chemical shift (δ) of the respective NMR spectra were recorded in parts per million (ppm) with respect to trimethylsilaneas internal standard. ESI-MS spectra were obtained from HRMS spectrometer (model, XEVO-G2QTOF#YCA351).

Quantum Yield and Limit of detection Calculation

Fluorescence quantum yields (Φ) were obtained by using the equation:

$$\Phi_{sample} = (OD_{std.} \times A_{sample})/(OD_{sample} \times A_{std.}) \times \Phi_{std.}$$

Where, A_{sample} and A_{std} represent the areas under the fluorescence spectral curves for sample and standard respectively. OD_{sample} and OD_{std} represents the optical densities of the sample and standard respectively at the excitation wavelength.[3] In this work, acidic quinine sulfate (0.1(N) H_2SO_4 solution) was taken as the

standard with known quantum yield, Φstd. = 0.54 for the quantum yield calculation of ligand HL and the complex with Zn^{2+} and CN^-.

LOD determination was calculated from fluorescence Titration Experiment on gradual addition of Zn^{2+} and CN^- ion to the solution of ligand HL. Standard Deviation measured from emission intensity of ligand with addition of varying concentration of Zn^{2+} and CN^-. Limit of Detection for Zn^{2+} and CN^- determined from the equation LOD = (3σ/Slope) where σ represents standard deviation and m is the slope acquired from the plot of fluorescence titration experiment.

Solution for Spectral Measurement

For UV-Vis and Fluorescence study, the ligand H_2L with concentration of 1x 10^{-3}(M) was prepared in DMSO. All the cationic and anionic solutions of 1 x 10^{-3}(M) were arranged in deionized water. The Spectroscopic experiment was carried out in acetonitrile medium. A 25 μM of HL solution was prepared in2 mL CH_3CN/H_2O (99:1, v/v) (HEPES Buffer, pH 7.5) for sensing study. To this solution 1 equivalent of metal cations were added and the sensitivity and selectivity was checked by UV-vis and Fluorescence measurement of the probe HL solution. The absorption and emission path length of cell used were 1 cm. fluorescence measurement experiments were done on excitation and emission of 12 nm x 7 nm (For Zn^{2+}) and 12 nm x 3 nm (For CN^-) slit width.

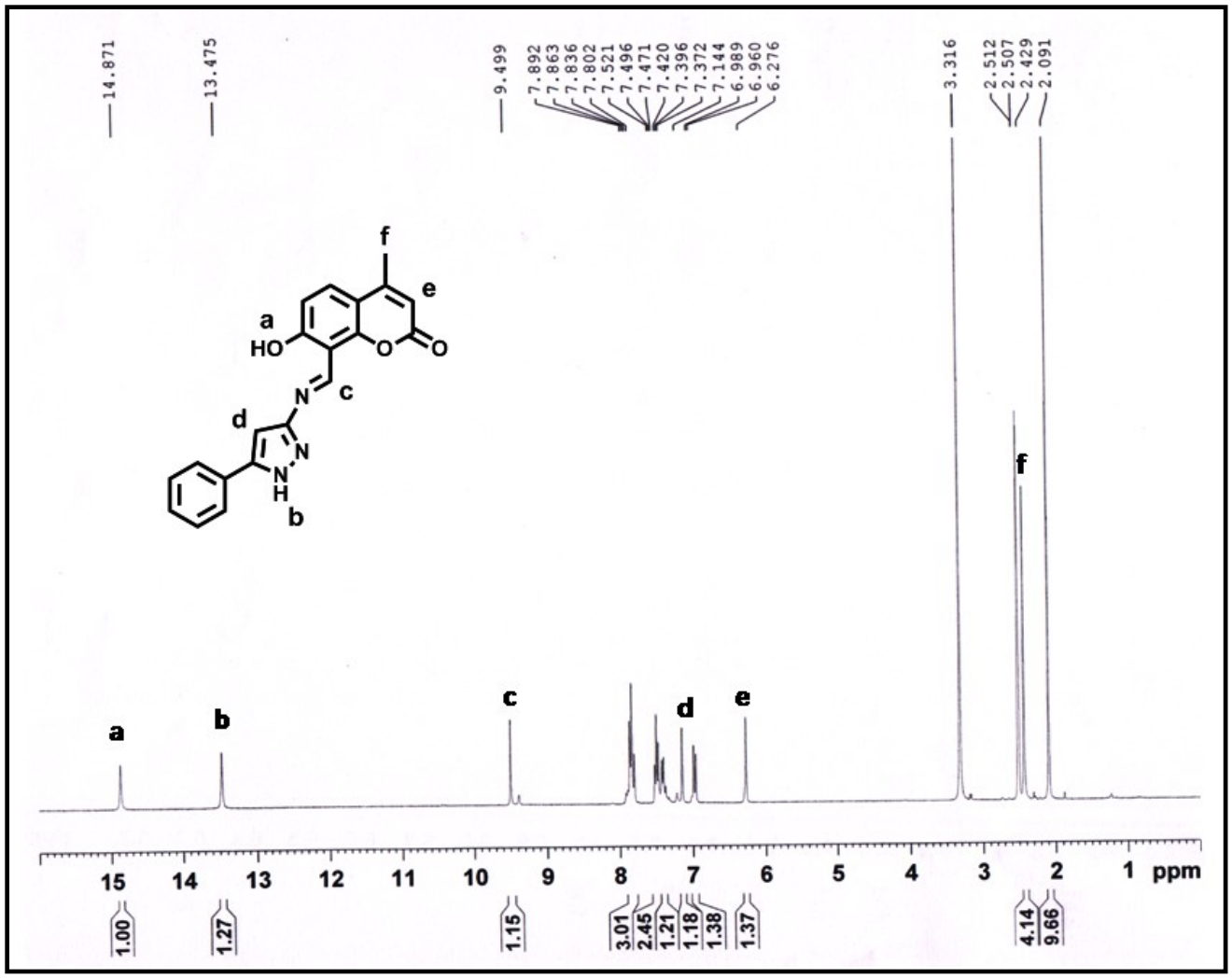


Fig.S1.[1]HNMR Spectrum (300 MHz) of the probe H_2L in DMSO-d_6

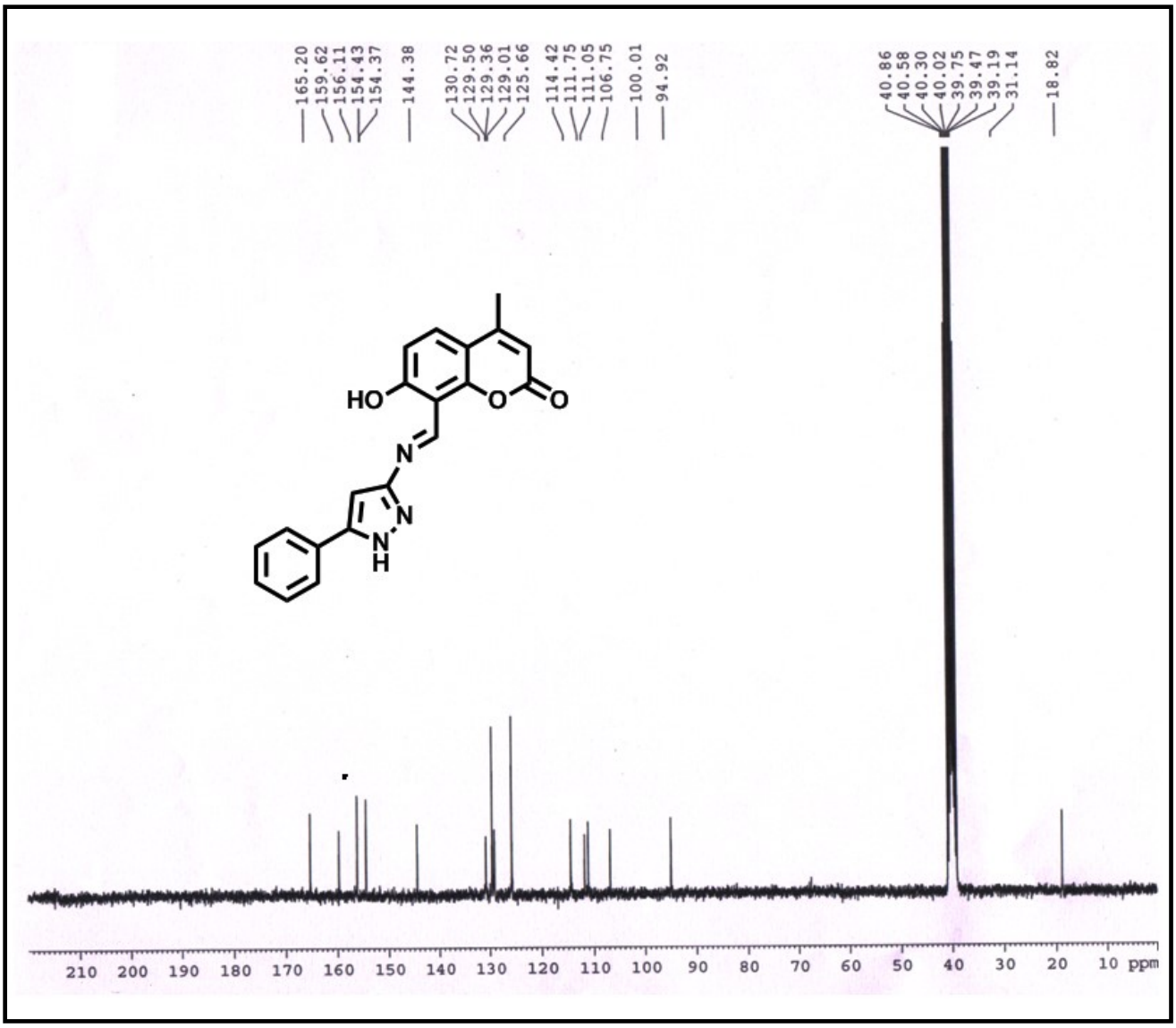


Fig.S2.[13]CNMR Spectrum (75 MHz) of H_2L in DMSO-d_6

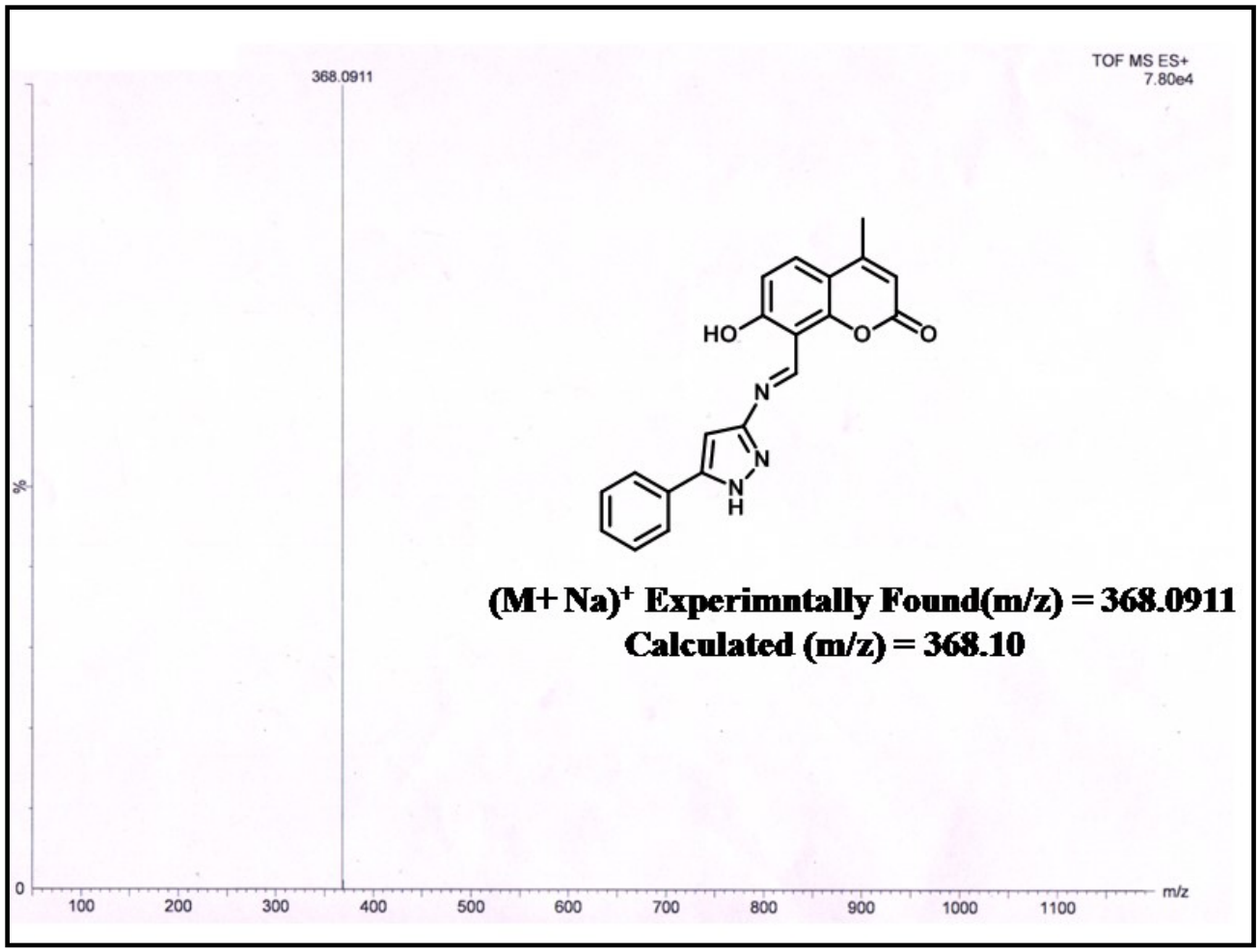


Fig. S3. ESI-MS Spectrum of H_2L

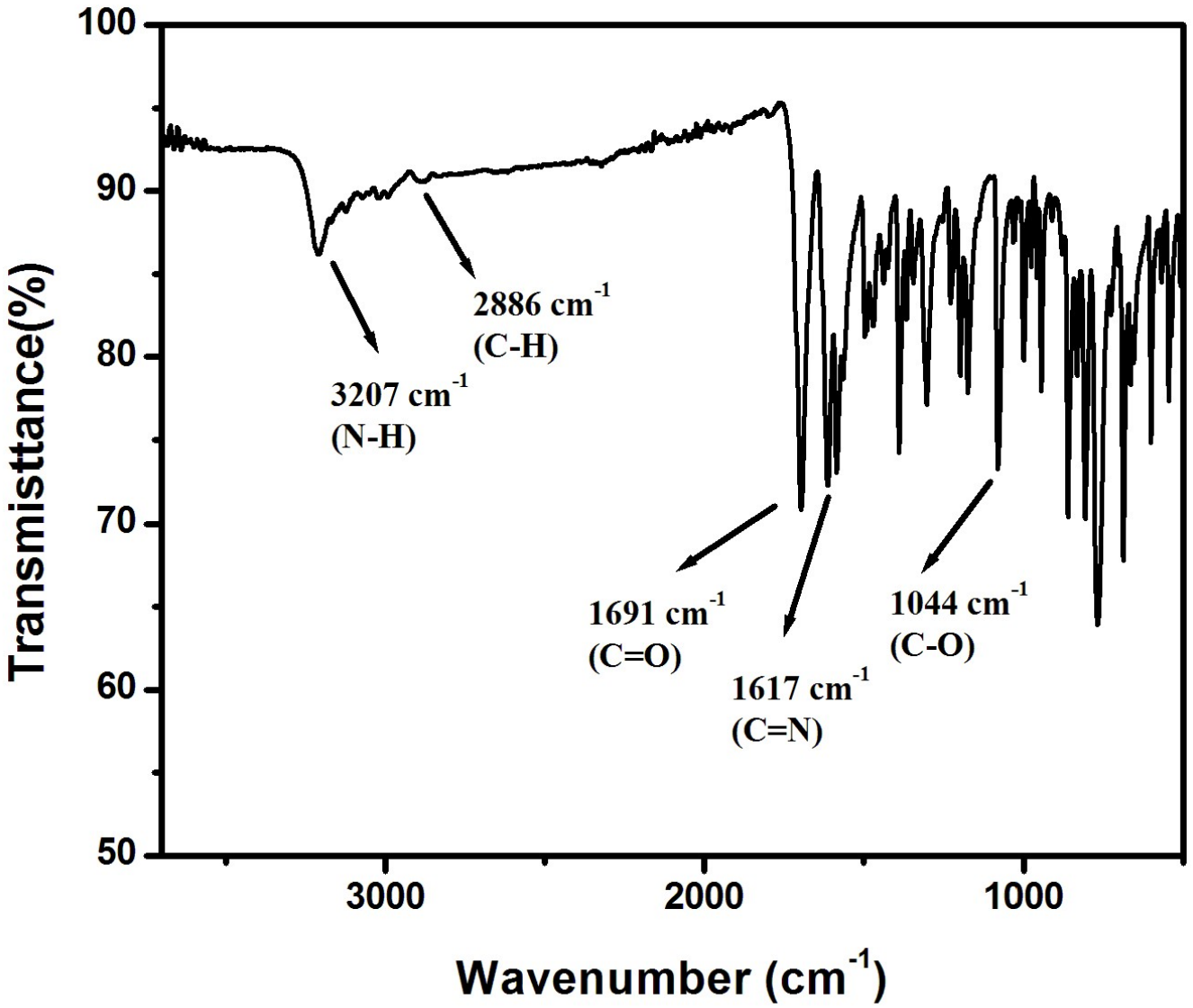


Fig.S4. IR Spectrum of the probe H_2L

Table S1. Crystal Data and Refined Parameters for H_2L

Empirical formula	$C_{20}\ H_{15}\ N_3\ O_3$
CCDC No.	2109503
Formula weight	345.35
Temperature (K)	273(2)
System	Monoclinic
Space group	*P 21/c*
a (Å)	11.8984(10)
b (Å)	12.8002(10)
c (Å)	11.1706(9)
α/°	90
β/°	101.831(2)
γ/°	90
V $(Å)^3$	1665.2(2)
Z	4
D(cal) /g cm^{-3}	1.378
μ/mm^{-1}	0.095
λ(Å)	0.71073
Data[I >2σ(I)]/param	3676/237
R_1[a][I>2σ(I)]	0.0530
wR_2[b]	0.1595
GOF[c]	1.215

Table S2: Crystal Data and Refined parameter for hexanuclear zinc complex $[Zn_6L_6]$

Empirical formula	$C_{245.78}H_{168.66}N_{36}O_{39.78}S_{1.57}Zn_{12}$
CCDC No.	2109502
Formula weight	5097.43
Temperature (K)	100.4
System	Triclinic
Space group	P -1
a (Å)	17.5384(14)
b (Å)	17.6613(12)
c (Å)	24.5865(16)
α/°	97.163(2)
β/°	100.276(2)
γ/°	106.205(2)
V $(Å)^3$	7072.1(9)
Z	1
D(cal) /g cm^{-3}	1.197
μ/mm^{-1}	1.076
λ(Å)	0.71073
Data[I >2σ(I)]/param	26162/ 1546
R_1[a][I>2σ(I)]	0.0612
wR_2[b]	0.1656
GOF[c]	1.043

[a]$R_1 = \Sigma||F_o|-|F_c||/\Sigma|F_o|$; [b]$wR_2 = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F^2_o)^2]\}^{1/2}$; $w = [\sigma^2(F_o)^2 + (0.1003P)^2 + 4.9693P]^{-1}$ $(F_o^2 + 2F_c^2)/3$; [c] Goodness-of-fit.

Table S3: Selected Bond length (Ă) and Bond Angles (°) of H_2L

Bond	Length (Ă)	Bond	Angle (°)
N(3)-H(3A)	0.86	N(3)-N(2)-H(3A)	123.2
N(3)-N(2)	1.345(2)	N(3)-N(2)-C(11)	103.60(16)
N(2)-C(11)	1.339(2)	N(2)-C(11)-N(1)	116.66(17)
C(11)-N(1)	1.401(2)	C(11)-N(1)-C(10)	120.49(16)
N(1)-C(10)	1.285 (2)	N(1)-C(10)-H(10)	120
C(10)-C(8)	1.452(2)	N(1)-C(10)-C(8)	119.72(17)
C(8)-C(7)	1.406 (2)	C(10)-C(8)-C(7)	121.75(15)
C(7)-O(3)	1.342(2)	C(8)-C(7)-O(3)	121.15(17)
O(3)-H(3)	0.82	C(7)-O(3)-H(3)	109.5

Table S4: Selected Bond length (Ă) and Bond Angles (°) for $[Zn_6L_6]$

Bond Length	Length (Ă)	Bond Length	Angle (°)
Zn(1)-O(1)	1.932(4)	Zn(4)-O(12)	1.945(3)
Zn(1)-N(12)	1.973(4)	Zn(4)-O(20)	2.539(4)
Zn(1)-N(13)	2.010(4)	Zn(4)-N(5)	2.087(4)
Zn(1)-N(18)	1.979(4)	Zn(4)-N(7)	1.983(4)
Zn(2)-O(6)	1.986(4)	Zn(4)-N(14)	2.007(4)
Zn(2)-O(19)	2.253(4)	Zn(5)-O(18)	1.960(4)
Zn(2)-N(1)	2.027(4)	Zn(5)-O(20)	2.360(4)
Zn(2)-N(4)	2.025(4)	Zn(5)-N(8)	2.023(4)
Zn(2)-N(16)	2.124(4)	Zn(5)-N(10)	2.126(4)
Zn(3)-O(13)	1.944(3)	Zn(5)-N(15)	2.012(4)
Zn(3)-O(19)	2.597(4)	Zn(6)-O(7)	1.938(3)

Zn(3)-N(2)	1.991(5)	Zn(6)-N(9)	2.001(4)
Zn(3)-N(3)	2.014(5)	Zn(6)-N(11)	1.983(4)
Zn(3)-N(6)	2.070(5)	Zn(6)-N(17)	1.985(4)

Bond Angles	Degrees(°)	Bond Angles	Degrees (°)	Bond Angles	Degrees
O1 - Zn1 - N12	118.25(16)	O1 - Zn1 - N13	92.96(16)	O1 - Zn1 - N18	107.72(16)
N12 - Zn1 - N13	115.22(16)	N12 - Zn1 - N18	107.7(17)	N13 - Zn1 - N18	114.52(15)
O6 - Zn2 - O19	82.42(14)	O6 - Zn2 - N1	113.83(16)	O6 - Zn2 - N4	134.84(15)
O6 - Zn2 - N16	86.57(14)	O19 - Zn2 - N1	86.31(15)	O19 - Zn2 - N4	81.62(15)
O19 - Zn2 - N16	167.20(14)	N1 - Zn2 - N4	107.03(16)	N1 - Zn2 - N16	104.21(16)
N4 - Zn2 - N16	101.32(19)	O13 - Zn3 - O19	77.46(15)	O13 - Zn3 - N2	126.67(17)
O13 - Zn3 - N3	111.32(16)	O13 - Zn3 - N6	90.47(15)	O19 - Zn3 - N2	76.26(17)
O19 - Zn3 - N3	82.44(17)	O19 - Zn3 - N6	167.36(17)	N2 - Zn3 - N3	110.20(16)
N2 - Zn3 - N6	108.78(16)	N3 - Zn3 - N6	105.81(17)	O12 - Zn4 - O20	77.26(15)
O12 - Zn4 - N5	89.82(15)	O12 - Zn4 - N7	127.43(17)	O12 - Zn4 - N14	111.09(19)
O20 - Zn4 - N5	165.09(17)	O20 - Zn4 - N7	74.55(15)	O20 - Zn4 - N14	83.57(17)
N5 - Zn4 - N7	108.47(16)	N5 - Zn4 - N14	108.56(16)	N7 - Zn4 - N14	108.64(16)
O18 - Zn5 - O20	80.48(13)	O18 - Zn5 - N8	115.94(16)	O18 - Zn5 - N10	87.83(15)
O18 - Zn5 - N15	130.84(16)	O20 - Zn5 - N8	82.63(14)	O20 - Zn5 - N10	168.04(14)
O20 - Zn5 - N15	82.70(15)	N8 - Zn5 - N10	105.00(15)	N8 - Zn5 - N15	107.12(16)
N10 - Zn5 - N15	103.34(17)	O7 - Zn6 - N9	93.58(15)	O7 - Zn6 - N11	110.73(15)
O7 - Zn6 - N17	117.06(15)	N9 - Zn6 - N11	113.73(17)	N9 - Zn6 - N17	113.04(16)
N11 - Zn6 - N17	108.30(16)	Zn1 - O1 - C1	127.5(3)	Zn2 - O6 - C22	133.3(3)
Zn6 - O7 - C63	126.8(3)	Zn3 - O13 - C103	128.8(4)	Zn4 - O12 - C92	131.1(4)
Zn5 - O18 - C52	131.4(3)	Zn2 - O19 - Zn3	87.62(15)	Zn2 - O19 - C02S	123.7(3)

Zn3 - O19 - C02S	128.1(3)	Zn4 - O20 - Zn5	87.89(15)	Zn4 - O20 - S1	118.2(2)
Zn5 - O20 - S1	139.3(2)	Zn2 - N1 - N2	120.4(3)	Zn2 - N1 - C12	126.8(3)
Zn3 - N2 - N1	112.8(4)	Zn3 - N2 - C14	137.7(3)	Zn3 - N3 - N4	119.8(4)
Zn3 - N2 - C14	137.7(3)	Zn3 - N3 - N4	119.8(4)	Zn3 - N3 - C89	126.9(3)
Zn2 - N4 - N3	115.2(3)	Zn2 - N4 - C81	135.8(3)	Zn3 - N6 - C101	124.1(4)
Zn3 - N6 - C112	119.2(3)	Zn4 - N7 - N8	113.9(3)	Zn4 - N7 - C72	135.8(3)
Zn5 - N8 - N7	121.5(3)	Zn5 - N8 - C80	128.0(3)	Zn6 - N9 - C61	124.0(3)
Zn6 - N9 - C80	117.0(4)	Zn5 - N10 - C49	120.6(3)	Zn5 - N10 - C50	123.9(4)
Zn6 - N11 - N12	123.9(3)	Zn6 - N11 - C49	125.9(4)	Zn1 - N12 - N11	115.9(3)
Zn1 - N12 - C41	134.0(4)	Zn1 - N13 - C11	124.4(3)	Zn1 - N13 - C12	116.4(3)
Zn4 - N14 - N15	123.1(4)	Zn4 - N14 - C112	126.3(3)	Zn5 - N15 - N14	113.5(3)
Zn5 - N15 - C114	137.7(3)	Zn2 - N16 - C31	126.0(4)	Zn2 - N16 - C32	120.1(3)
Zn6 - N17 - N18	114.6(3)	Zn6 - N17 - C34	134.5(4)	Zn1 - N18 - N17	126.0(3)
Zn1 - N18 - C32	124.8(4)	Zn2 - O19 - H19	119.8(2)	Zn3 - O19 - H19	98.8(2)

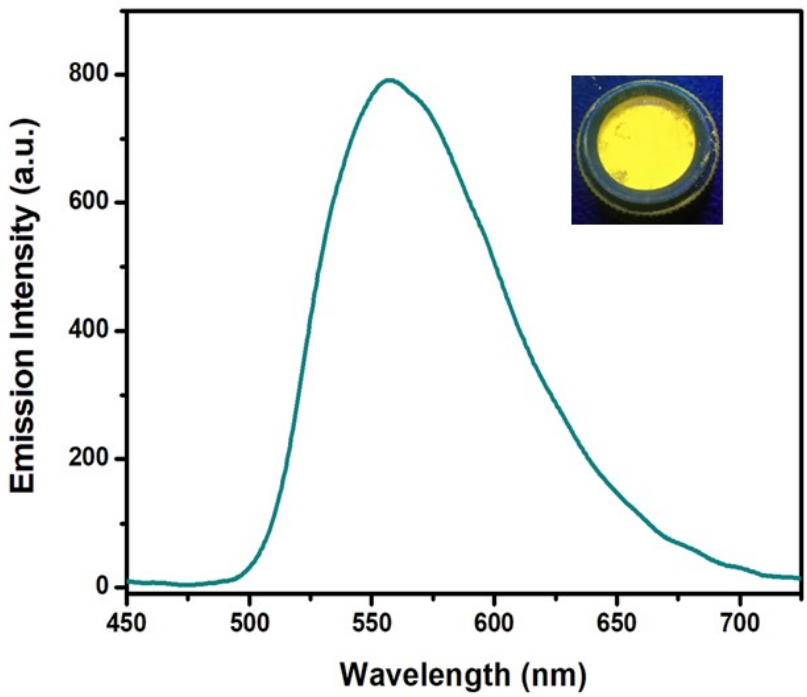


Fig. S5 Solid State Emission Spectrum of H_2L (Inset: images)(λ_{ex}= 390 nm).

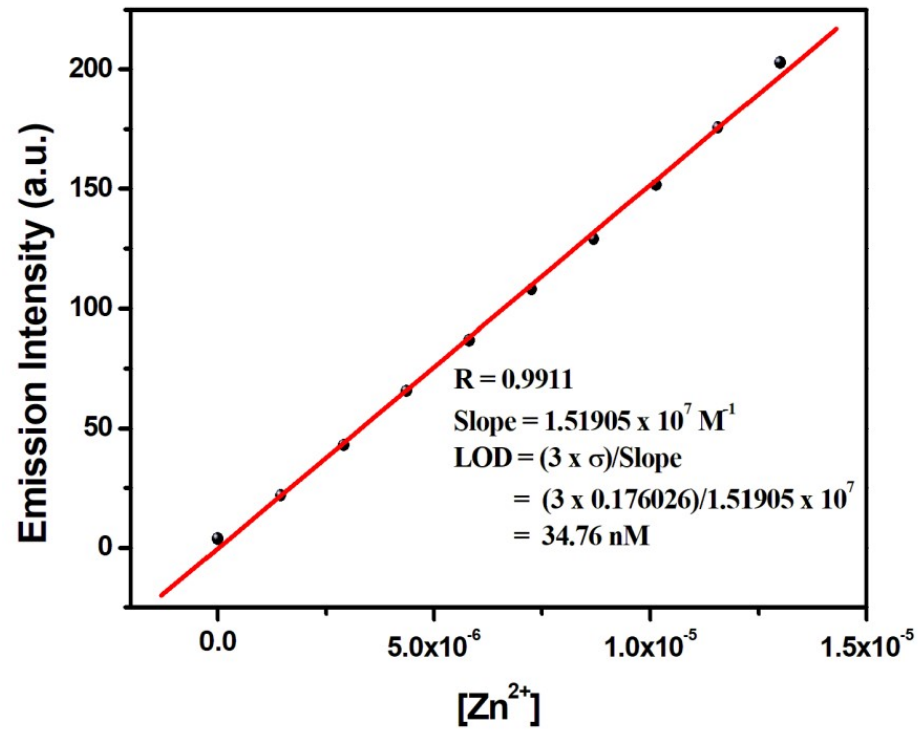


Fig. S6 Detection limit of H_2L for Zn^{2+} sensing.

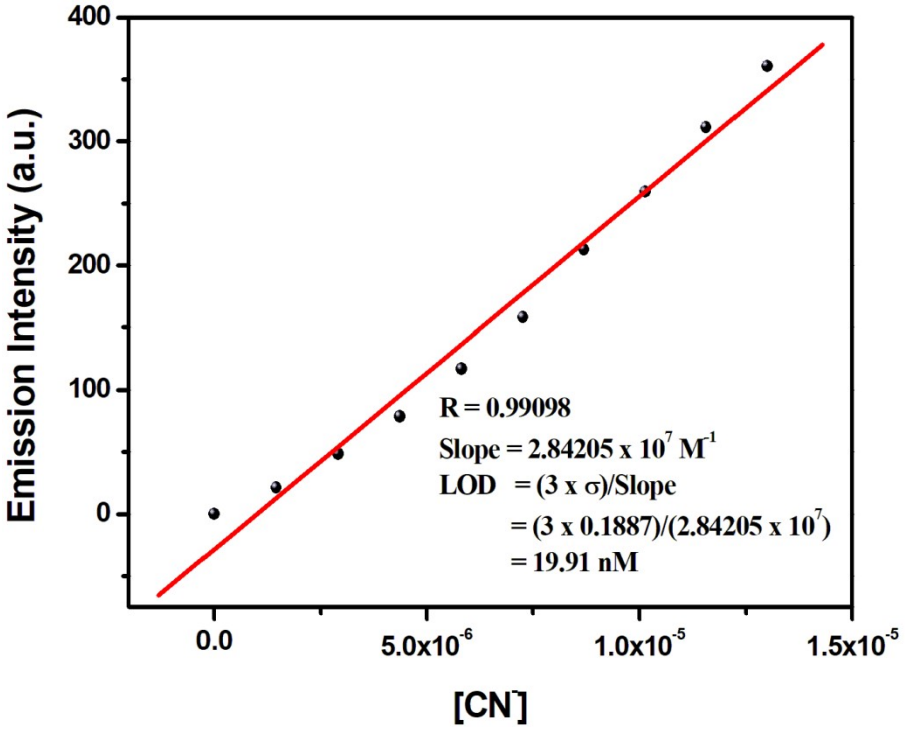
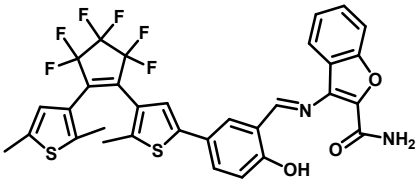
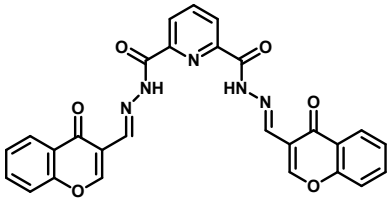
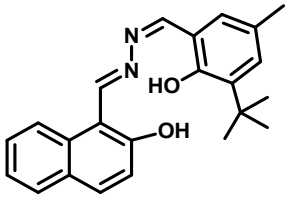
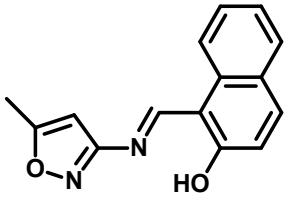
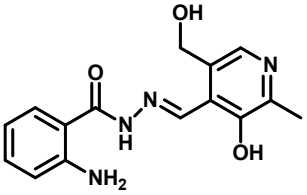
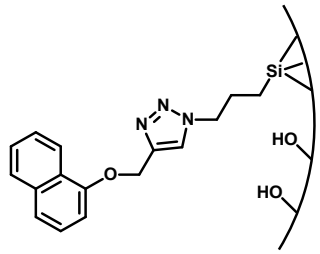


Fig. S7 Detection limit of H_2L towards CN^- sensing

Table S5 : Reported Zn^{2+} and CN^- selective probe with their LOD value

Sl. No.	Probe	Selectivity (LOD)	Solvent	Reference
1.		Zn^{2+} (32 nM) CN^-(13 nM)	CH_3CN	[4]
2.		Zn^{2+} (0.87 μM) CN^- (1.56 μM)	DMSO:H_2O (9:1)	[5]
3.		Zn^{2+} (13 nM) Cu^{2+} (1.6 μM) CN^- (0.81 μM)	H_2O	[6]
4.		Zn^{2+}(1.29 μM) CN^- (12.3 μM)	DMF H_2O	[7]
5.		Zn^{2+} (0.302 μM) CN^-(0.153 μM)	DMSO	[8]

6.		Zn^{2+} (0.23 μM) CN^- (0.39 μM)	H_2O	[9]

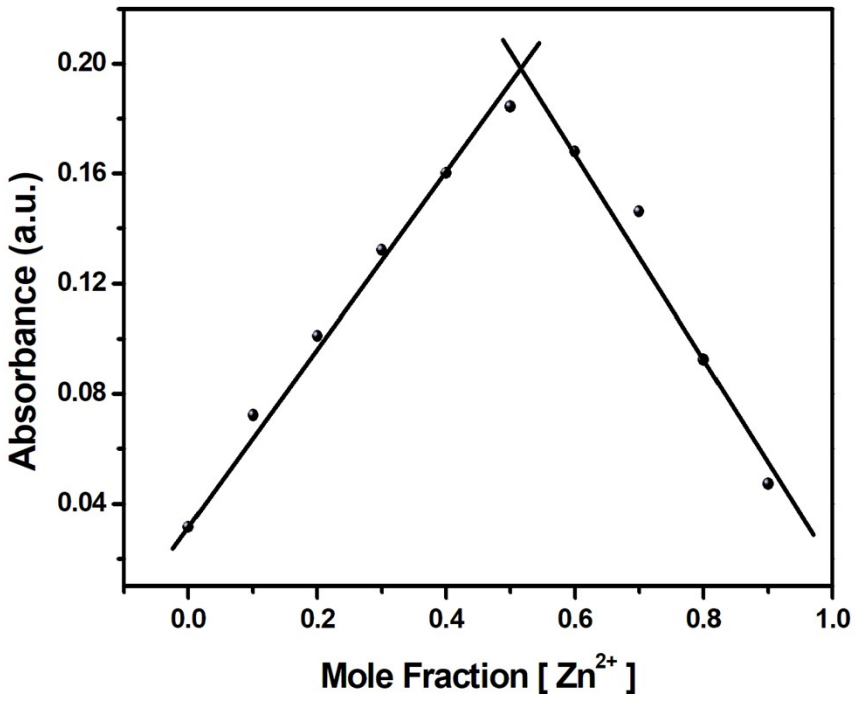


Fig. S8 Job's Plot for stoichiometric binding to Zn^{2+}

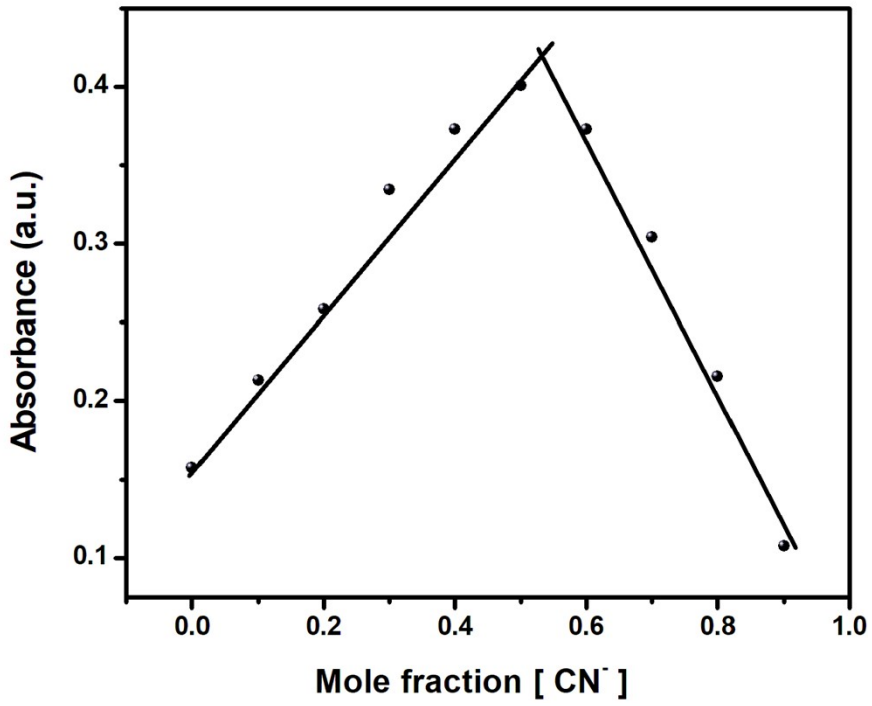


Fig. S9 Job's Plot for stoichiometry binding to CN^-.

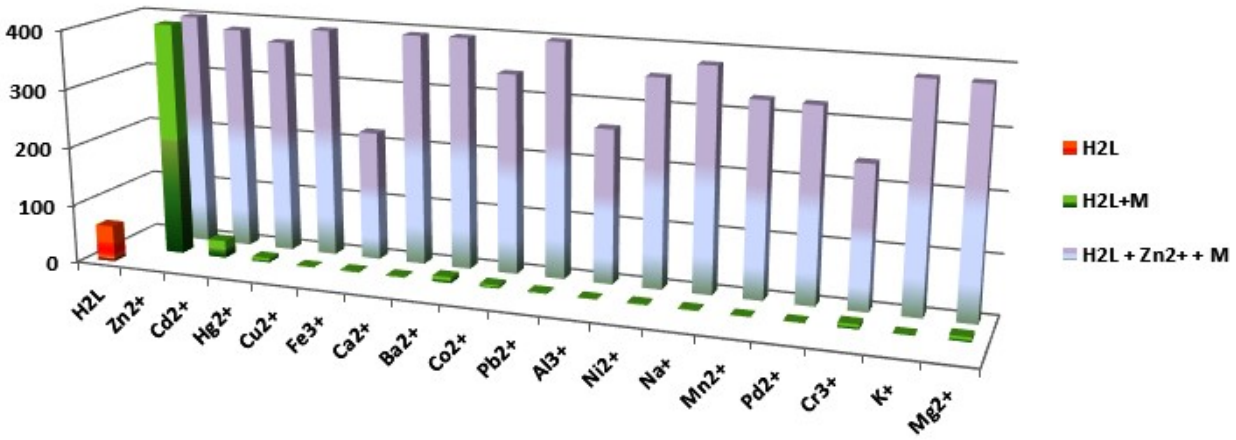


Fig. S10 Interference Study on Zn^{2+} Sensing.

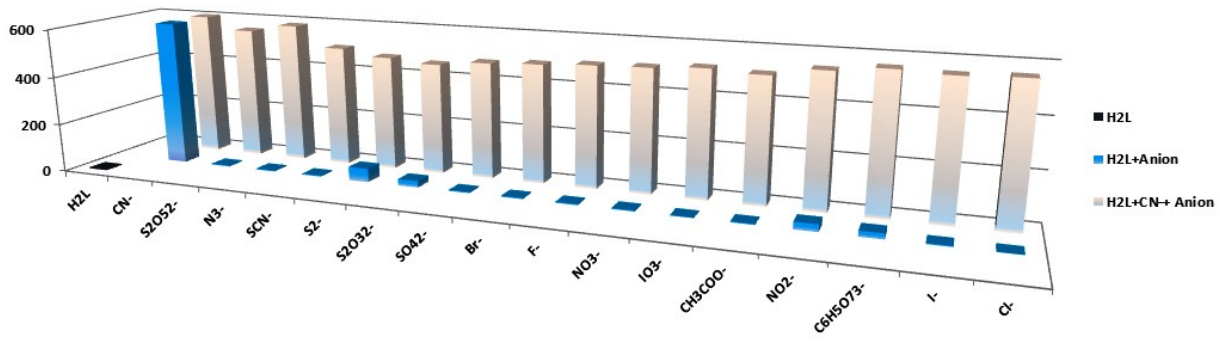


Fig. S11 Interference study on CN^- Sensing.

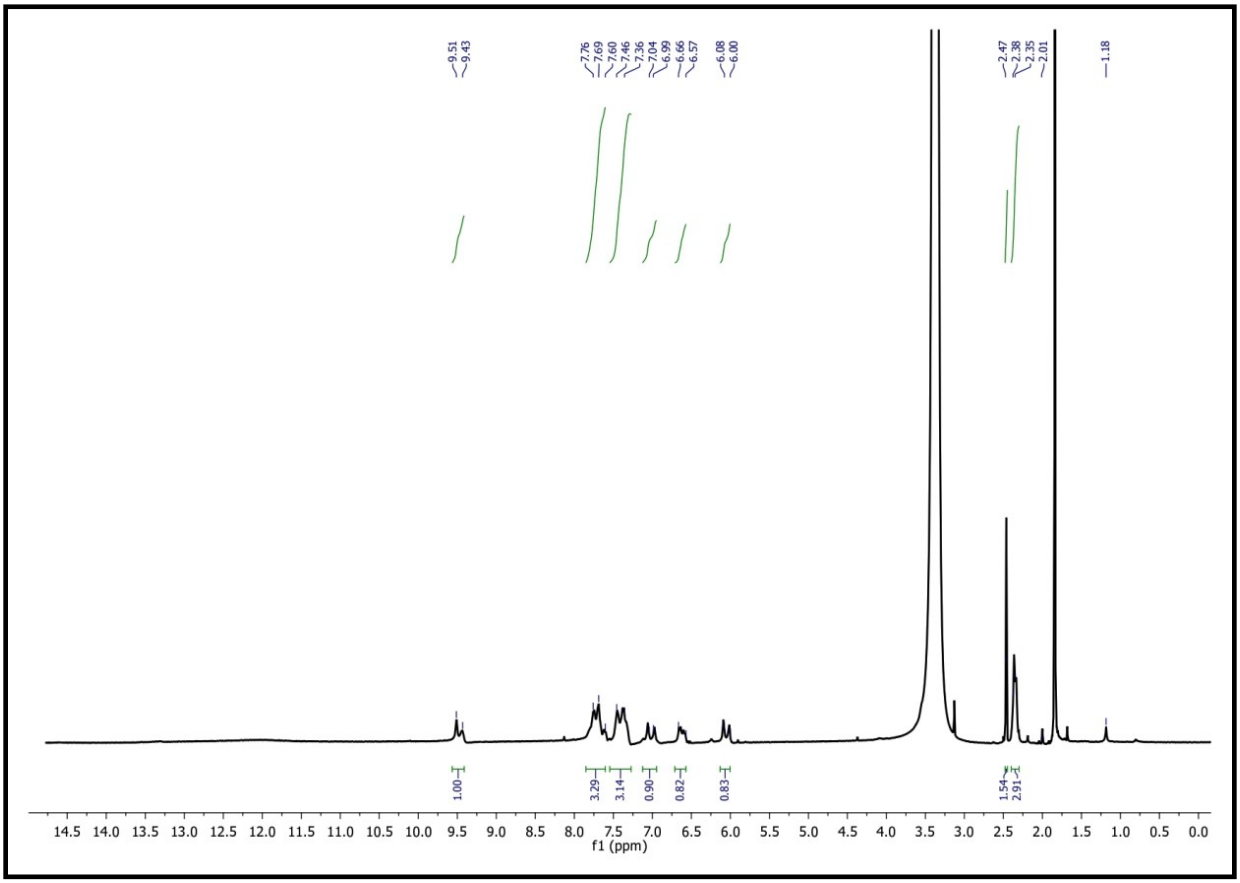


Fig. S12 ^{1}HNMR Spectra of Zn^{2+} Complex in DMSO-d_6

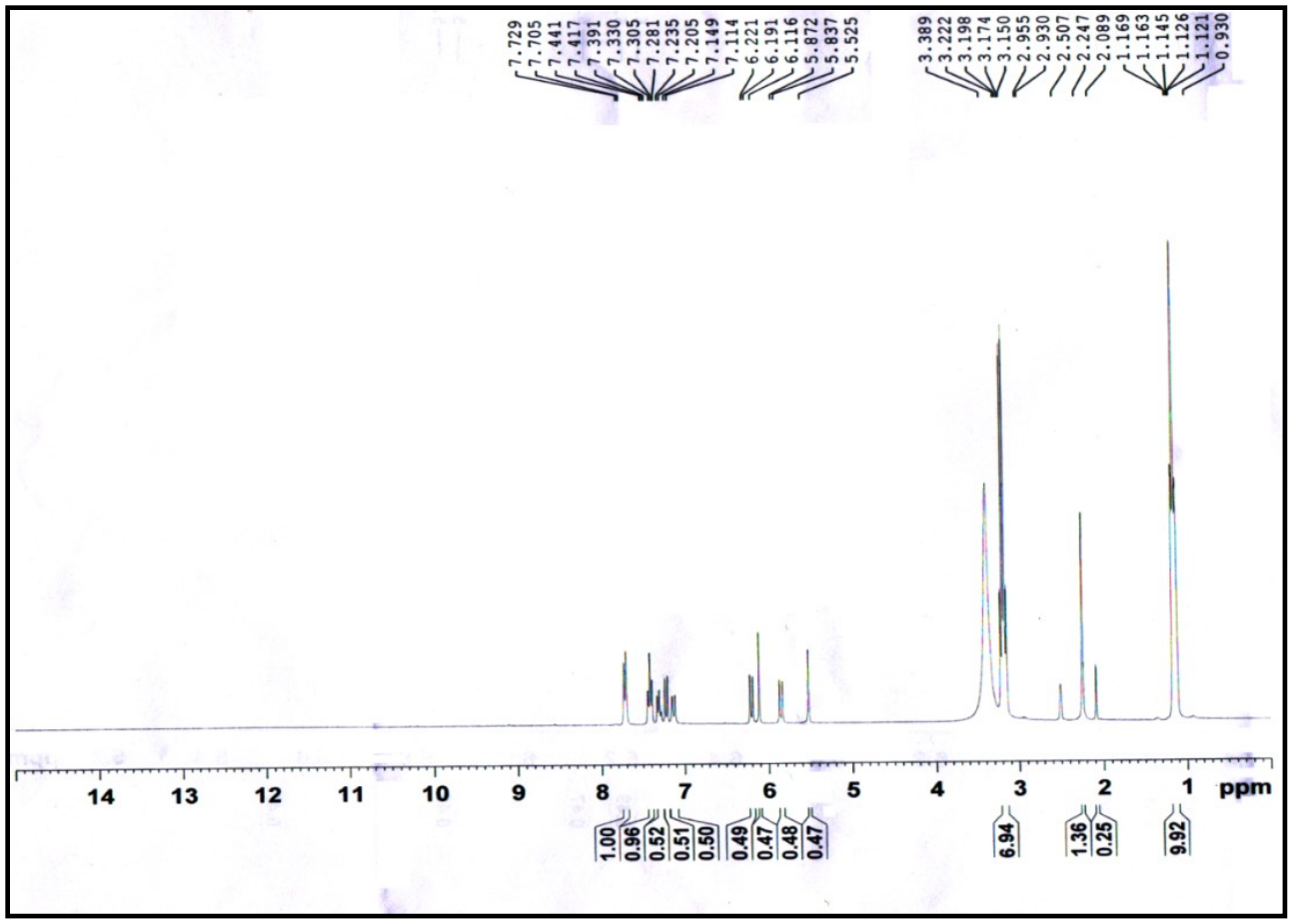


Fig. S13 ^{1}HNMR Spectra of CN^{-} Complex in DMSO-d_6.

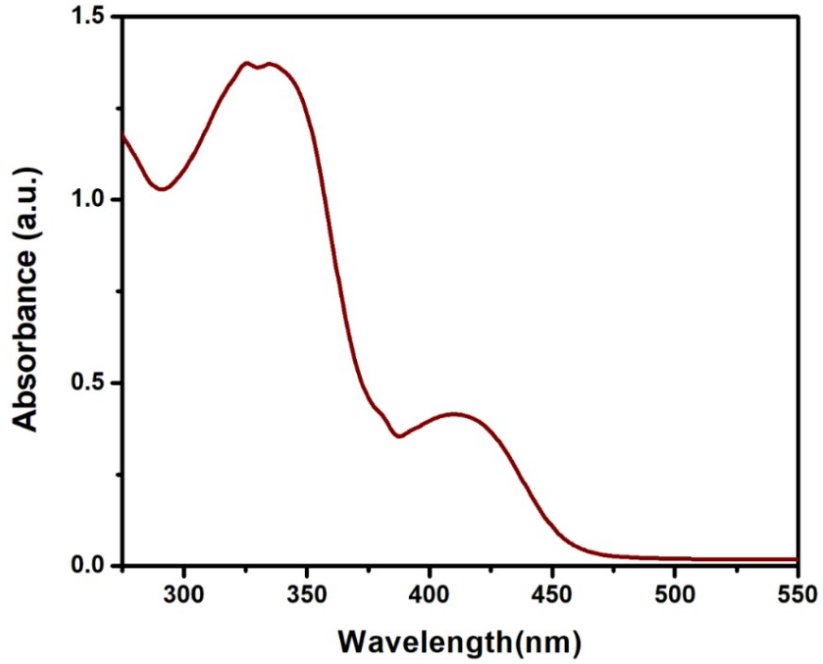


Fig. S14 UV-Vis Absorption Spectrum of $[Zn_6L_6]$ in (99:1, v/v) (HEPES Buffer, pH 7.5) medium.

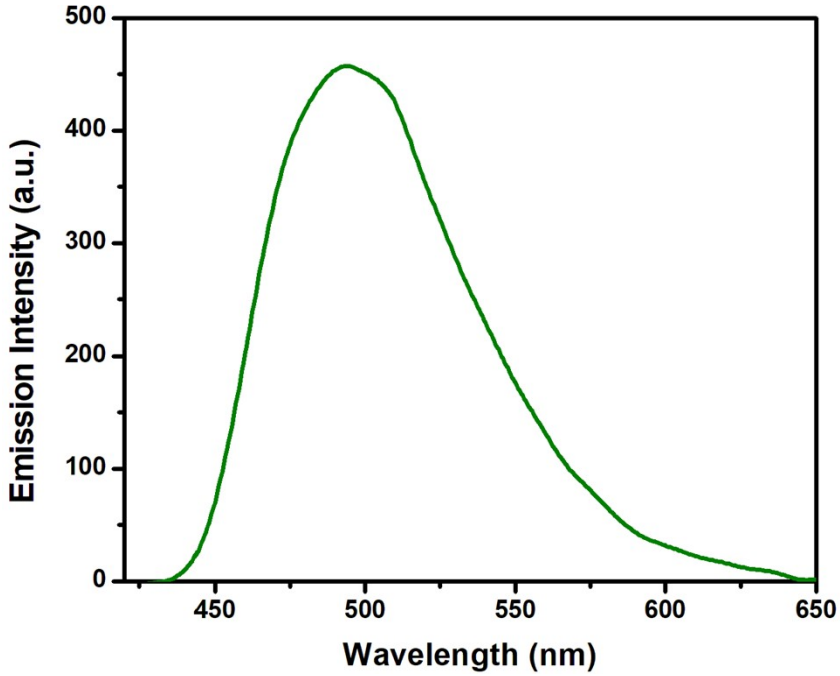


Fig. S15 Fluorescence Spectrum of $[Zn_6L_6]$ in (99:1, v/v) (HEPES Buffer, pH 7.5) medium.

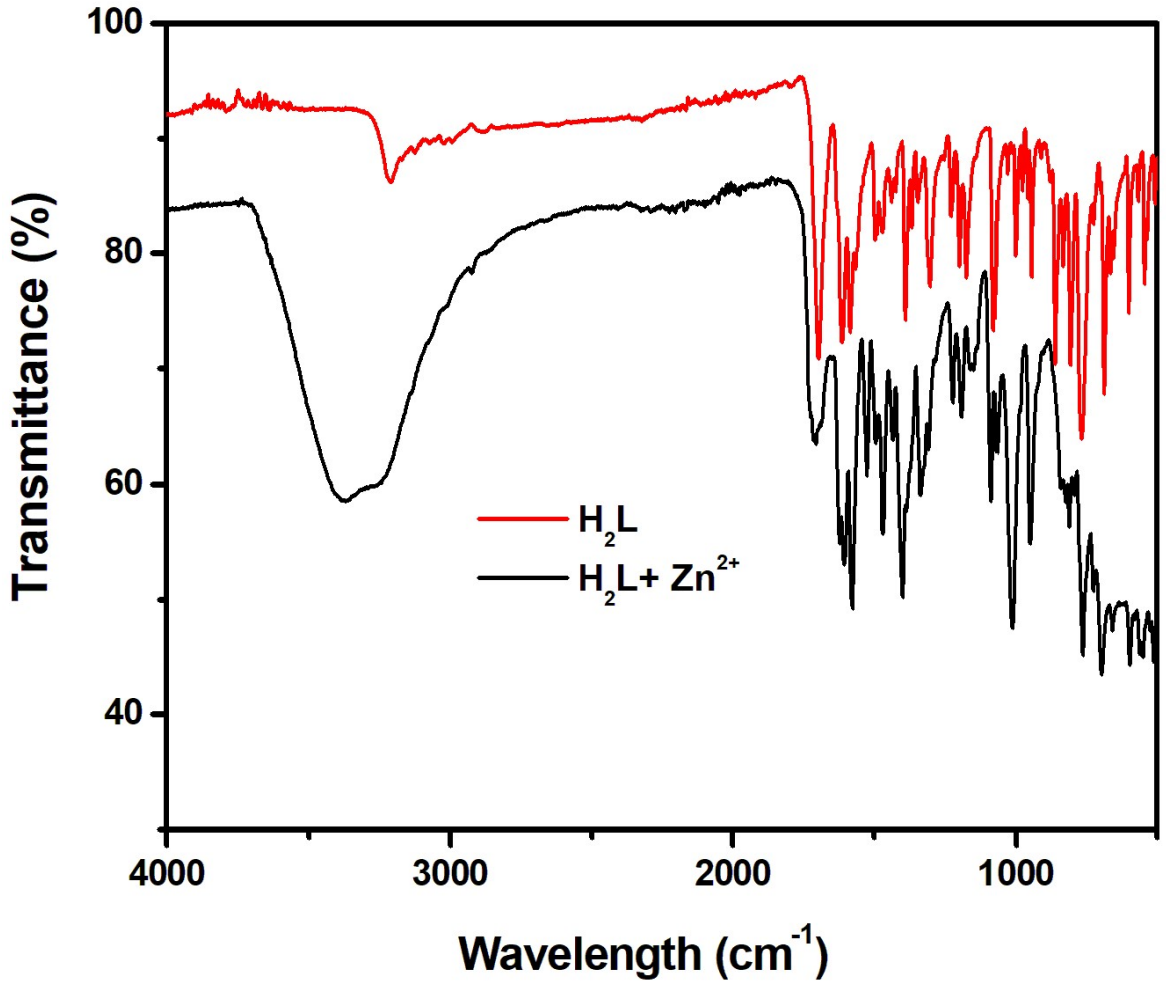


Fig. S16 IR Spectrum of Zn^{2+} Complex.

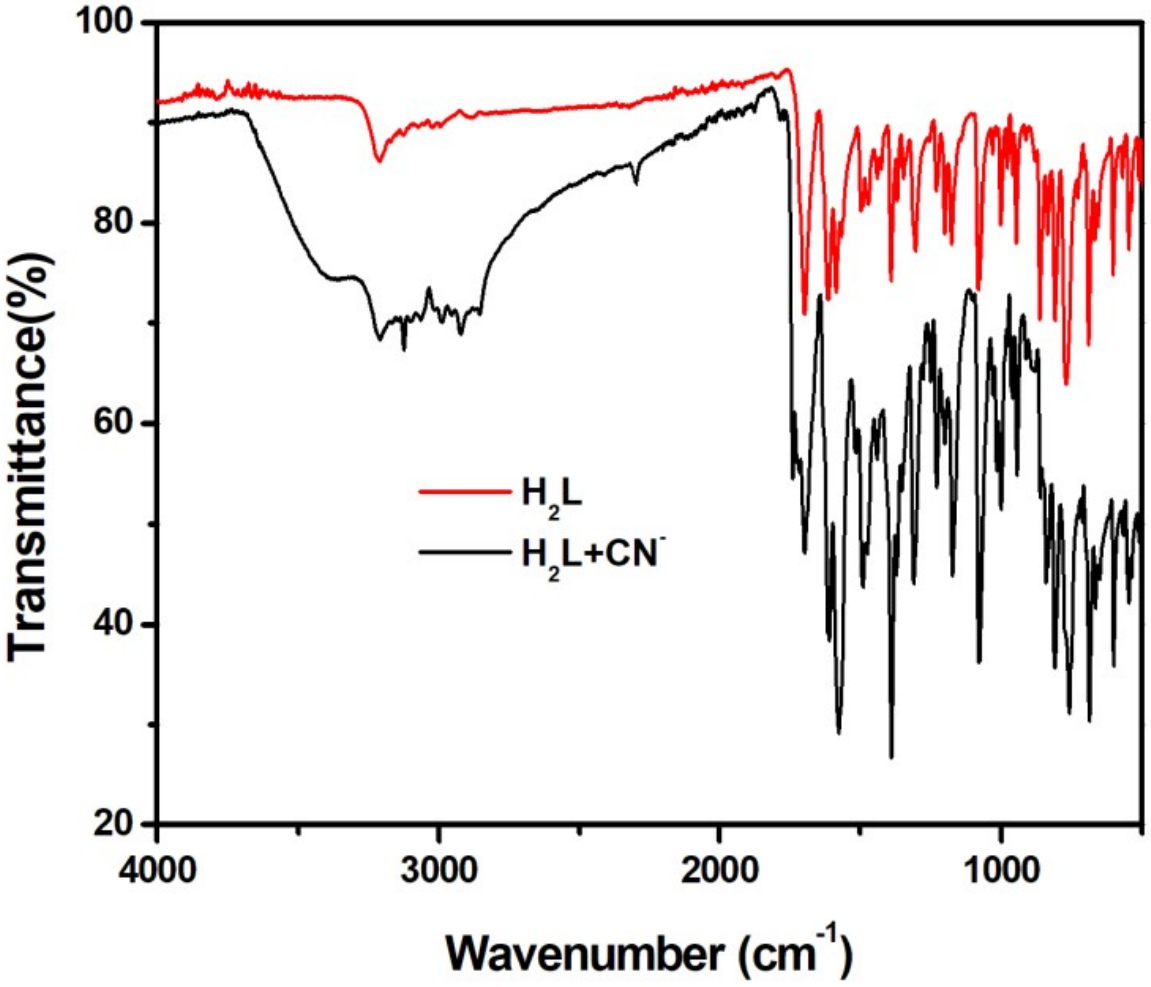


Fig. S17 IR spectrum of CN^- Complex

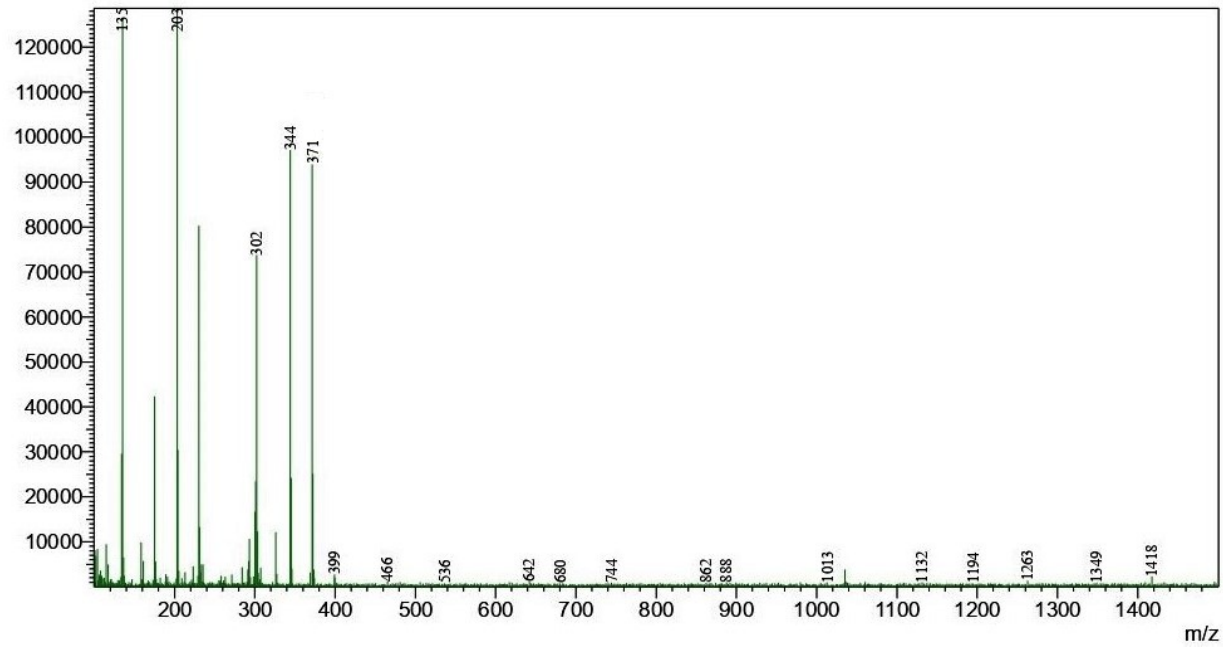


Fig. S18 ESI-MS of CN^- Complex.

Tables S6: Optimized Geometry of H_2L, $[Zn_6L_6]$ and $[L\text{-}CN^-]$

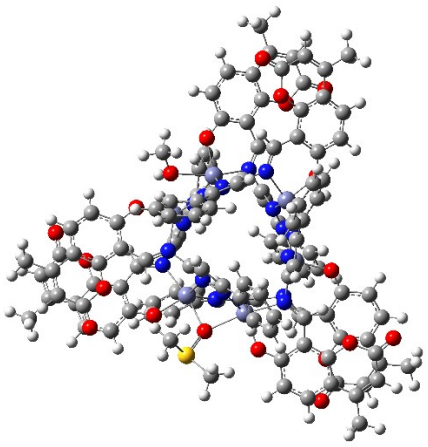
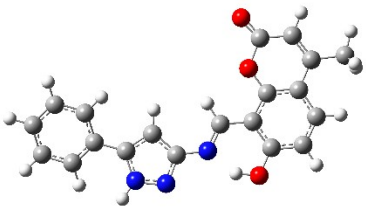
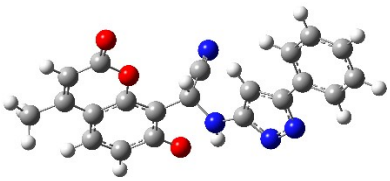
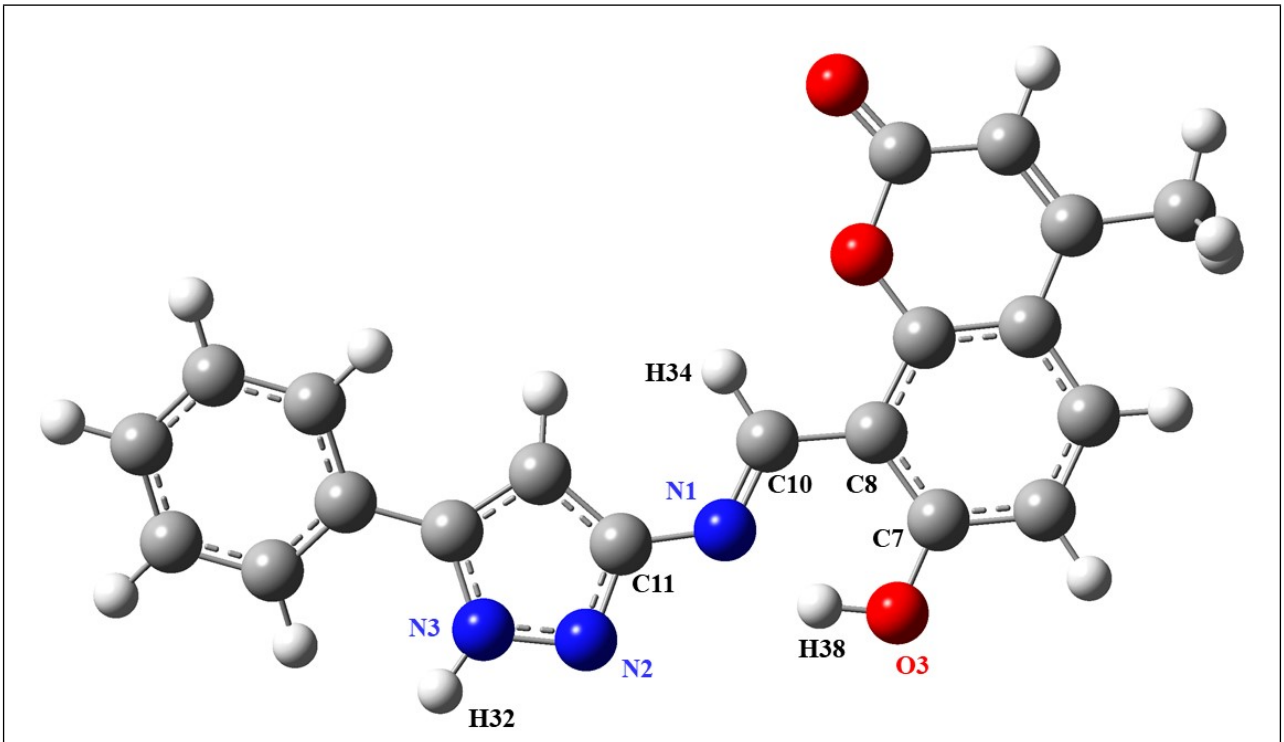
		
$[Zn_6L_6]$	H_2L	$L\text{-}CN^-$

Table S7: Calculated Bond Parameters obtained from DFT calculation of the optimized geometry of H_2L



Bond Length	Theoretical Value (Ă)	Bond Angle	Theoretical Value (°)
C11-N1	1.392	N2-C11-N1	117.53
C11-N2	1.353	N2-N3-H32	118.63
N3-N2	1.367	N3-N2-C11	103.97
N1-C10	1.305	C10-C8-C7	120.95
C7-O3	1.349	C8-C7-O3	120.57
O3-H38	1.034	C7-O3-H38	108.56
N3-H32	1.005	C11-N1-C10	123.19
C10-C8	1.440	N1-C10-C8	118.91
C8-C7	1.424	N1-C10-H34	120.22

Table S8: Selected MO's of H_2L along with their energy.

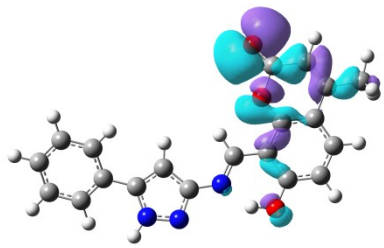
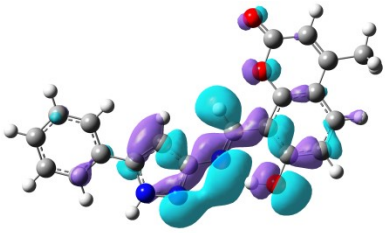
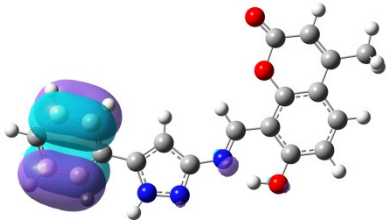
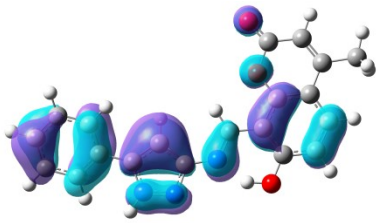
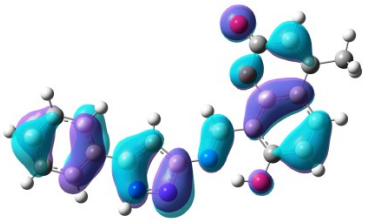
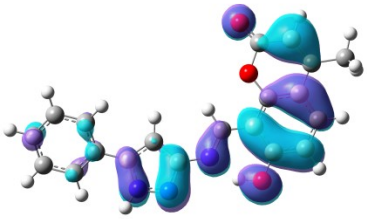
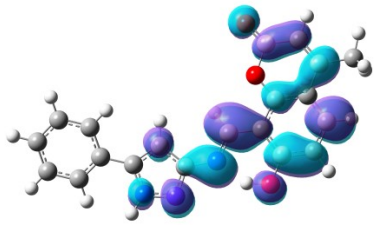
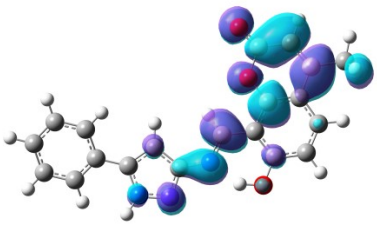
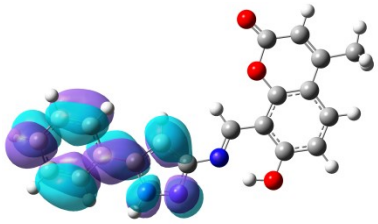
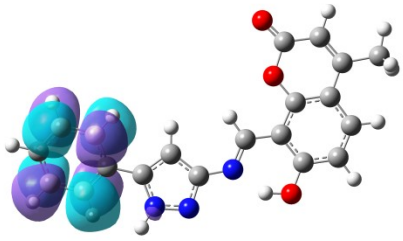
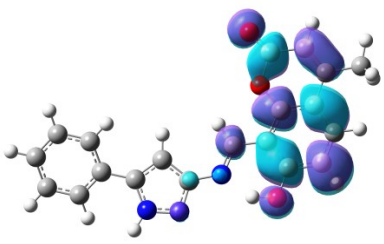
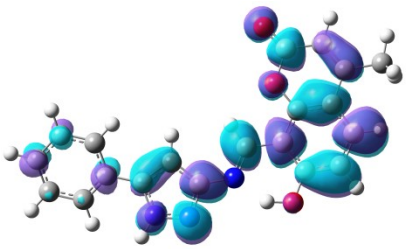
		
HOMO-5 -7.49 eV	HOMO-4 -7.42 eV	HOMO-3 -7.3 eV
		
HOMO-2 -6.59 eV	HOMO-1 -6.28 eV	HOMO -5.98 eV
		
LUMO -2.06 eV	LUMO+1 -1.64 eV	LUMO+2 -1.12 eV
		
LUMO+3 -0.44 eV	LUMO+4 -0.1 eV	LUMO+5 0.63 eV

Table S9: Selected MO's of $[Zn_6L_6]$ Complex with their energy state

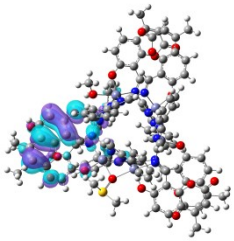
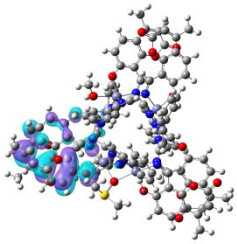
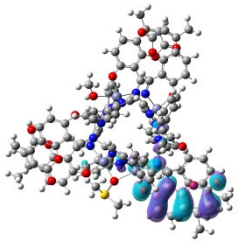
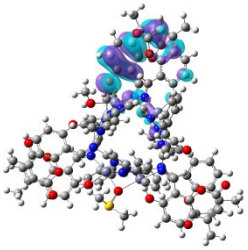
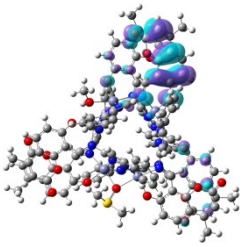
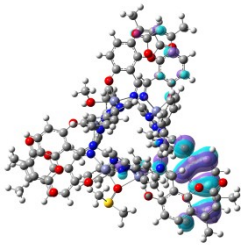
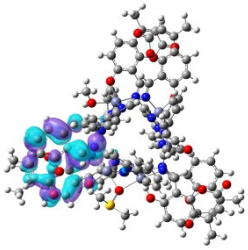
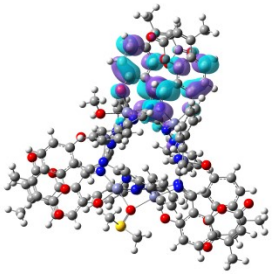
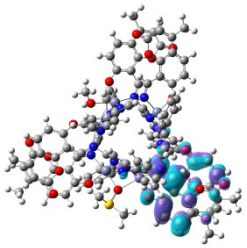
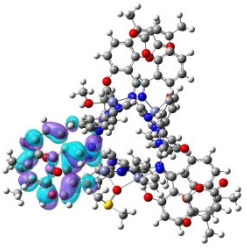
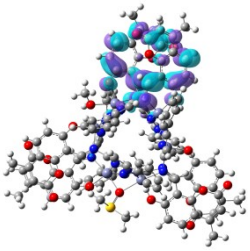
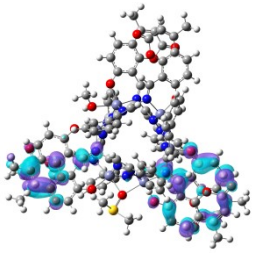
		
HOMO-5 -5.98 eV	HOMO-4 -5.93 eV	HOMO-3 -5.85 eV
		
HOMO-2 -5.84 eV	HOMO-1 -5.75 eV	HOMO -5.72 eV
		
LUMO -2.24 eV	LUMO+1 -2.16 eV	LUMO+2 -2.13 eV
		
LUMO+3 -2.05 eV	LUMO+4 -2.00 eV	LUMO+5 -1.95 eV

Table S10: Selected MO's of L-CN^- complexes with their energy level.

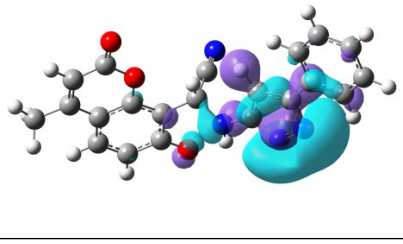
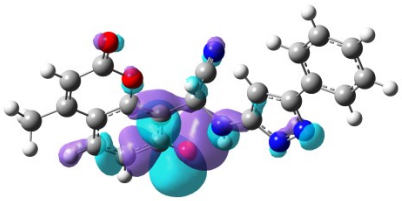
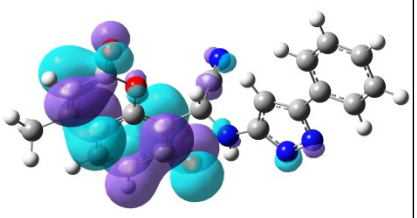
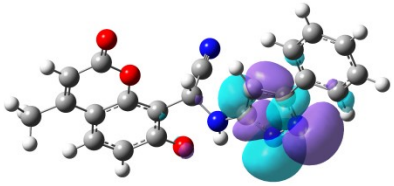
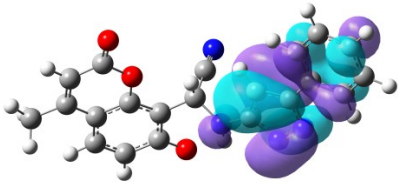
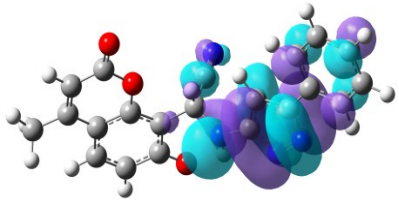
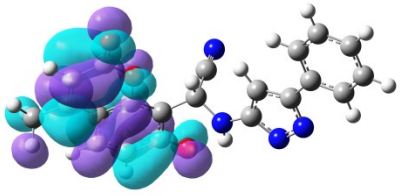
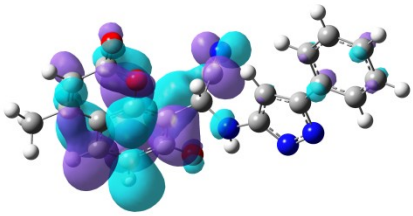
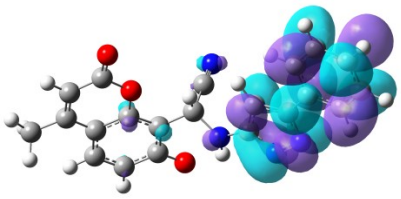
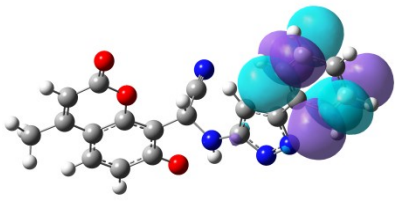
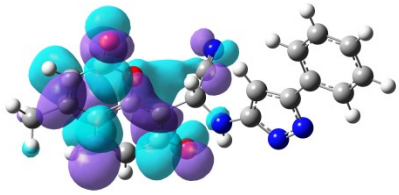
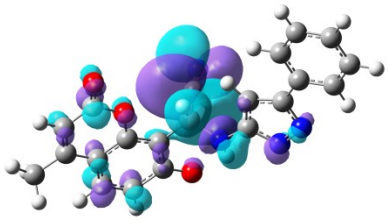
		
HOMO-5 -1.03 eV	HOMO-4 -0.75 eV	HOMO-3 0.05 eV
		
HOMO-2 0.43 eV	HOMO-1 0.61 eV	HOMO 1.55 eV
		
LUMO 3.34 eV	LUMO+1 4.92 eV	LUMO+2 4.95 eV
		
LUMO+3 5.09 eV	LUMO+4 5.42 eV	LUMO+5 6.4 eV

Table S11: TD-DFT transition of H_2L, $[Zn_6L_6]$ and $[L\text{-}CN^-]$ complex.

System	Excitation Energy (eV)	Exp. Wavelength (nm)	Theor. Wavelength (nm)	Oscillation Frequency	Key Transition
H_2L	3.9591	316	313.16	0.8159	HOMO-2→LUMO
$[Zn_6L_6]$	3.2772	339	378.32	0.0018	HOMO-1→LUMO
	3.0436	412	407.05	0.0509	HOMO-1→LUMO+1
$[L\text{-}CN^-]$	3.3103	379	374.55	0.3658	HOMO-1→LUMO

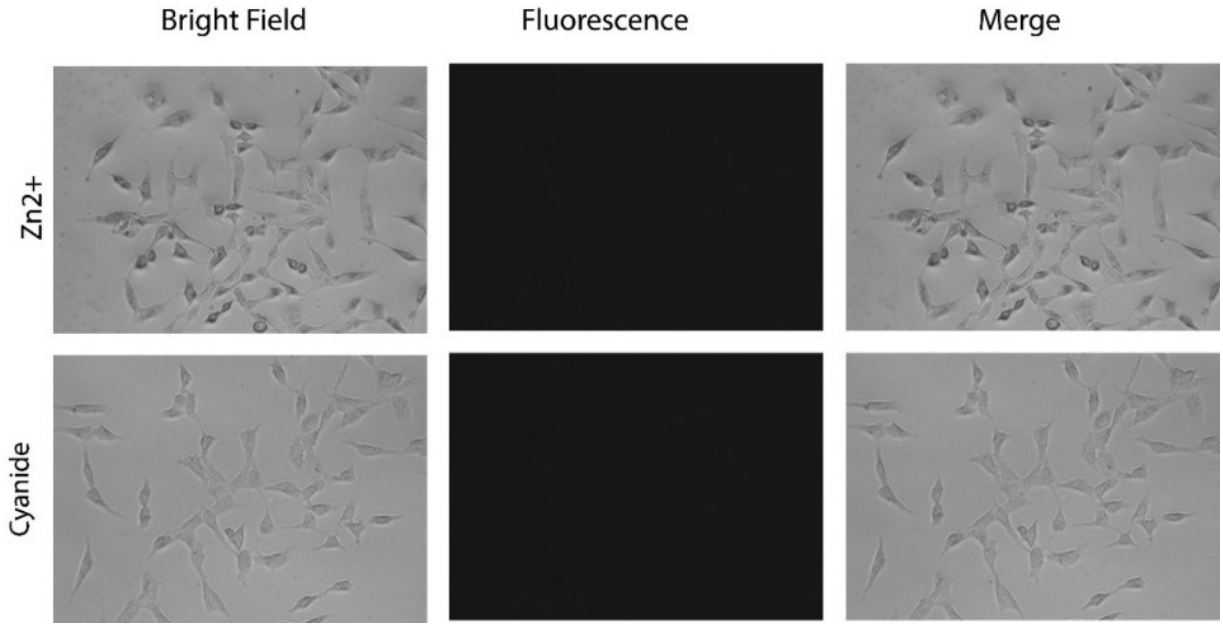


Fig. S19 Microscopic images of MDA-MB 231 cells treated with Zn^{2+}(10μM) and CN^-(10μM) after 30 min incubation period under bright, fluorescence and merged field.

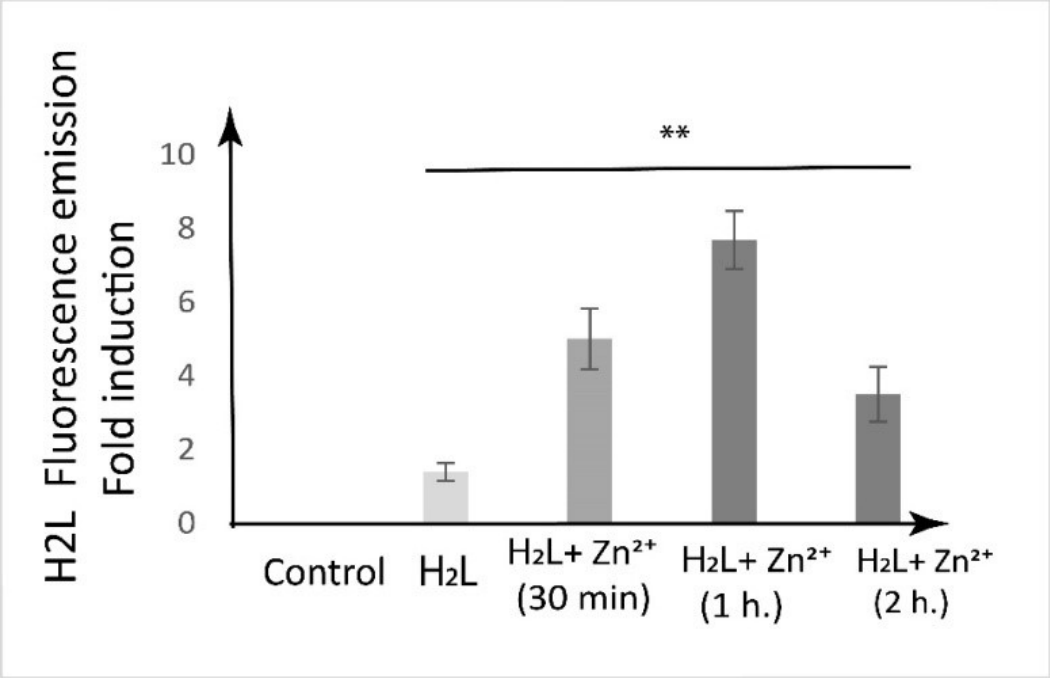


Fig. S20 H_2L Fluorescence emission fold induction in untreated MDA-MB 231 cells (Control), cells treated with H_2L (10μM), H_2L (10μM) + Zn^{2+} (10μM) after 30 min, 1h and 2h

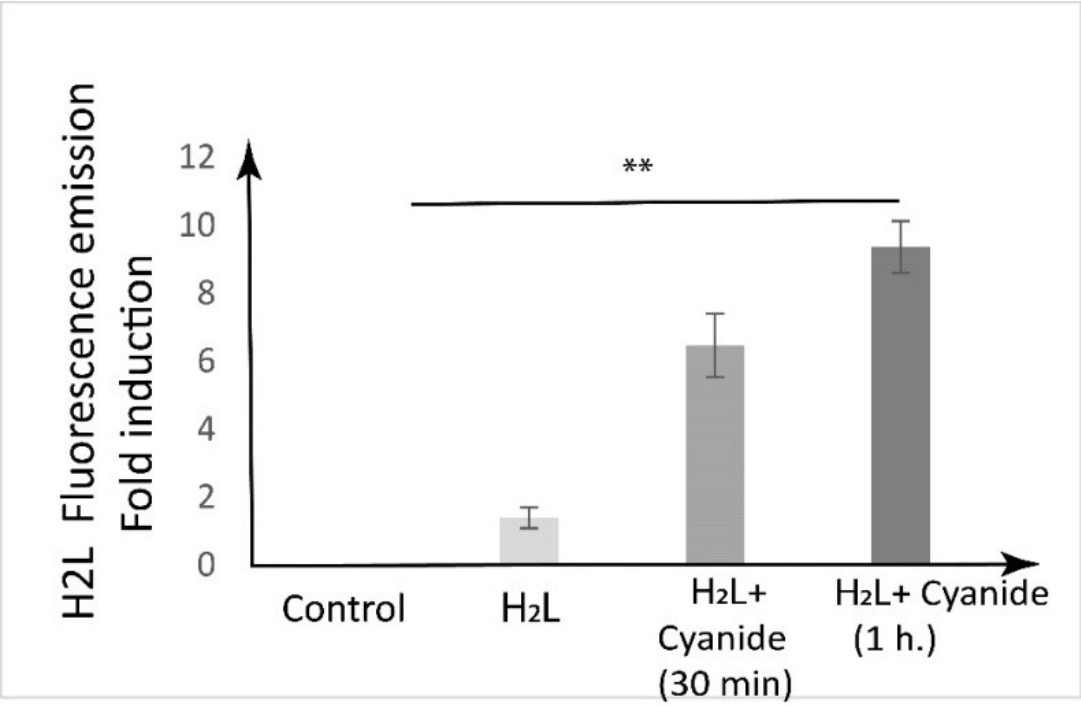


Fig. S21 H_2L Fluorescence emission fold induction inuntreated MDA-MB 231 cells (Control), cells treated with H_2L (10μM), H_2L (10μM) + Cyanide (10μM) after 30 min, 1hr.

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