Electronic Supporting Information (ESI)

Tuning of uracil derivative for AIE based detection of pyrene at nano-molar level: Single crystal X-ray structure of the probe and DFT support

Mahuya Banerjee[a], Milan Ghosh,[a] Sabyasachi Ta,[a] Subhasis Ghosh[a] and Debasis Das*[a]
Department of Chemistry, The University of Burdwan, Burdwan, 713104, West Bengal, India.
Correspondence: <ddas100in@yahoo.com2>; phone, +91-342- 2533913; fax, +91-342-2530452

INDEX

1. Materials and equipment

High-purity HEPES buffer, 6-amino-1, 3-dimethyl uracil, 2-thiouracil, benzoic acid, p-nitroaniline, p-nitrophenol and Sodium Nitrate have been purchased from Sigma−Aldrich (India). The spectroscopic grade solvents have been used whenever it required. Other chemicals are of analytical reagent grade and used without further purification unless specified otherwise. Milli-Q Millipore water (18.2 MΩ cm^{-1}) is used whenever required. A Shimadzu spectrophotometer (Model No-2450) is used for recording UV−vis. spectra. FTIR spectrum is carried out by Shimadzu FTIR (model IR Prestige 21 CE) spectrophotometer. Mass spectra in positive mode is carried out by QTOF 60 Micro YA 263 mass spectrometer. The steady state fluorescence spectra have been recorded with a Hitachi F-4500 spectrofluorimeter. Time-resolved fluorescence lifetime measurements are performed with a picosecond pulsed diode laser-based time-correlated single-photon counting (TCSPC) spectrometer (IBH, UK, λ_{ex} = 384 nm) coupled to MCP-PMT detector (Model FL-1057). A Systronics digital pH meter (model 335) is used for pH measurement. ^{1}HNMR spectra are recorded on a Bruker Avance III HD (400 MHz) spectrometer. The GAUSSIAN-09 revision C.01 program package is used for all geometries and energies calculations.

2. General method of UV-Vis and fluorescence titration

Path length of the cells used for absorption and emission studies has 1 cm. For UV-Vis and fluorescence titrations, stock solution of L, L1, L2, and L3 are prepared (20 μM) in DMSO/H_2O (4/1, v/v, pH 7.4) HEPES (20 mM) buffer. Working solutions of L, L1, L2 and pyrene have been prepared from their respective stock solutions. Fluorescence measurements are performed using 5 nm x 5 nm slit width.

3. Job's experiment using fluorescence method

A series of solutions containing L and pyrene are prepared such that the total concentration of pyrene and the probes remained constant (20 μM) in each set. The mole fraction (X) of the probes are varied from 0.1 to 0.9. The fluorescence intensity at its emission point is plotted against the mole fraction of the ligands (L) in solution.

4. Real sample analysis

Among all PAHs compounds, pyrene is moderately water soluble. It can easily contaminate water bodies as an effluent from various sources. Hence, development of appropriate optical probe that can detect and estimate traces of pyrene in real samples is a social demand. For analysis, the real samples have been collected from pond and tap and analyzed by the present method. To assess the precision of the method, studies have been performed at different concentrations of pyrene.

5. Solid phase extraction of pyrene using silica immobilized L

The immobilization of L on silica gel (100-120 mesh) is achieved following reported process.[1-2] L (3.5g) and silica (15.5g) are mixed together in 30 mL MeOH and refluxed for 4h. The solvent is removed under vacuum and air dried. A glass column (10 cm × 1 cm) is packed with the immobilized probe with a bed volume, 10 mL This packed column is now ready for sorption-desorption studies of pyrene.

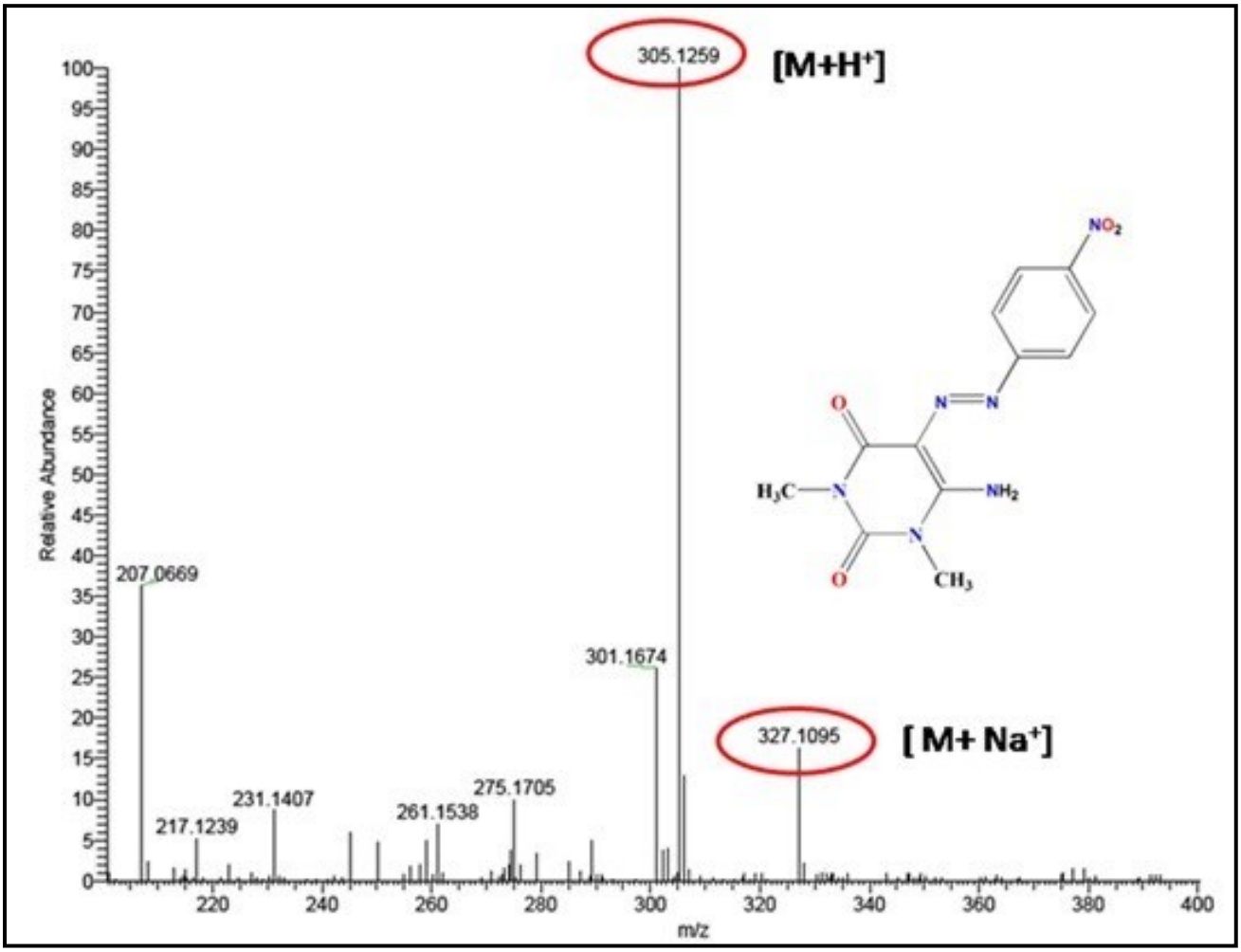


Figure S1 QTOF-MS (ES^+) spectrum of L in MeOH.

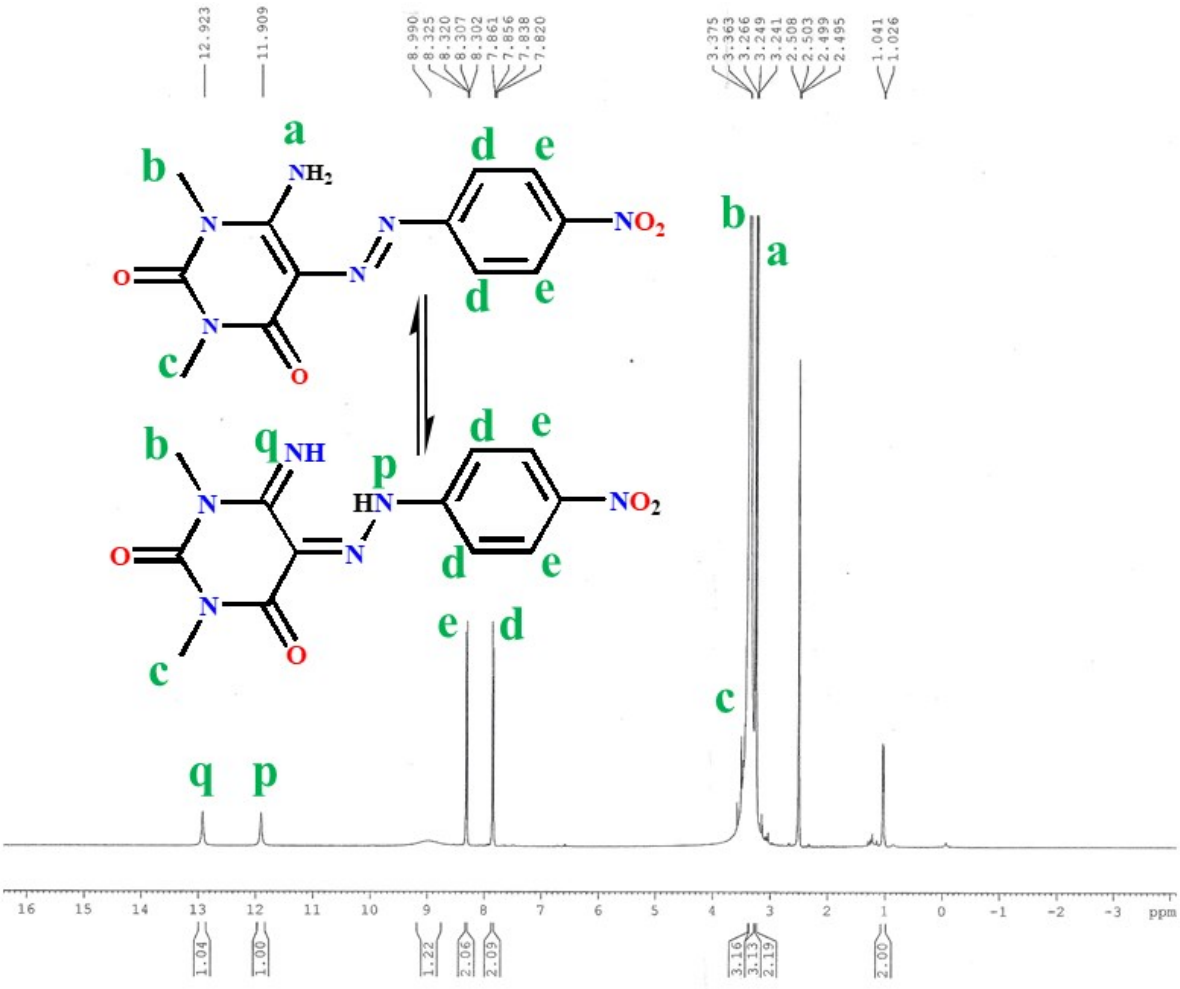


Figure S2 ^{1}HNMR spectrum of L in DMSO-d_6.

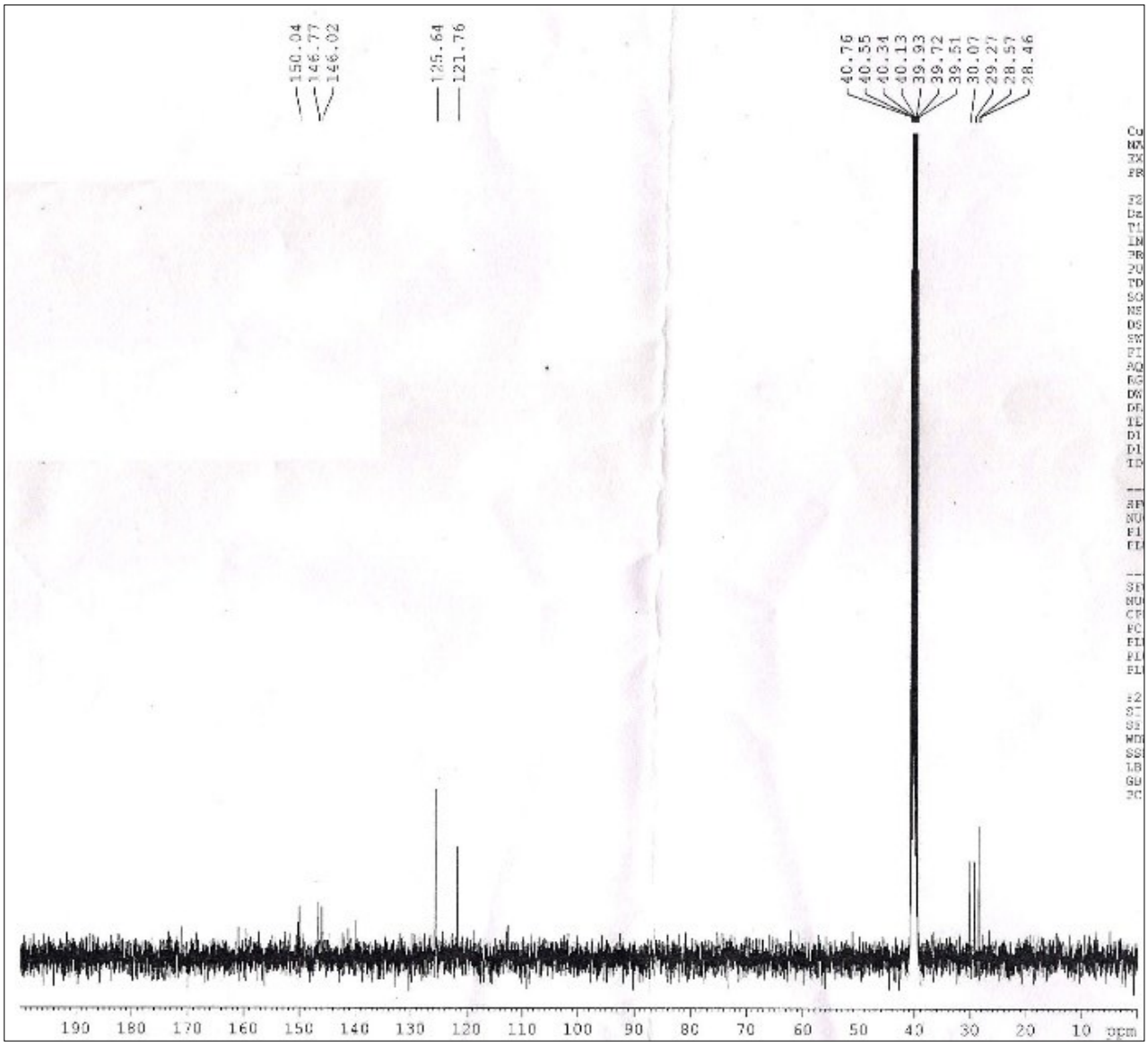


Figure S3 ^{13}CNMR spectrum of L in DMSO-d_6.

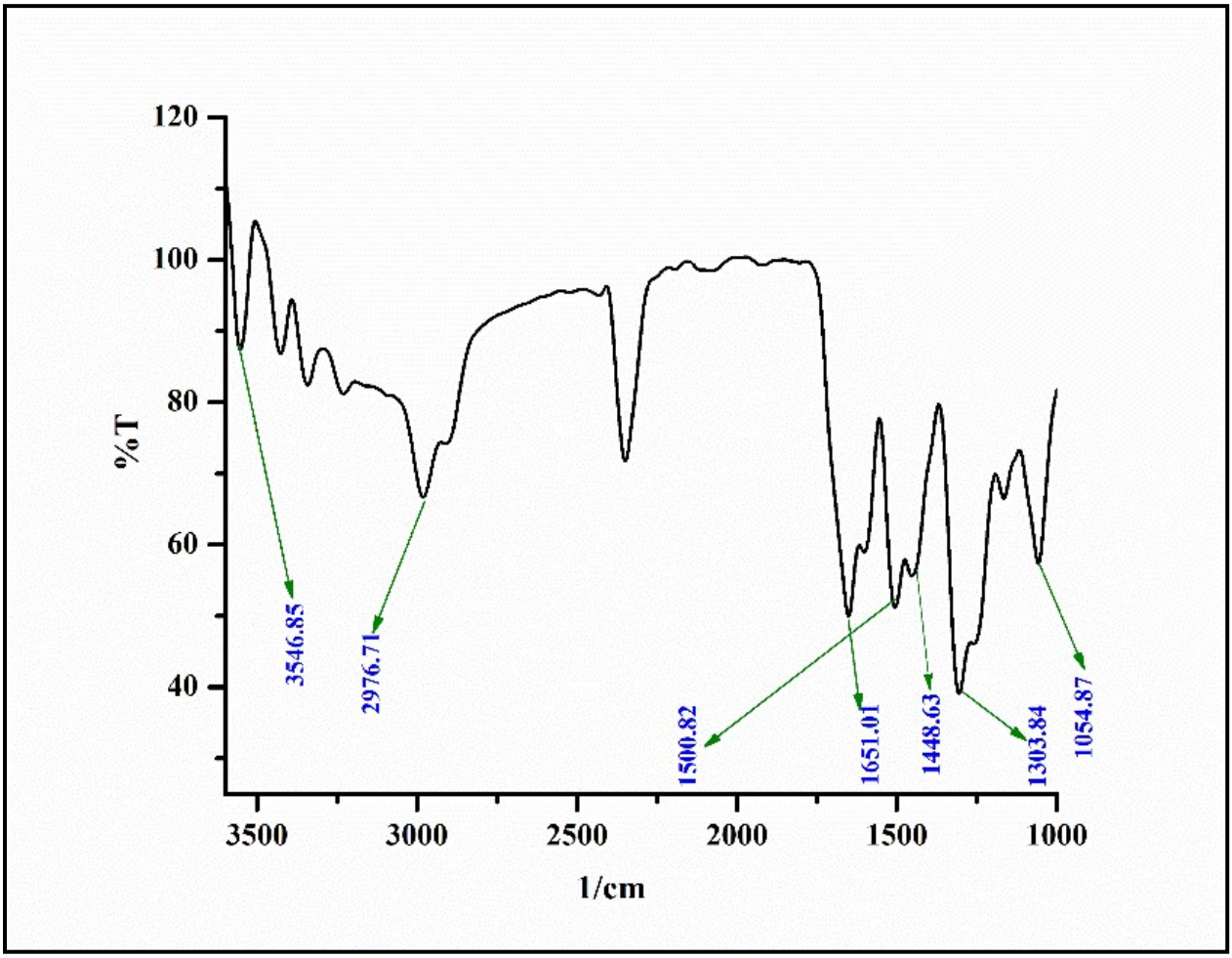


Figure S4 FTIR spectrum of L.

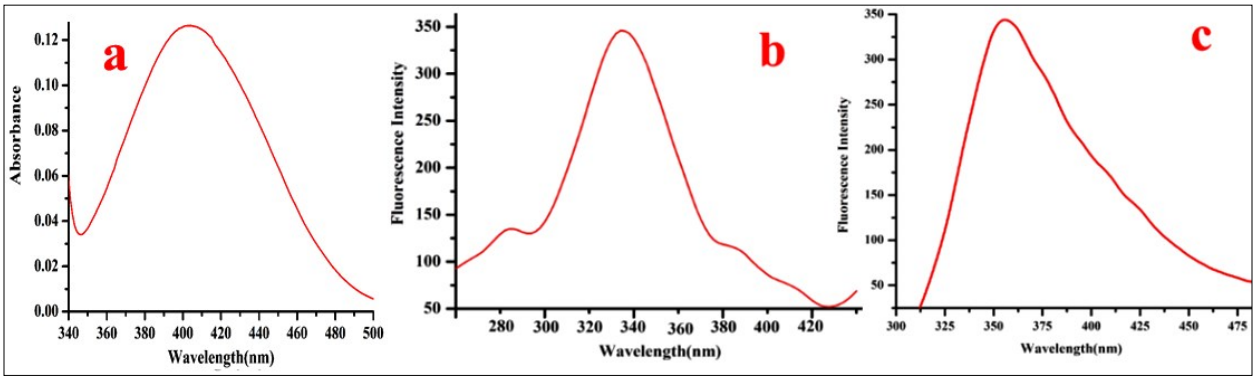


Figure S5 (a) Absorption; (b) excitation and (c) emission spectra of L (DMSO/ H_2O, 4/1, v/v, 20 μM, pH 7.4).

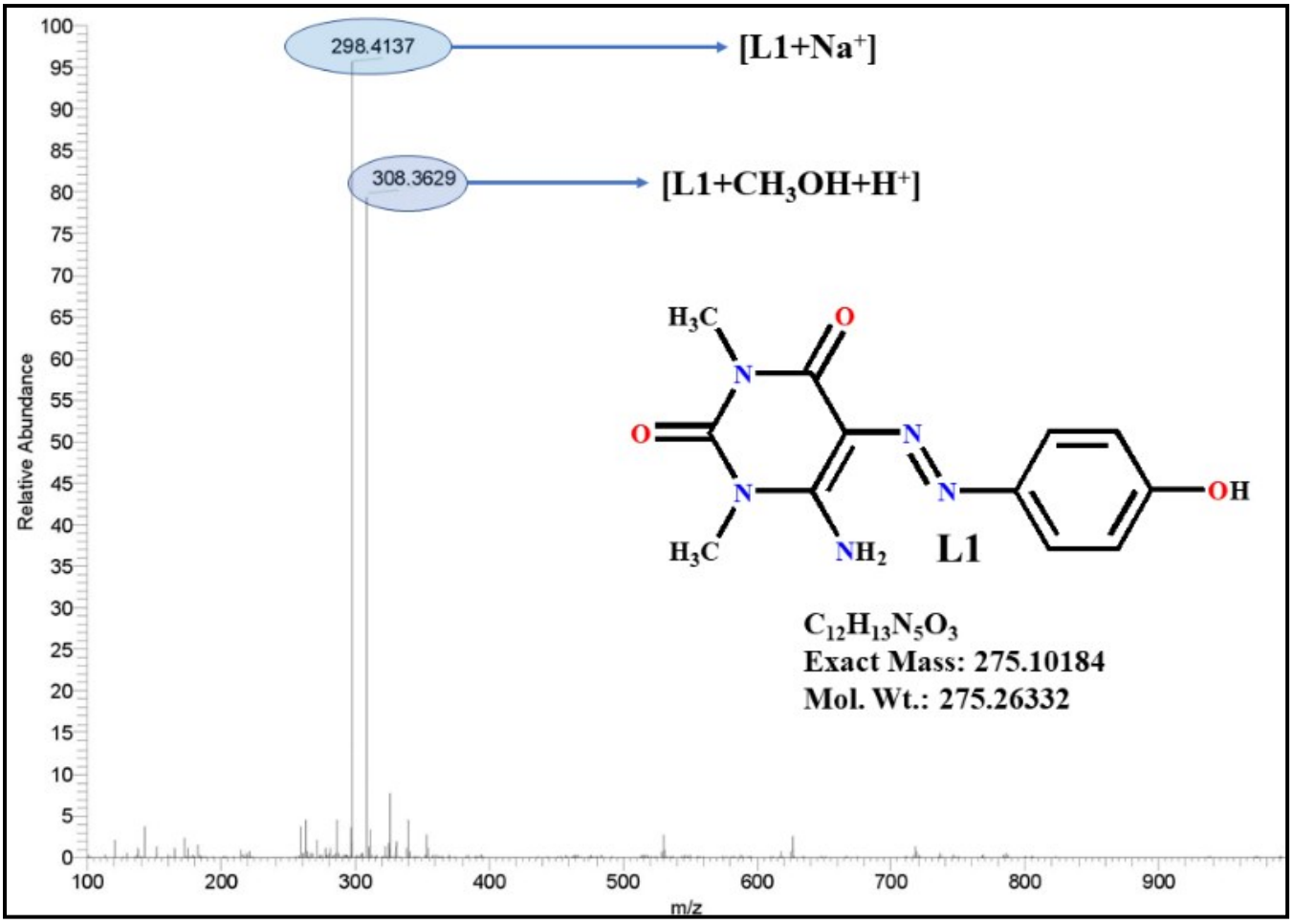


Figure S6 QTOF-MS (ES^+) spectrum of L1 in MeOH.

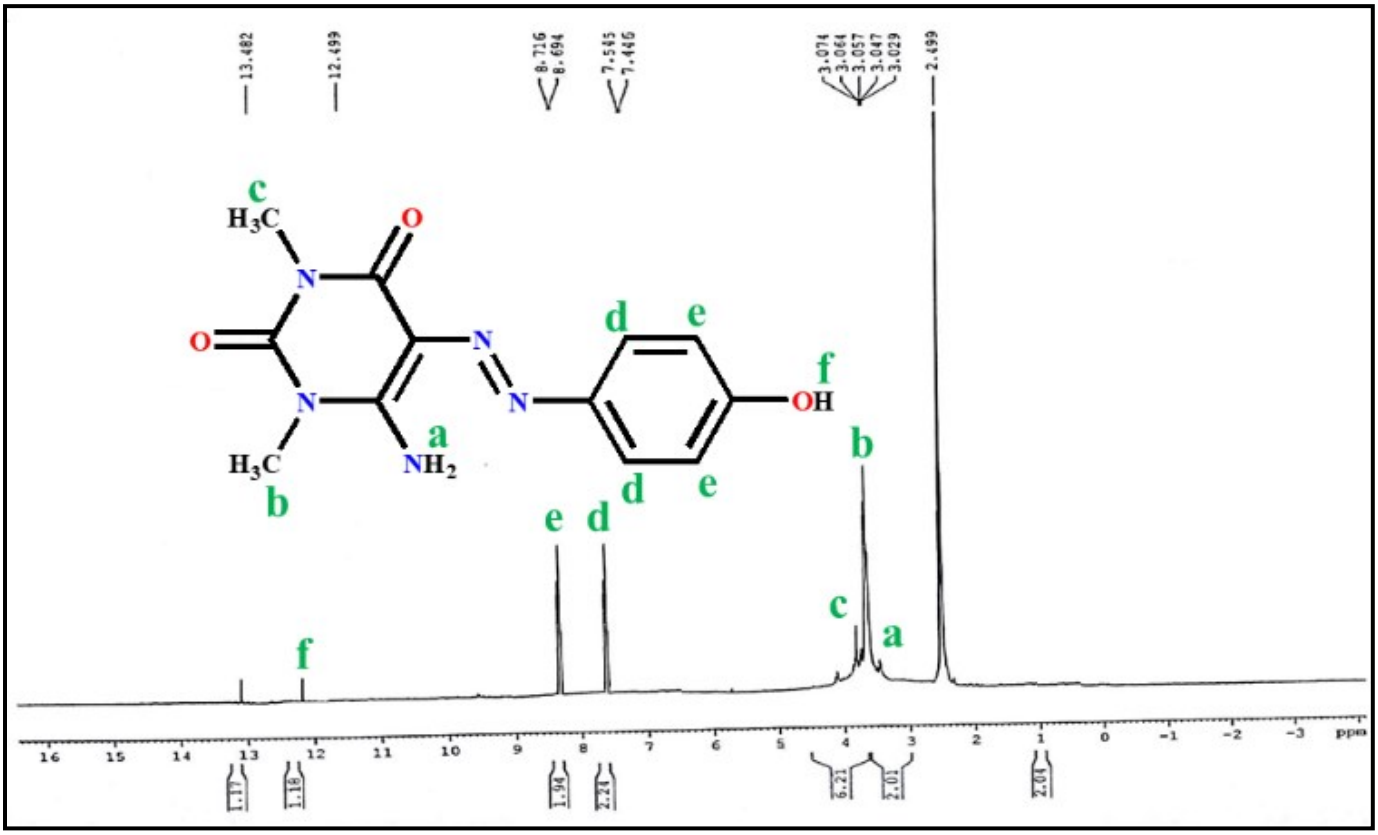


Figure S7 ^{1}HNMR spectrum of L1 in DMSO-d_6.

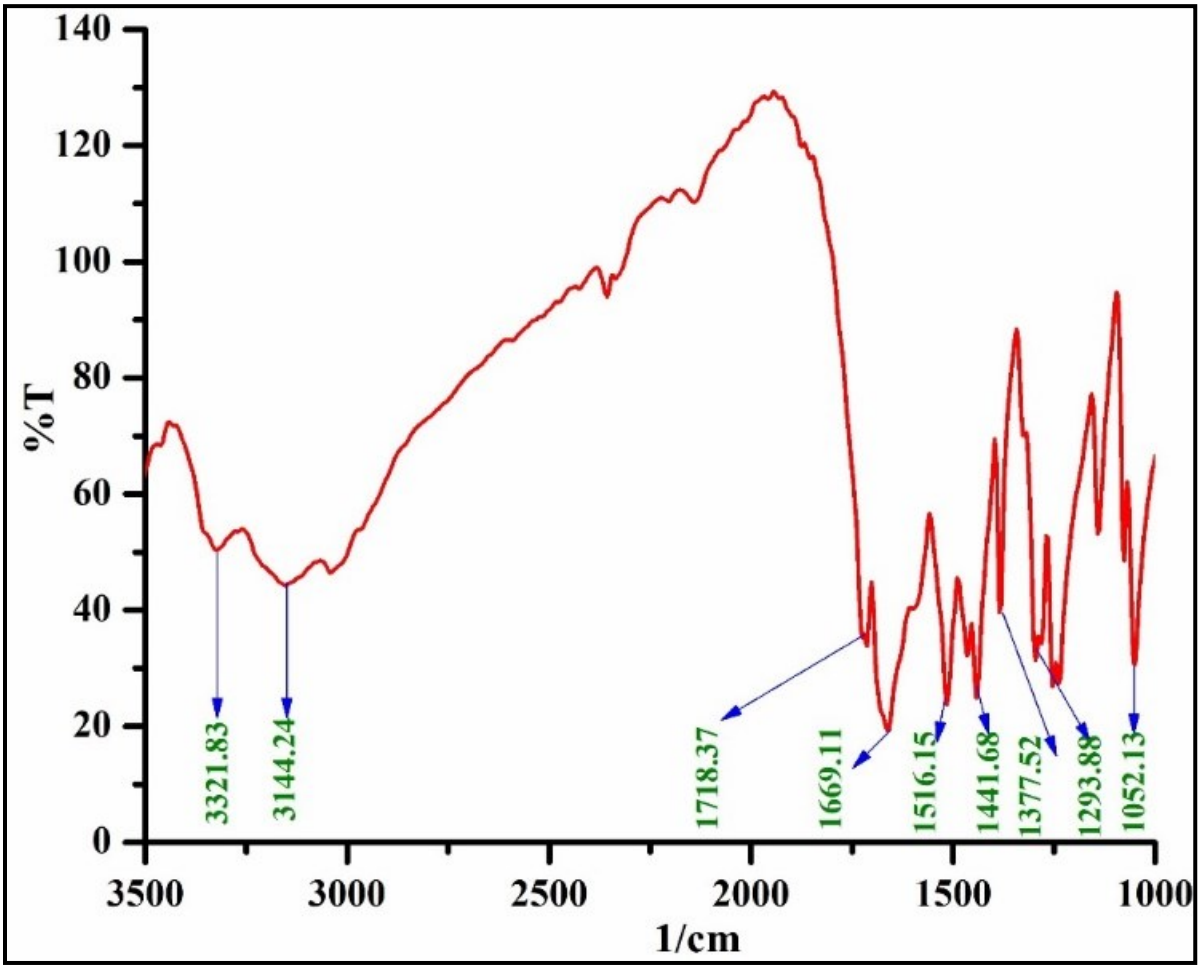


Figure S8 FTIR spectrum of L1.

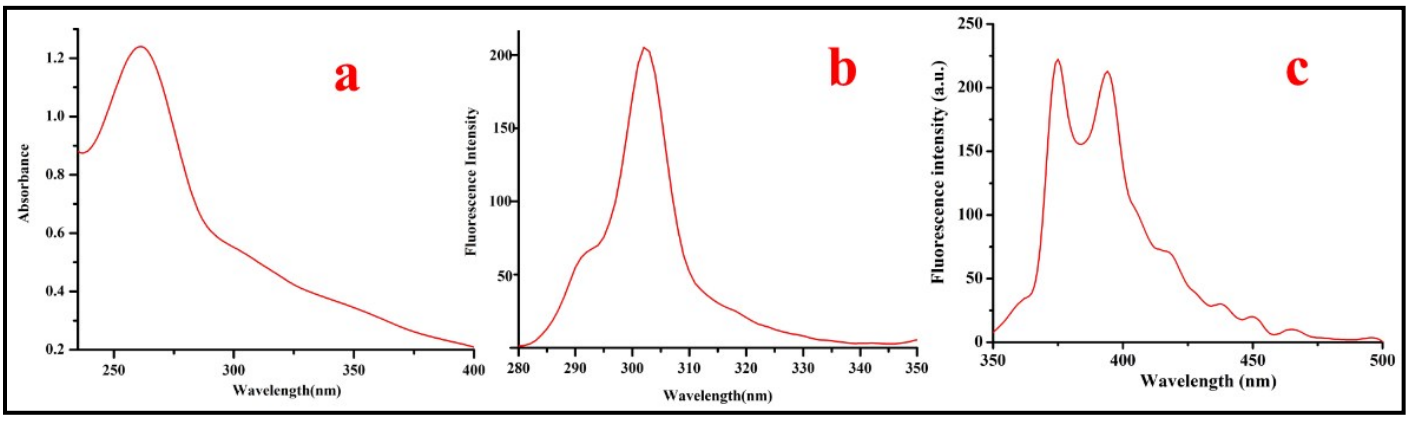


Figure S9 (a) Absorption; (b) excitation and (c) emission spectra of L1 (DMSO/ H_2O, 4/1, v/v, 20 μM, pH 7.4).

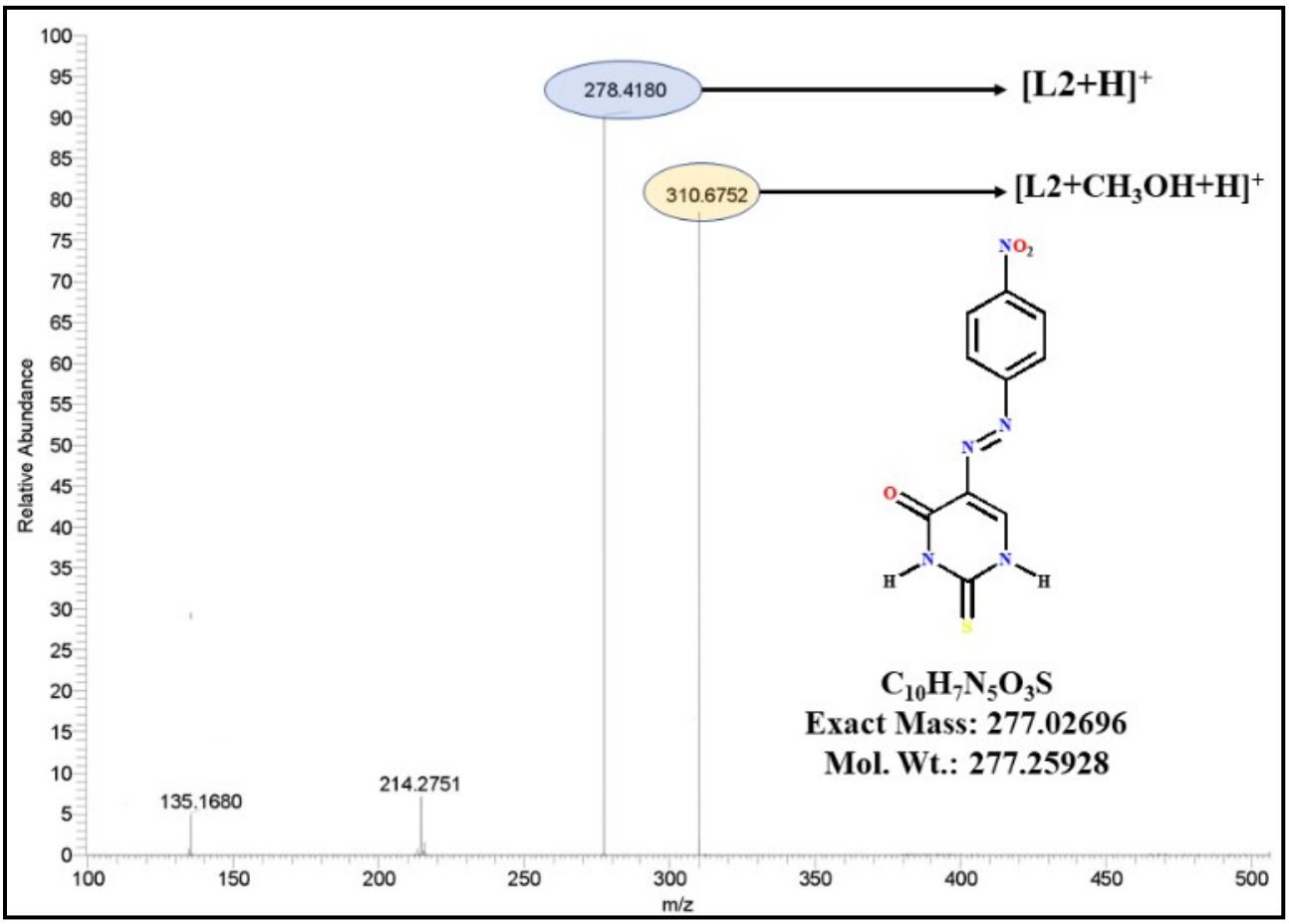


Figure S10 QTOF-MS (ES^+) spectrum of L2 in MeOH.

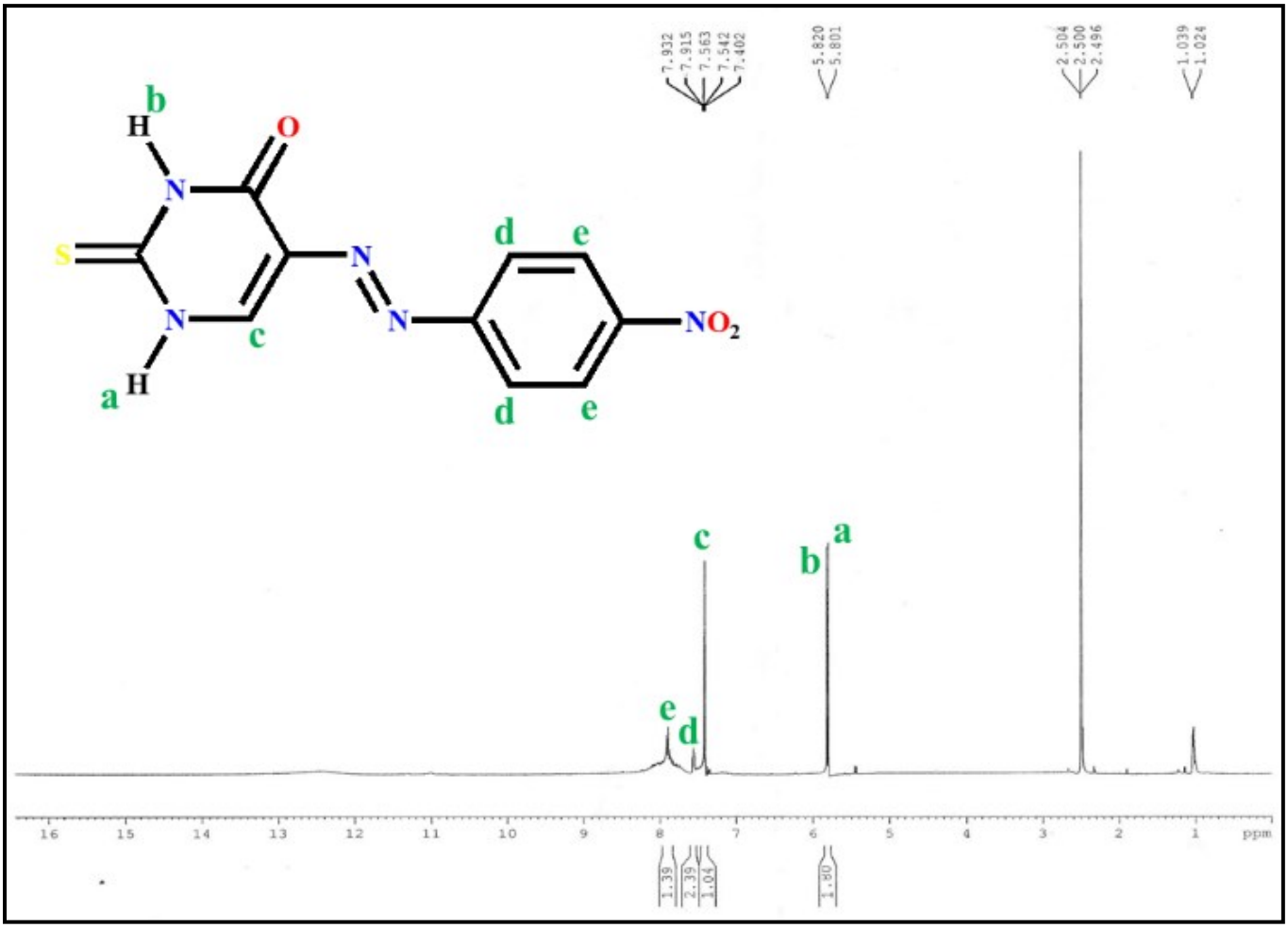


Figure S11 ^{1}H NMR spectrum of L2 in DMSO-d_6.

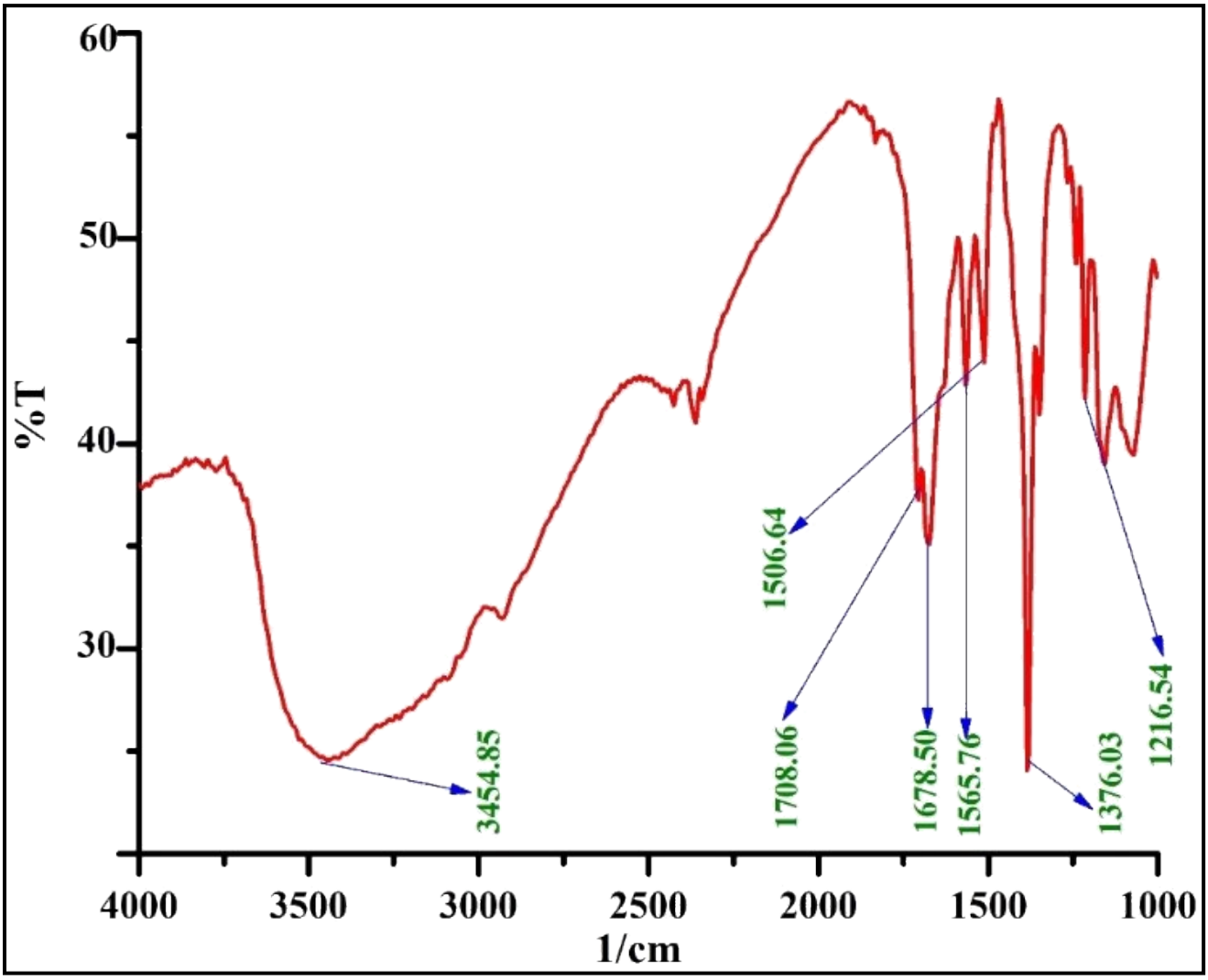


Figure S12 FTIR spectrum of L2.

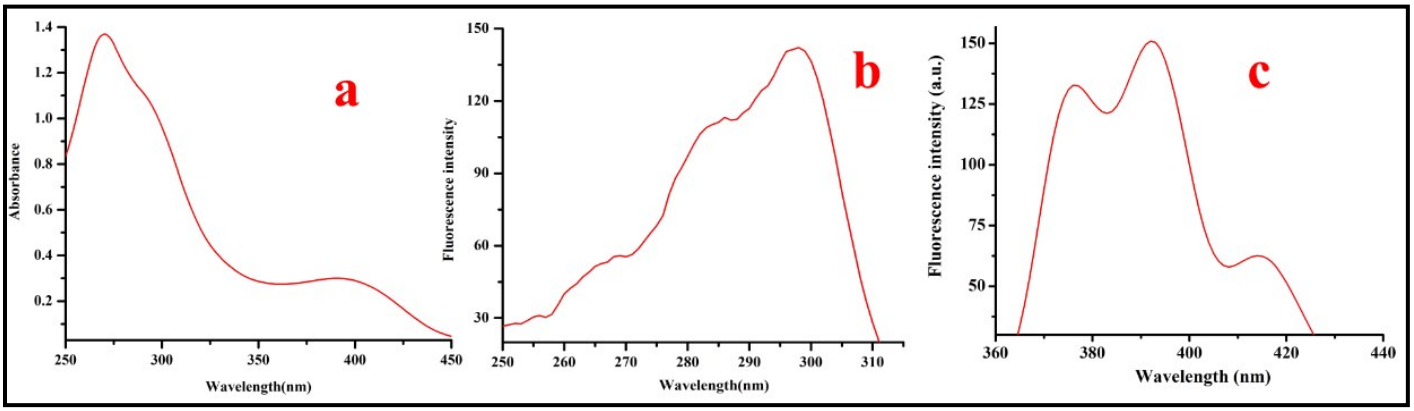


Figure S13 (a) Absorption; (b) excitation and (c) emission spectra of L2 (in DMSO/ H_2O, 4/1, v/v, 20 μM, pH 7.4).

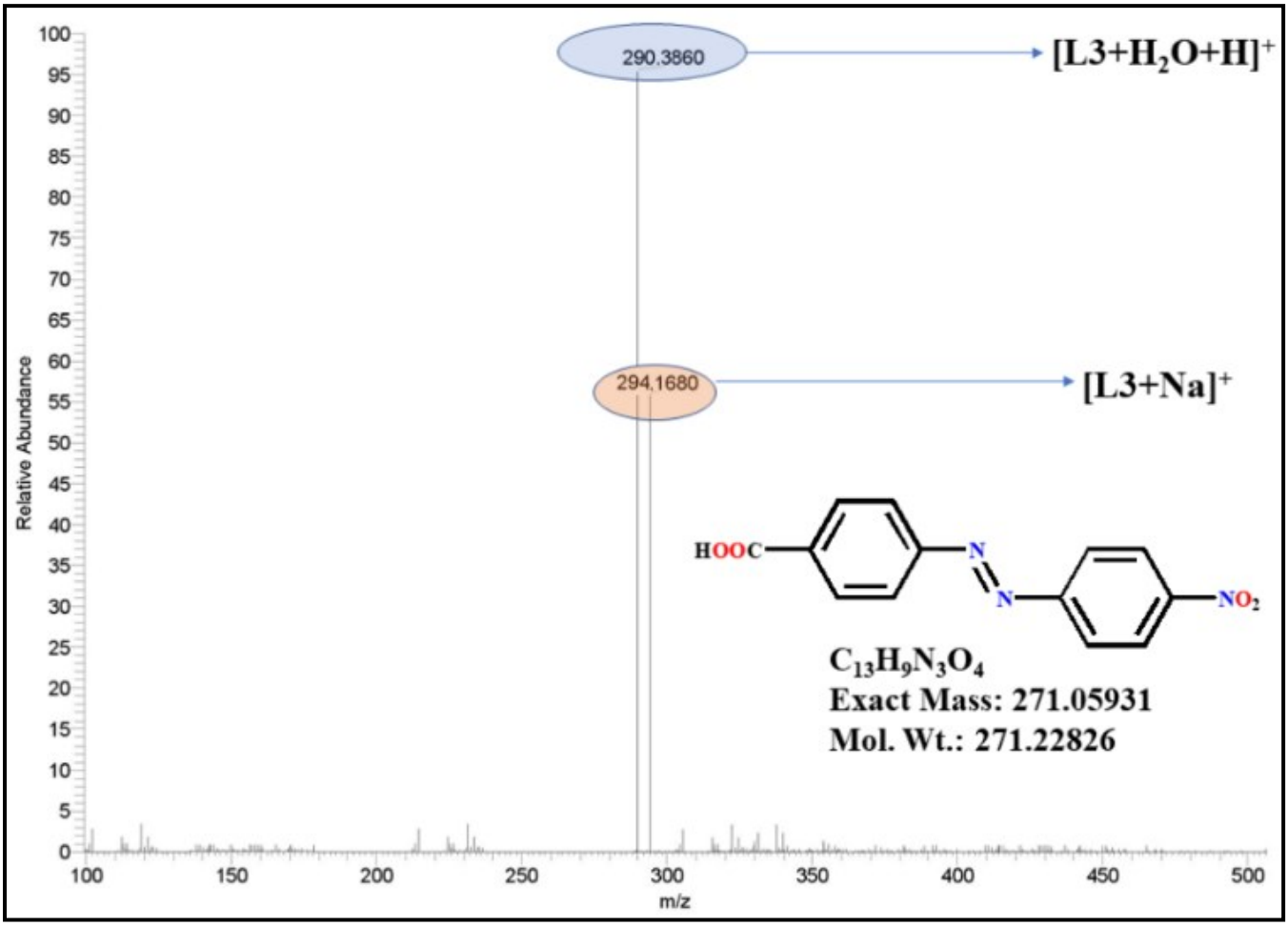


Figure S14 QTOF-MS (ES^+) spectrum of L3 in MeOH.

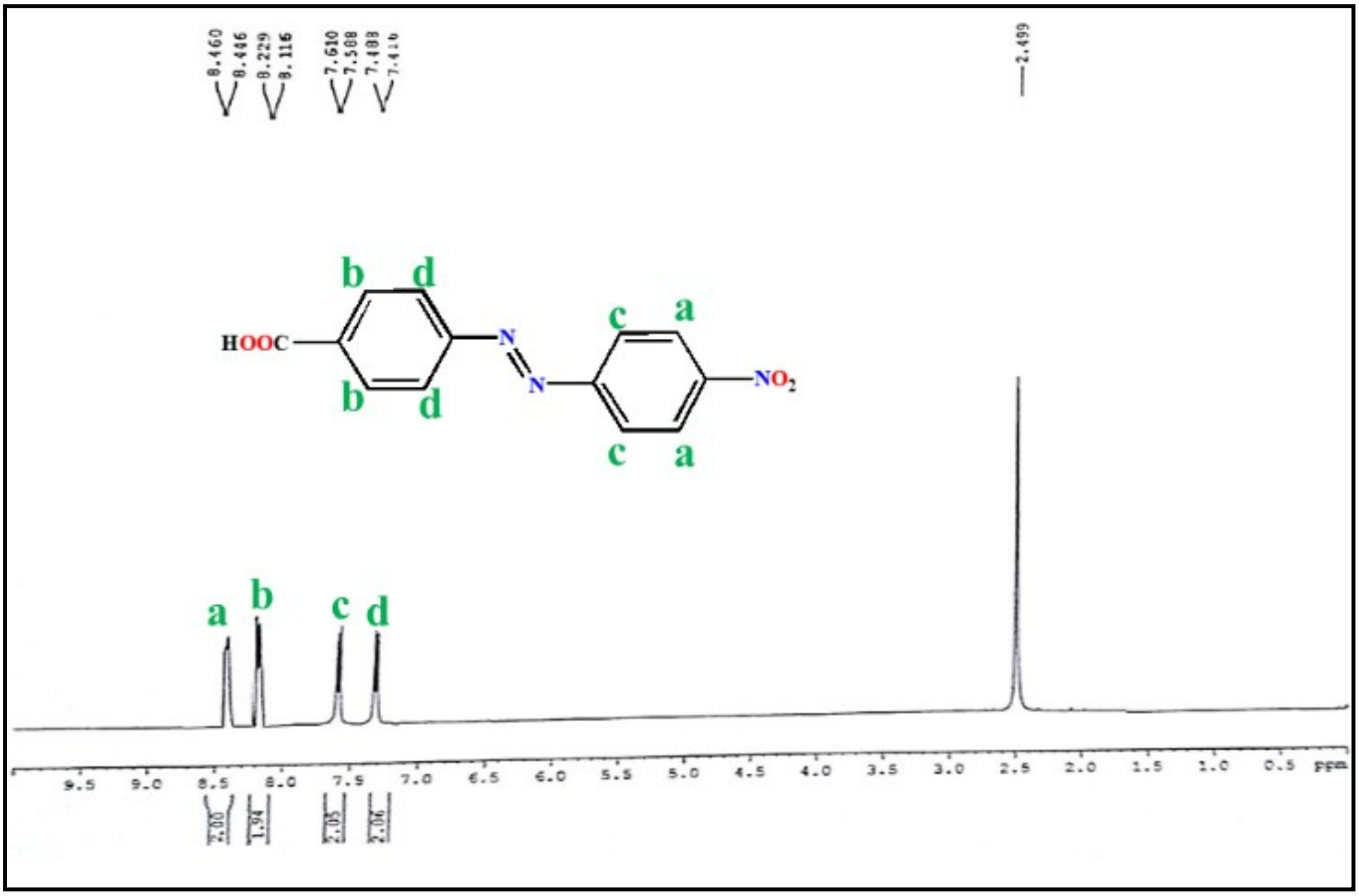


Figure S15 ^{1}HNMR spectrum of L3 in DMSO-d_6

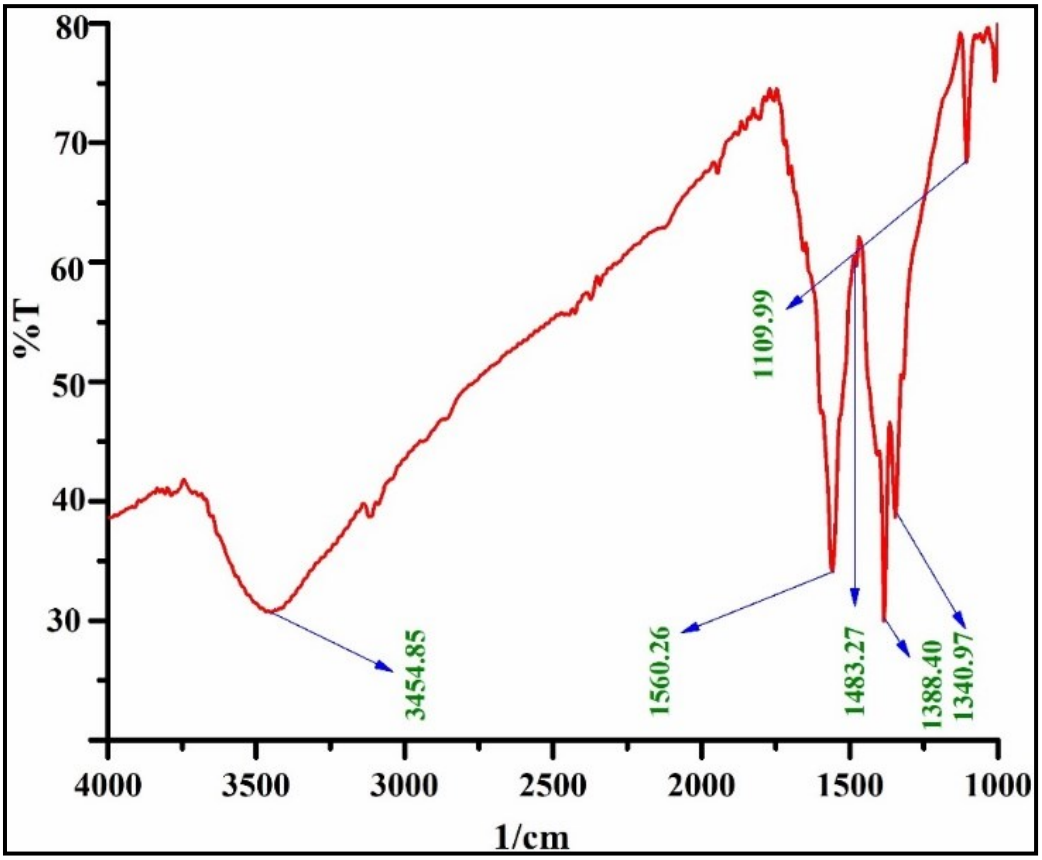


Figure S16 FTIR spectrum of L3.

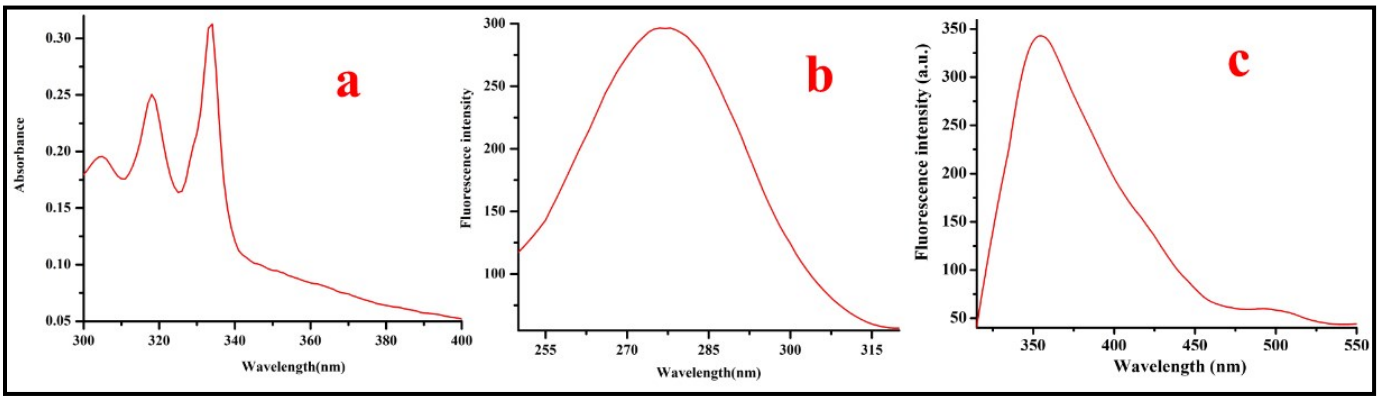


Figure S17 (a) Absoption; (b) excitation and (c) emission spectra of L3 (DMSO/ H_2O, 4/1, v/v, 20 μM, pH 7.4).

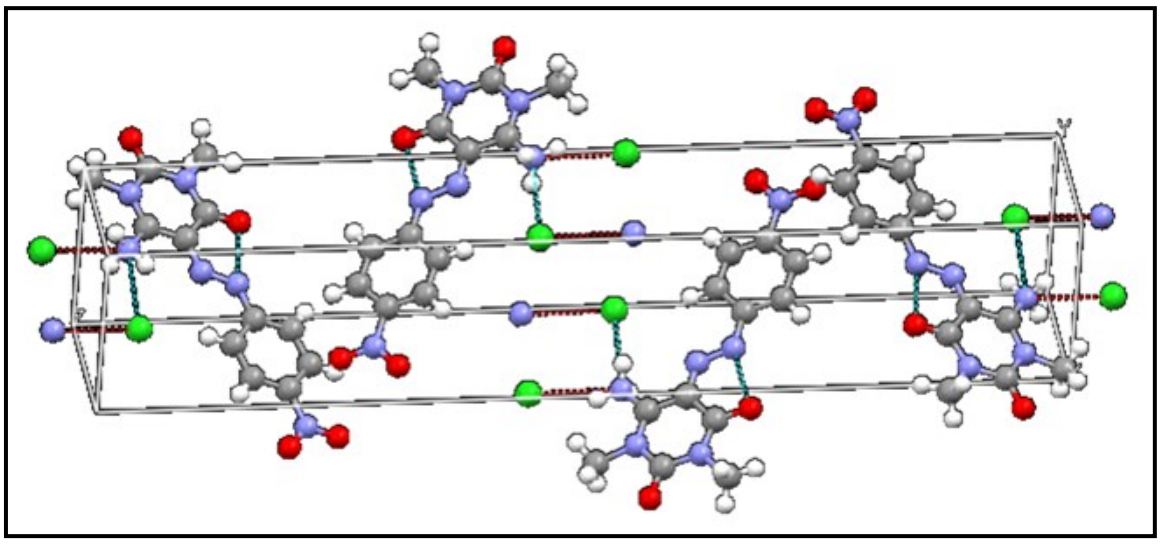


Figure S18 Packing diagram of L

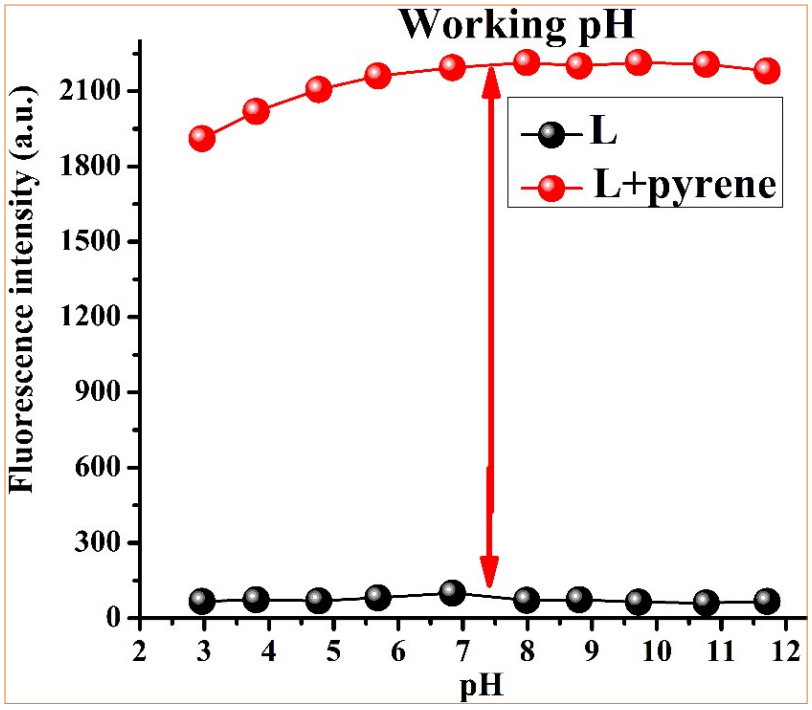


Figure S19 Effect of pH on the emission intensities of free L and its pyrene adduct.

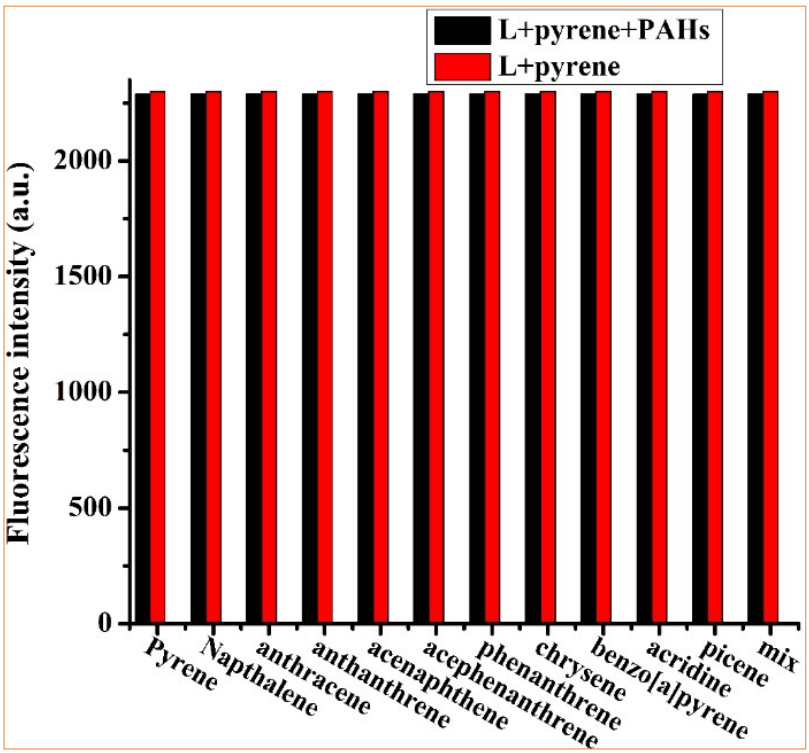


Figure S20 Relative emission intensities of [L-pyrene] adduct (1:1, 20 μM, red bar) in presence of other PAHs (20 μM, black bar) in DMSO/H_2O (4/1; v/v, pH 7.4) (λ_{Ex} = 336 nm).

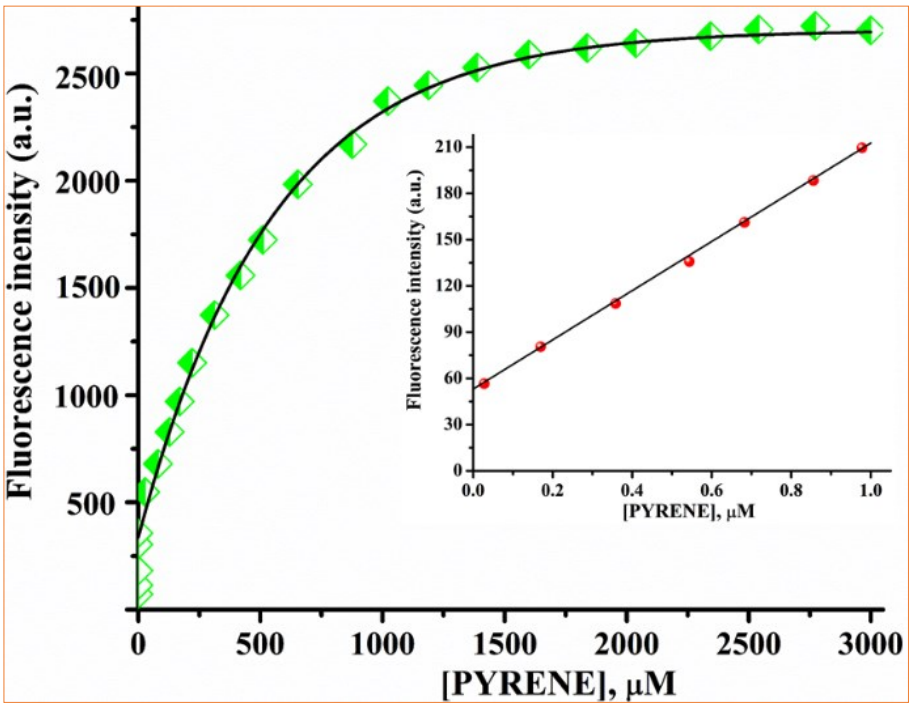


Figure S21 Plot of emission intensities of L (20 μM; DMSO/H_2O, 4/1, v/v; 20 mM HEPES buffer, pH 7.4) as a function of added pyrene (0.0001-3000 μM).

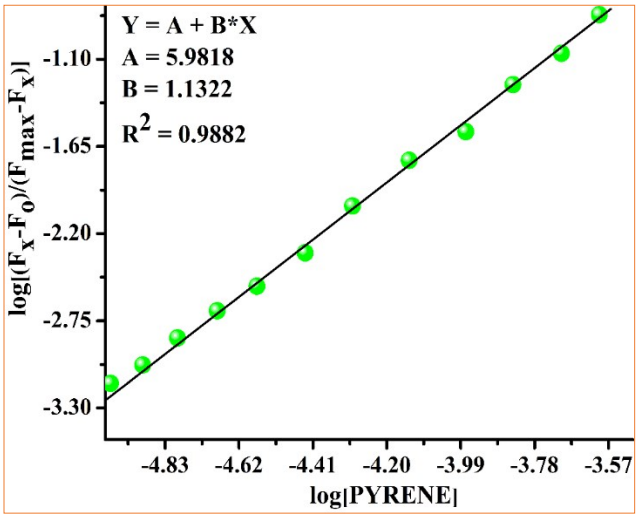
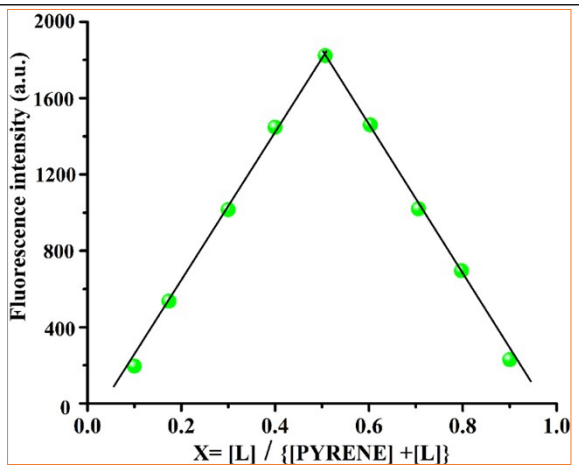
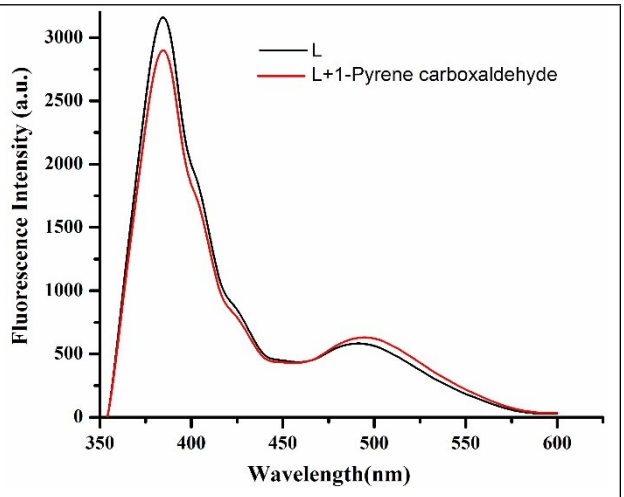


Figure S22 Hill plot for determination of binding constant of L to pyrene (20 μM; DMSO/H_2O, 4/1, v/v; 20 mM HEPES buffer, pH 7.4).

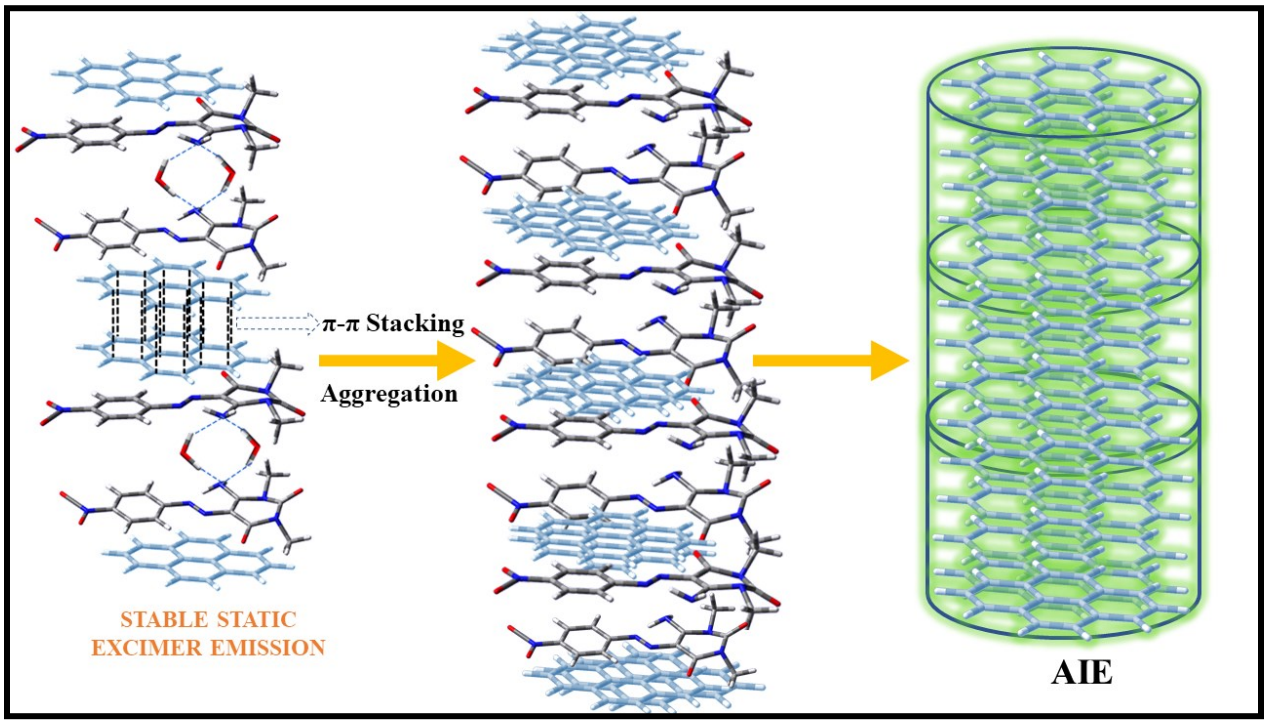


(a)

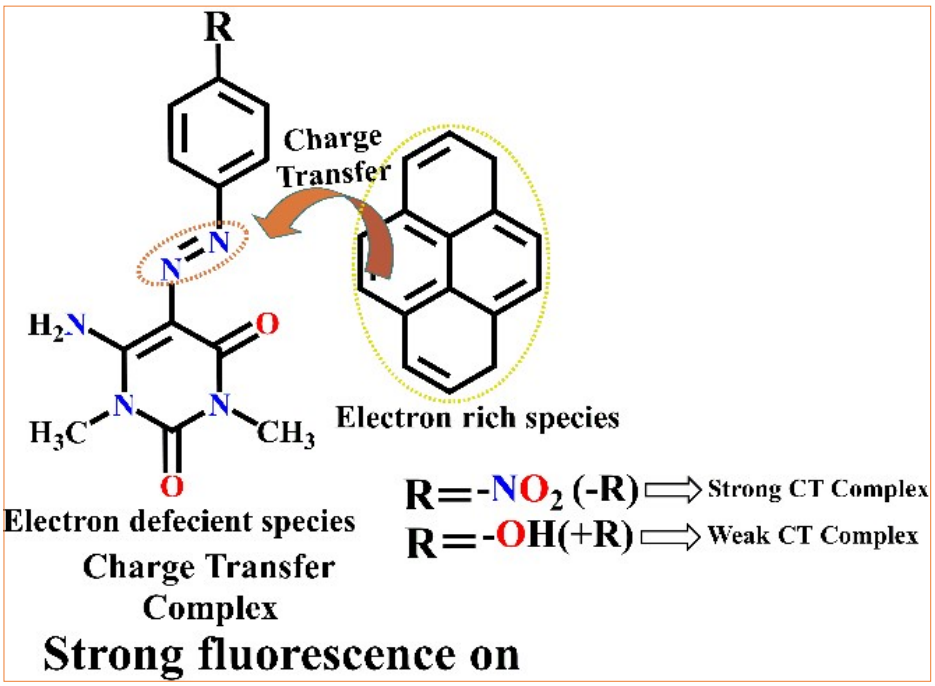


(b)

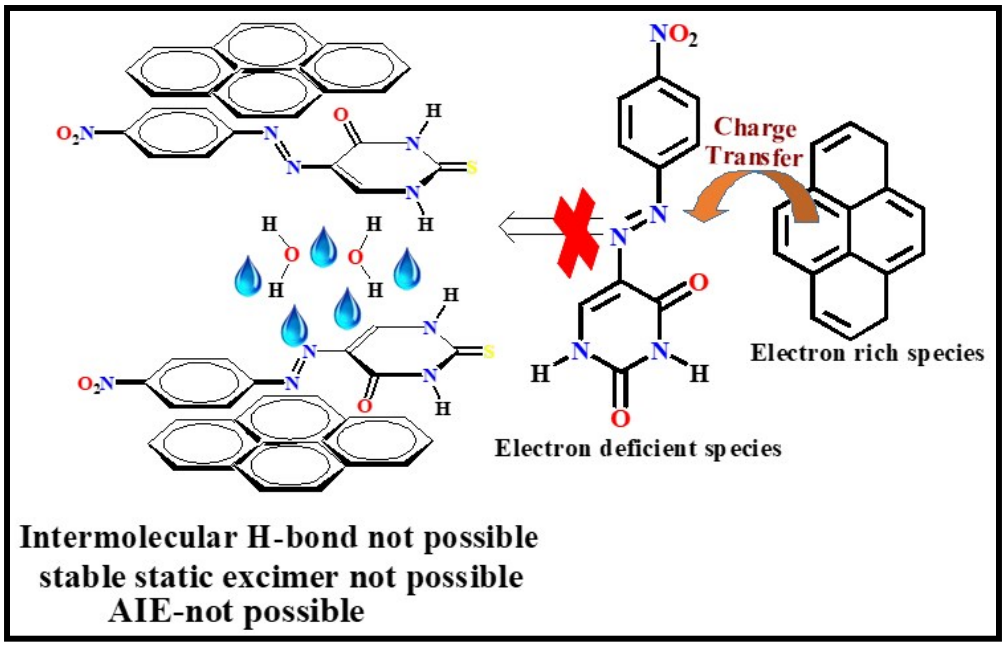
Figure S23 (a) Job's plot for determination of interaction stoichiometry between L and pyrene; (b) Emission intensity of L upon interaction with pyrene 1-carboxaldehyde (DMSO, 20 mM HEPES buffer, pH 7.4, λ_{Ex} = 336 nm)



Scheme S1 Aggregation induced emission of L guided by π–π stacking.



Scheme S2 Fluorescence enhancement through charge transfer (CT) complex.



Scheme S3 Necessity of $-NH_2$ in azo-moiety to stabilize AIE process.

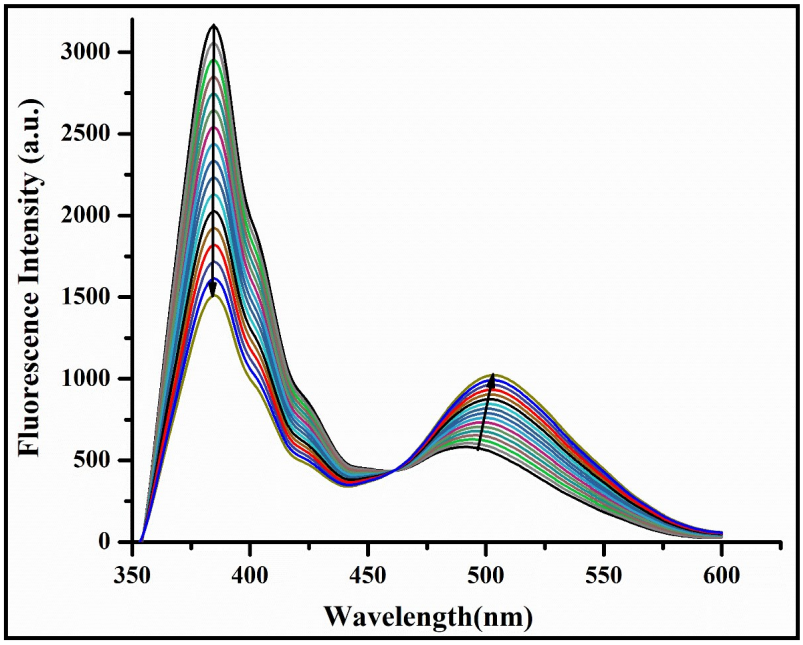


Figure S24 Changes in the emission spectra of L (20 μM) *vs.* [pyrene] at room temperature in DMSO medium (with no added water); [pyrene] = 0.0001, 0.001, 0.01, 0.1, 1, 10, 100, 400, 600, 800, 1000, 1200, 1400, 1500, 2000, 2500 and 3000 μM), λ_{Ex} = 336 nm.

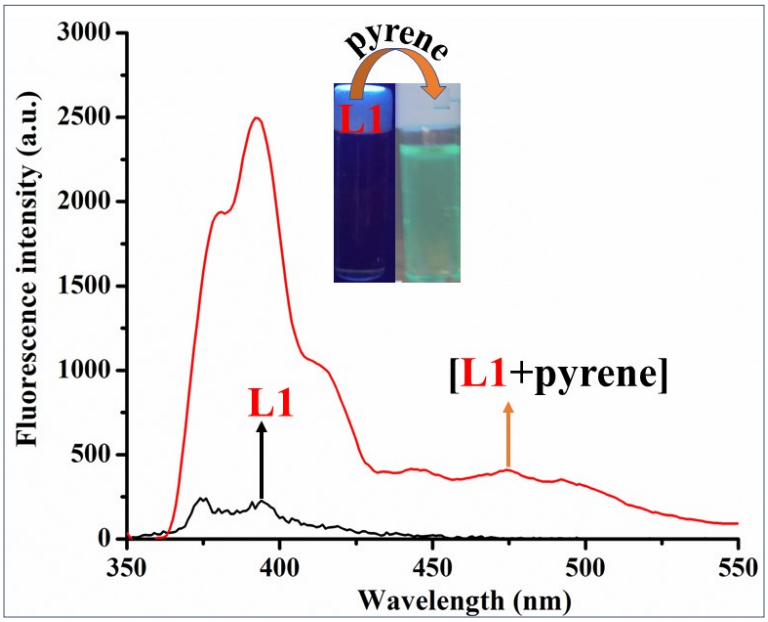


Figure S25 Fluorescence spectra of L1 (20 μM, DMSO/H_2O, 4/1, v/v, 20 mM HEPES buffer, pH 7.4) in presence of pyrene (λ_{Ex} = 302 nm).

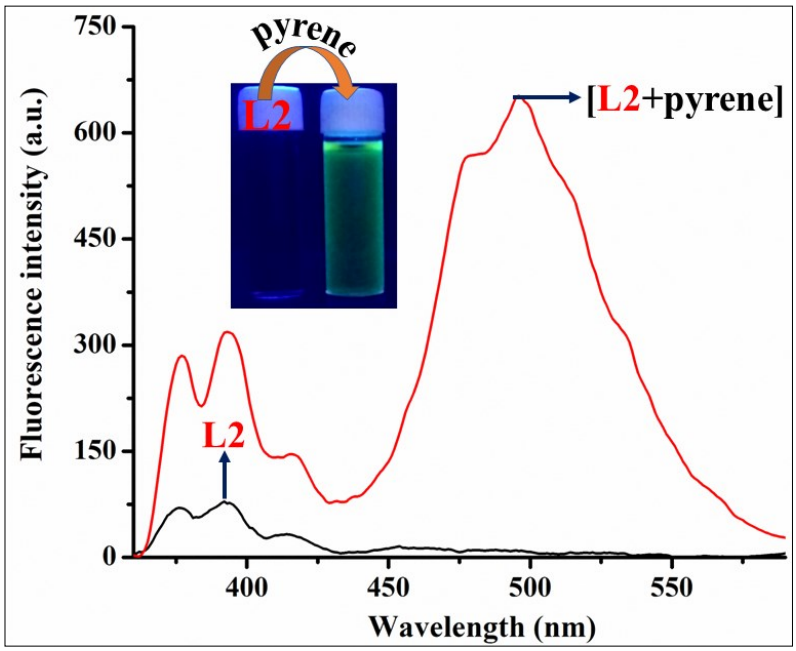


Figure S26 Fluorescence spectra of L2 (20 μM, DMSO/H_2O, 4/1, v/v, 20 mM HEPES buffer, pH 7.4) in presence of pyrene (λ_{Ex} = 300 nm).

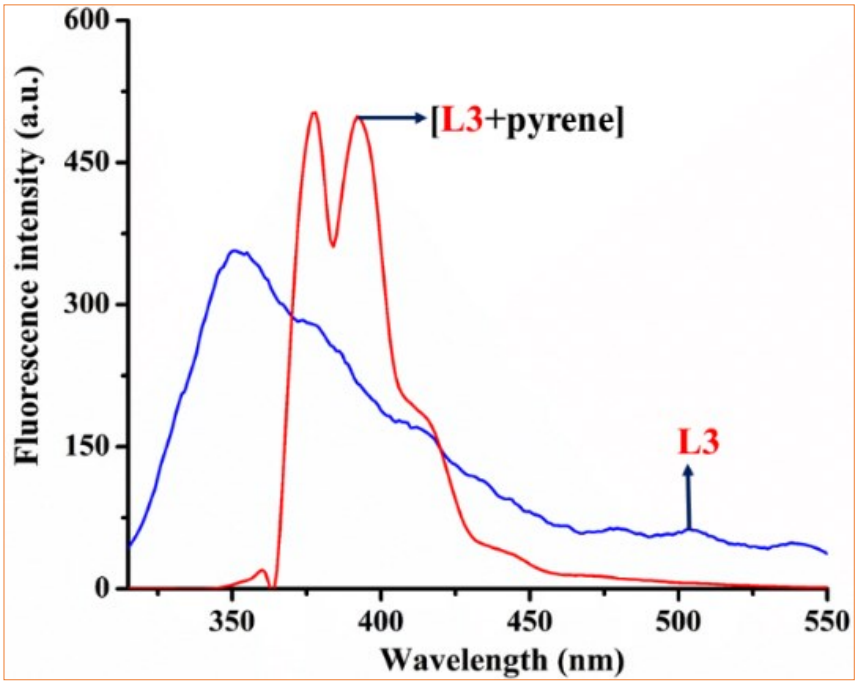


Figure S27 Fluorescence spectra of L3 (20 μM, DMSO/H_2O, 4/1, v/v, 20 mM HEPES buffer, pH 7.4) in presence of pyrene (λ_{Ex} = 282 nm).

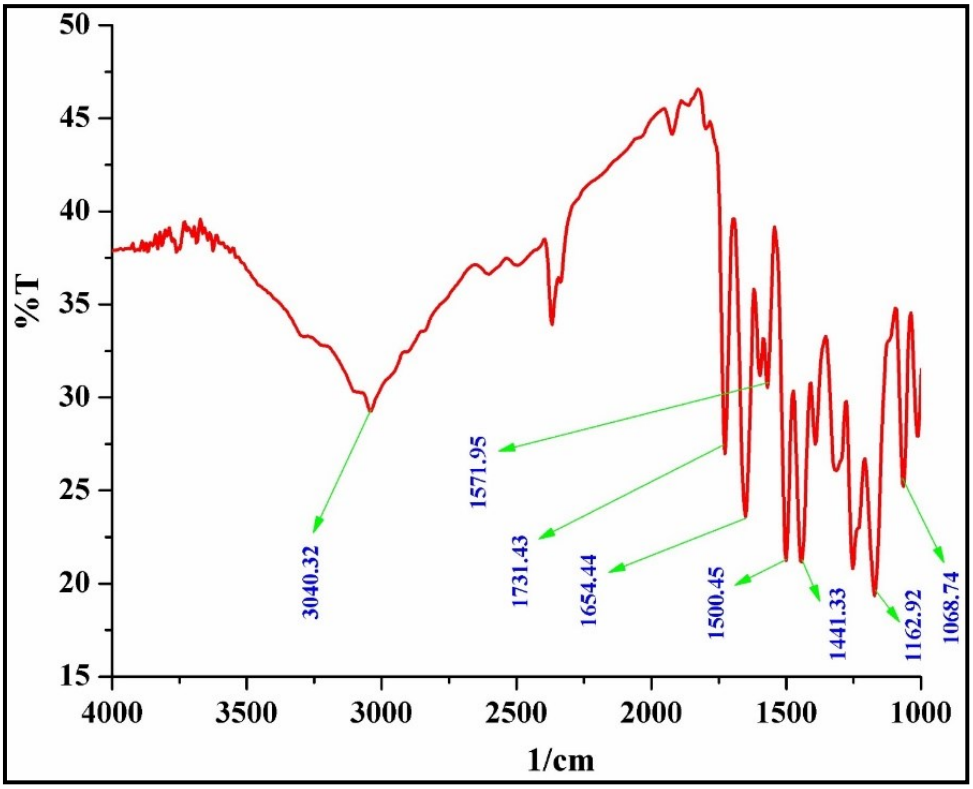


Figure S28 FTIR spectrum of [L-pyrene] adduct.

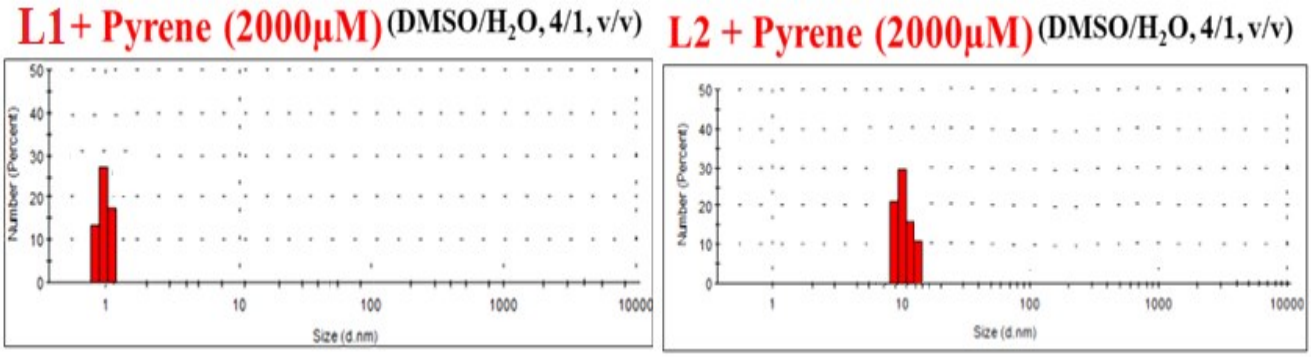


Figure S29 DLS of [L1- pyrene] and [L2- pyrene] in (DMSO/H_2O, 4/1, v/v)

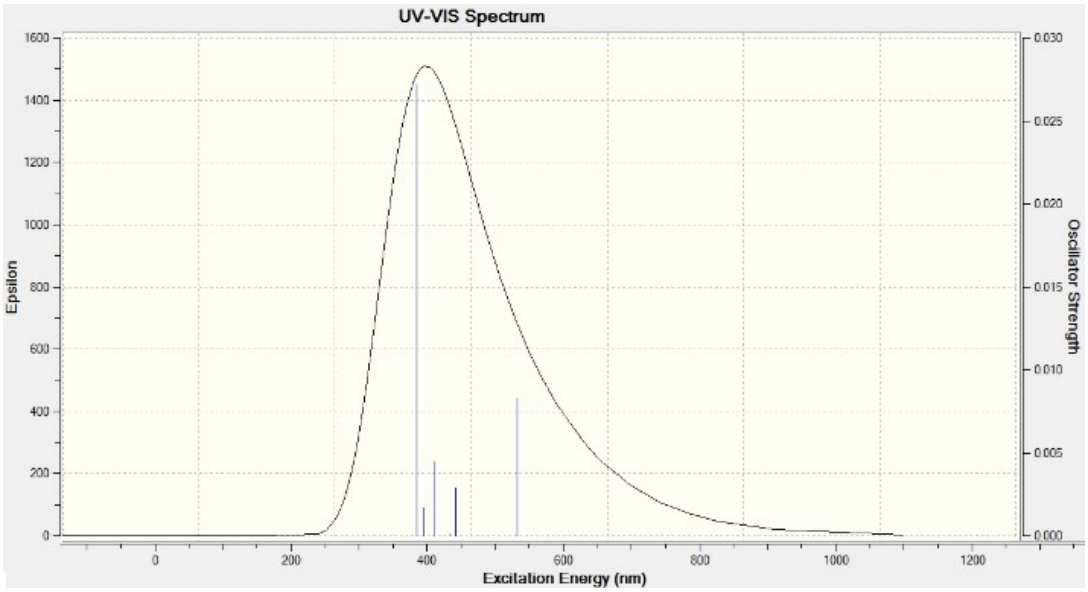


Figure S30 Theoretical absorption spectrum of L.

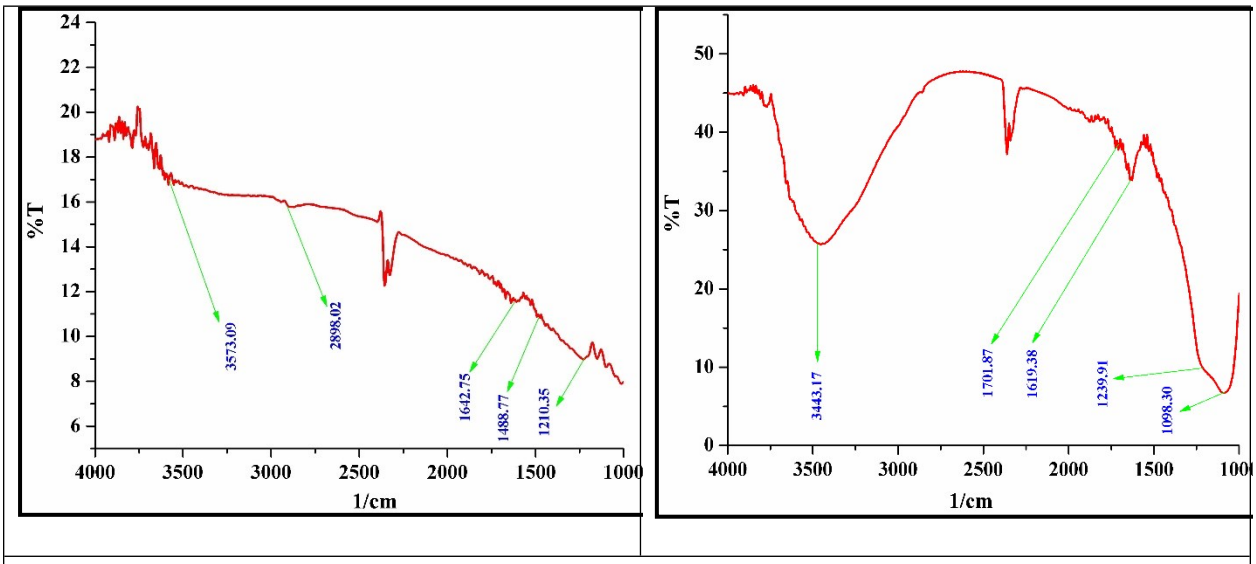


Figure S31 FTIR spectra of silica immobilized L (left) and its pyrene sorbed form (right).

Table S1 Crystal refinement parameters for L

Parameters	**Crystal data for L** (CCDC No: 1869304)
Empirical Formula	$C_{12}H_{13}ClN_6O_4$
Moiety Formula	$C_{12}H_{13}N_6O_4$, Cl
Molecular weight	340.73
Crystal color	Red
Crystal description	rectangular
Crystal system	triclinic
Cell parameter a α b β c γ	 6.9402 89.801 7.3544 89.818 32.837 70.483
Density	1.432
Volume	1580.1(7)
Space group	'P -1'
Hall group	'-P 1'
Temperature	293(2)
Z	4
Absorption coefficient(mm-1)	0.271
F000	704.0
h, k, l max	9,10,45
Reflections threshold expression	I > 2\s(I)
Wavelength	0.71073
Theta (min)	2.481
Theta (max)	29.801
Theta (full)	25.242

Table S2 Selected bond lengths [Å] and angles [°] for L

ATOMS	ANGLE	ATOMS	LENGTH
C00P N007 C00Q	122.2(6)	O003 C00Q	1.238(9)
C00P N007 C018	124.2(6)	O004 C015	1.236(8)
C00Q N007 C018	113.6(6)	O005 C00Z	1.229(9)
C00X N008 C00Q	123.0(6)	O006 C00X	1.256(8)
C00X N008 C01C	116.8(6)	N007 C00P	1.346(9)
C00Q N008 C01C	120.1(6)	N007 C00Q	1.410(8)
C00R N009 N4	120.2(6)	N007 C018	1.489(9)
N3 N00A C00U	120.2(6)	N008 C00X	1.367(9)
C00T N00B C00Z	122.4(6)	N008 C00Q	1.375(9)
C00T N00B C01B	124.0(6)	N008 C01C	1.477(9)
C00Z N00B C01B	113.6(6)	N009 C00R	1.319(9)
C015 N9 C00Z	124.1(6)	N009 N4	1.316(8)
C015 N9 C01A	116.3(6)	N00A N3	1.327(8)
C00Z N9 C01A	119.4(6)	N00A C00U	1.319(9)
C00P N8 H4A	109.5	N00B C00T	1.342(9)
C00P N8 H4B	109.5	N00B C00Z	1.424(9)
H4A N8 H4B	109.5	N00B C01B	1.481(9)

C00P N8 H4C	109.5	O1 N1	1.241(8)
H4A N8 H4C	109.5	N9 C015	1.362(9)
H4B N8 H4C	109.5	N9 C00Z	1.383(9)
N00A N3 C00W	120.2(6)	N9 C01A	1.492(9)
N009 N4 C011	120.7(6)	N8 C00P	1.327(9)
C00T N7 H3A	109.5	N8 H4A	0.8901
C00T N7 H3B	109.5	N8 H4B	0.8901
H3A N7 H3B	109.5	N8 H4C	0.8901
C00T N7 H3C	109.5	N3 C00W	1.407(9)
H3A N7 H3C	109.5	N4 C011	1.418(10)
H3B N7 H3C	109.5	O00H N1	1.224(8)
O00H N1 O1	122.3(7)	N7 C00T	1.322(9)
O00H N1 C00S	118.7(6)	N7 H3A	0.8900
O1 N1 C00S	119.0(6)	N7 H3B	0.8900
O00L N2 O2	122.4(7)	N7 H3C	0.8900
O00L N2 C00Y	118.9(7)	O2 N2	1.246(8)
O2 N2 C00Y	118.8(6)	N1 C00S	1.462(9)
N8 C00P N007	120.9(6)	O00L N2	1.215(9)
N8 C00P C00R	120.2(6)	N2 C00Y	1.450(10)
N007 C00P C00R	118.9(6)	C00P C00R	1.438(10)
O003 C00Q N008	121.6(6)	C00R C00X	1.467(9)
O003 C00Q N007	119.1(6)	C00S C00V	1.382(10)

N008 C00Q N007	119.2(6)	C00S C010	1.392(10)
N009 C00R C00P	116.9(6)	C00T C00U	1.447(10)
N009 C00R C00X	123.8(6)	C00U C015	1.484(10)
C00P C00R C00X	119.3(6)	C00V C012	1.386(11)
C00V C00S C010	123.5(7)	C00V H00V	0.9300
C00V C00S N1	118.6(6)	C00W C019	1.394(10)
C010 C00S N1	117.9(6)	C00W C014	1.411(10)
N7 C00T N00B	120.6(6)	C00Y C016	1.388(10)
N7 C00T C00U	120.9(6)	C00Y C017	1.414(10)
N00B C00T C00U	118.5(6)	C010 C013	1.405(11)
N00A C00U C00T	116.7(6)	C010 H010	0.9300
N00A C00U C015	123.2(6)	C011 C013	1.378(10)
C00T C00U C015	120.0(6)	C011 C012	1.407(9)
C00S C00V C012	118.3(6)	C012 H012	0.9300
C00S C00V H00V	120.9	C013 H013	0.9300
C012 C00V H00V	120.9	C014 C016	1.372(11)
C019 C00W N3	115.8(6)	C014 H014	0.9300
C019 C00W C014	121.1(7)	C016 H016	0.9300
N3 C00W C014	123.1(6)	C017 C019	1.386(11)
O006 C00X N008	121.3(6)	C017 H017	0.9300
O006 C00X C00R	121.4(6)	C018 H01A	0.9600
N008 C00X C00R	117.3(6)	C018 H01B	0.9600

C016 C00Y C017	122.4(7)	C018 H01C	0.9600
C016 C00Y N2	119.7(7)	C019 H019	0.9300
C017 C00Y N2	117.9(6)	C01A H01D	0.9600
O005 C00Z N9	121.8(6)	C01A H01E	0.9600
O005 C00Z N00B	119.5(6)	C01A H01F	0.9600
N9 C00Z N00B	118.7(6)	C01B H01G	0.9600
C00S C010 C013	117.8(6)	C01B H01H	0.9600
C00S C010 H010	121.1	C01B H01I	0.9600
C013 C010 H010	121.1	C01C H01J	0.9600
C013 C011 C012	121.8(7)	C01C H01K	0.9600
C013 C011 N4	115.7(6)	C01C H01L	0.9600
C012 C011 N4	122.4(6)		
C00V C012 C011	119.2(6)		
C00V C012 H012	120.4		
C011 C012 H012	120.4		
C011 C013 C010	119.3(6)		
C011 C013 H013	120.3		
C010 C013 H013	120.3		
C016 C014 C00W	119.3(7)		
C016 C014 H014	120.3		
C00W C014 H014	120.3		
O004 C015 N9	121.8(6)		

O004 C015 C00U	122.0(6)		
N9 C015 C00U	116.1(6)		
C014 C016 C00Y	119.3(7)		
C014 C016 H016	120.4		
C00Y C016 H016	120.4		
C019 C017 C00Y	117.7(7)		
C019 C017 H017	121.2		
C00Y C017 H017	121.2		
N007 C018 H01A	109.5		
N007 C018 H01B	109.5		
H01A C018 H01B	109.5		
N007 C018 H01C	109.5		
H01A C018 H01C	109.5		
H01B C018 H01C	109.5		
C017 C019 C00W	120.1(7)		
C017 C019 H019	119.9		
C00W C019 H019	119.9		
N9 C01A H01D	109.5		
N9 C01A H01E	109.5		
H01D C01A H01E	109.5		
N9 C01A H01F	109.5		
H01D C01A H01F	109.5		

H01E C01A H01F	109.5		
N00B C01B H01G	109.5		
N00B C01B H01H	109.5		
H01G C01B H01H	109.5		
N00B C01B H01I	109.5		
H01G C01B H01I	109.5		
H01H C01B H01I	109.5		
N008 C01C H01J	109.5		
N008 C01C H01K	109.5		
H01J C01C H01K	109.5		
N008 C01C H01L	109.5		
H01J C01C H01L	109.5		
H01K C01C H01L	109.5		

Table S3 Structural parameters of L derived from TDDFT studies

Multiplicity	1
Number of electrons	158
Number of alpha electrons	79
Number of beta electrons	79
Number of basic functions	222
Number of independent functions	222
Number of point charges in /Mol/	0

Number of translation vectors	0
Atomic numbers	34

Table S4 Structural parameters of [L-pyrene] derived from TDDFT studies

Multiplicity	1
Number of electrons	264
Number of alpha electrons	132
Number of beta electrons	132
Number of basic functions	386
Number of independent functions	386

Table S5 Experimental *vs.* theoretical FTIR data of **L**

Experimental (cm^{1})	**Theoretical (cm^{-1})**	**Assignments**
3546	3601, 3442	υ(–NH_2)
3295	3066, 3011	ν(aromatic C–H)
3295	3202	υ(sp^3C-H)
2976	2948	υ(–C=O)
1651	1656	υ(–C=C)
1500	1524, 1478, 1462	υ(–N=N–)
1426	1429, 1422	υ(–C–N)
1303	1352, 1347, 1312	νC-H (bending)
-	1295, 1287, 1263	νC-O (anti-symmetry stretching)
1106	1114, 1103, 1077	νC-O (symmetry stretching)

1054	1023, 1046, 1073, 1082	νC-N (symmetry stretching)

Table S6 Electronic transitions predicted from TDDFT calculations for L

Electronic transitions	Energy (eV)	f[b]	Wavelength (nm)	Transitions involved
S_0-S_1	2.7856 eV	f = 0.0041	445.09 nm	HOMO→LUMO HOMO →LUMO+2
S_0-S_2	3.1132 eV	f = 0.0028	398.25 nm	HOMO-2→LUMO+1 HOMO-3→LUMO+1
S_0-S_3	3.6694 eV	f = 0.0016	337.89 nm	HOMO-1→LUMO+1

Table S7 Electronic transitions predicted from TDDFT calculations for L-pyrene adduct

Electronic transitions	Energy (eV)	f[b]	Wavelength (nm)	Transitions involved
S_0-S_1	2.2099 eV	f = 0.0096	561.05 nm	HOMO→LUMO HOMO →LUMO+2
S_0-S_2	2.9617 eV	f = 0.0078	433.25 nm	HOMO-1→LUMO HOMO-1→LUMO+1 HOMO→LUMO+1
S_0-S_3	3.3929 eV	f = 0.0045	365.42 nm	HOMO-1→LUMO+1 HOMO→LUMO HOMO→LUMO+1 HOMO →LUMO+2

Reference

1. Malik, A.; Verma, P.; Singh A. K. and Singh, K. P. Distribution of polycyclic aromatic hydrocarbons in water and bed sediments of the Gomti River, India. *Environ. Monit. Assess.,* **2011**, *172*, 529–545.
2. Nandi, S.; Adhikari, S.; Mandal, S.; Banerjee A. and Das, D. Tuning FRET efficiency as a novel approach for improved detection of naphthalene: application to environmental samples. *J. Mol. Recognit*., **2016**, *29*, 303–307.