Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supplementary Information

Pyrano[3,2-*c*]julolidin-2-one: A Novel Class of Fluorescent Probe for Ratiometric Detection and Imaging of Hg^{2+} in Live Cancer Cells

Ajay Kumar Jha,[a] Shahida Umar,[a] Rakesh Kumar Arya,[b] Dipak Datta,[b] Atul Goel*[a,c]

[a]*Fluorescent Chemistry Lab, Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, Lucknow 226031, E-mail: atul_goel@cdri.res.in*

Fax: +91-522-2771941; Tel.: +91-522-2772450

[b]*Biochemistry Division, CSIR-Central Drug Research Institute, Lucknow 226031, India.*

[c]*Academy of Scientific and Innovative Research, New Delhi 110001, India*

**Correspondence should be addressed to A.G. (atul_goel@cdri.res.in).*

Table of Contents

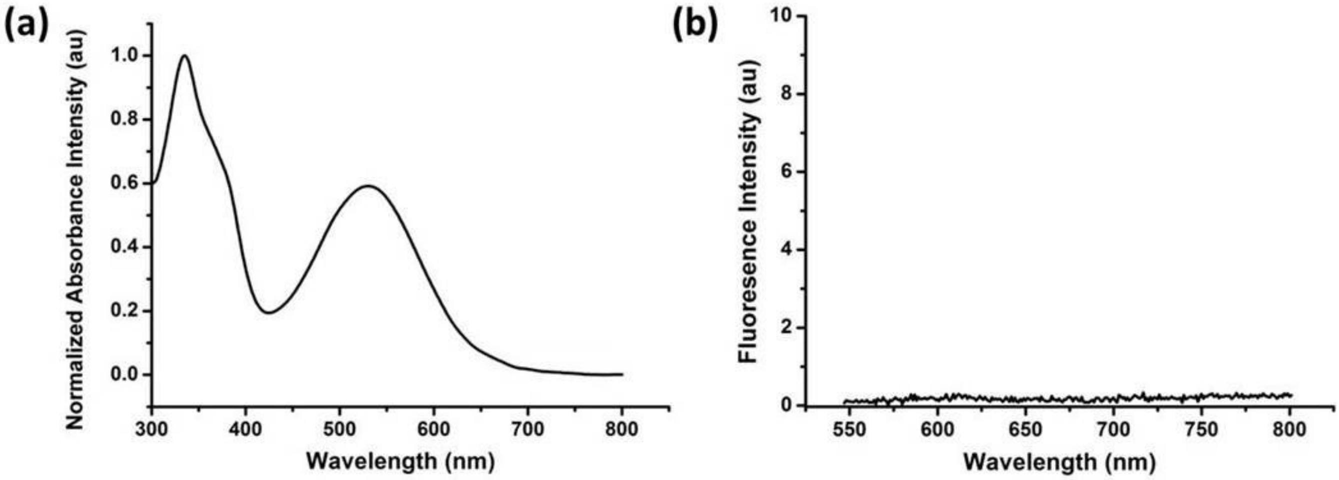


Figure S1 (a) Absorption and (b) Fluorescence spectra of compound **PYJO1** ($4x10^{-5}$ M) in methanol/HEPES buffer (7:3) at pH 7.2.

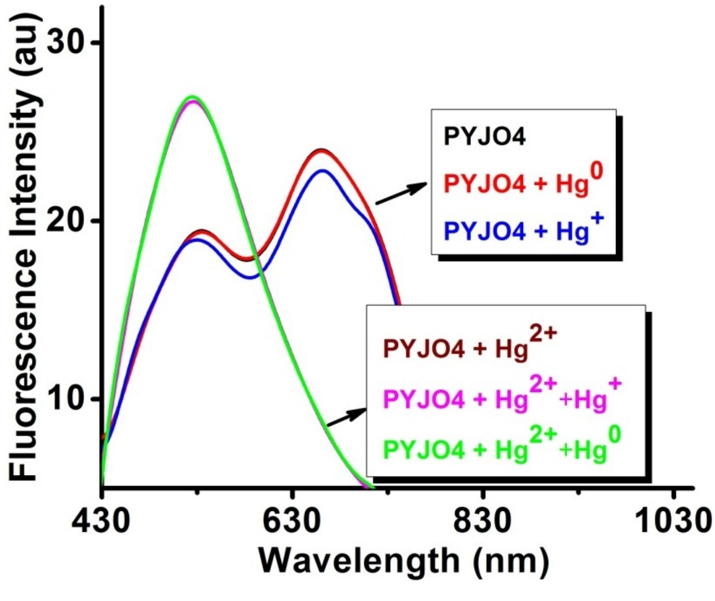


Figure S2 Fluorescence response of **PYJO4**($4x10^{-5}$M) in the presence of Hg^{2+} ($2x10^{-4}$M), Hg^{+} ($2x10^{-4}$M), and Hg^{0} ($2x10^{-7}$ M, poor solubility[1]) in methanol/HEPES buffer (7:3) at pH 7.2.

Table S1. Representative ratiometric fluorescent probes for Hg^{2+} based on organic fluorophores for sensing and bio-imaging.

Journal	Solvent system	LOD	Cell Imaging	Mechanism of sensing
Chem. Eur. J. 2015, **21**, 13201	HEPES/EtOH 6:4 v/v	60 nm	FL A549 cells	-- Au^{3+}& Hg^{2+}
Inorg. Chem. 2012, **51**, 1769	CH_3CN-aq.HEPES buffer (1 mM, pH=7.2; 1:1, v/v)	0.35 ppb	A431 cells	RET Cr^{3+} & Hg^{2+}
J. Am. Chem. Soc. 2007, **129**, 5910	(50 mM HEPES, pH 8)	50 nm	--	PET
Tetrahedron 2010, **66**, 9762	CH_3CN/H_2O (9:1, v/v)	--	MCF-7 cells	FRET
Analyst, 2015, **140**, 2778	10 mM PBS buffer (1% CH_3CN, pH=7.4)	7.8 nm	Hela cell	ESIPT
Org. Lett., 2009, **11**, 2740	H_2O-CH_3CN (1:1, v/v)	0.1 ppm	Pseudomonas putida	RET
J. Org. Chem. 2006, **71**, 4308	0.05 M phosphate-buffered water solution (pH =7.5)	--	--	ICT
Chem. Commun., 2009, 3175	water	10^{-6} M	--	ICT
J. Mater. Chem., 2005, **15**, 2778	(50 mM HEPES, 100 mM KCl)	--	--	PET
ACS Appl. Mater. Interfaces. 2010, **2**, 1066	THF	5×10^{-7}M	--	ICT
Spectrochimica Acta Part A: 2016, **165**, 99	H_2O/CH_3CN (9:1, v/v; TrisHCl 50 mmol L^{-1}; pH = 7.0)	0.008 µM	HeLa cells	FRET
Sensors and Actuators B: Chemical. 2016, **230**, 639	DMF–H_2O (3:1, v/v) with HEPES (pH=7.4, 10 mM)	5.22×10^{-7} M	--	ICT
Analyst, 2013, **138**, 2654	Tris–HCl/CH_3CN (10 mM, pH =7.4, 1 : 1, v/v)	2.8×10^{-8} M	HeLa cells	PET
Chem. Commun., 2012, **48**, 7292	(0.5% CH_3CN) pH = 7.4	--	C8D1A cells	ICT
Chem. Commun., 2012, **48**, 8371	PBS buffer solutions (pH = 7.4, containing 0.5% DMSO)	4.9 nM	Hep G2 cells	ICT
Dalton Trans., 2013, 42, 12093	CH_3CN–H_2O (98 : 2 v/v).	41 nM	--	LMCT/MLCT
New J.Chem., 2014, **38**, 109	PBS buffer solution (pH = 7.4, containing 1% CH_3CN	3.6 nM	SMMC-7721 cells	ICT
Org. Biomol. Chem., 2015, **13**, 3032	Water aggregate based	72.7 nM	--	ICT, Hg^{2+} & I^-
RSC Adv., 2014, **4**, 14919	2% EtOH, 1 mM, pH 7.4;	90 ppb	Hela cell	FRET, Hg^{2+} & S^{2-}
J. Mater. Chem., 2012, **22**, 4003	10 mM HEPES buffer solution (pH 7.0)	96 nM	HeLa cells	ICT
Org. Lett., 2011, **13**, 3422	PBS buffer (pH 7.4) containing ~1% CH_3CN.	20 ppb	--	ESIPT
Org. Lett., 2012, **14**, 1986	($MeOH/H_2O$, 2:98, v/v)	1.6×10^{-8} M	--	ICT/ ESIPT
Org. Lett., 2011, **13**, 5774	DMF/MeOH (98:2) v/v	--	--	FRET Hg^{2+}& Fe^{2+}
Org. Lett., 2010, **12**, 5310	HEPES buffer (0.01 M, pH =7.4) (0.05%DMSO, v/v)	10 ppb	--	--
Org. Lett., 2008, **10**, 4891	THF/H_2O (9:1, v/v)	1×10^{-7} M	--	ICT
Angew. Chem. Int. Ed., 2008, **47**, 8025	C_2H_5OH/H_2O (8:2) , pH= 7.0	--	MCF-7	FRET
Org. Lett., 2012, **14**, 2564	THF/H_2O (1/1) v/v)	--	--	ICT
This Work	**Methanol/HEPES (7/3,v/v) pH=7.2**	**1.14 ppb**	**Significant ratiometric bioimaging in MCF-7 cells**	**TICT Operated First ratiometric Hg^{2+} sensing rotor**

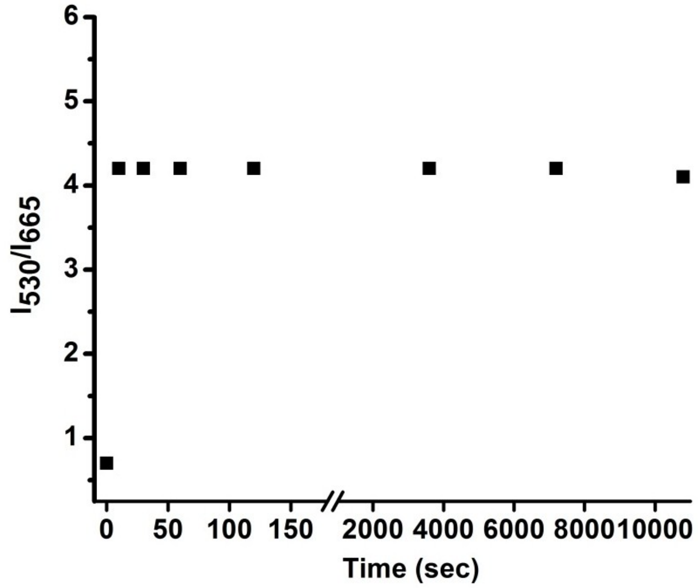


Figure S3 Time dependent fluorescence response[2] of **PYJO4** (4x10^{-5} M) in the presence of Hg^{2+} (5 equiv.) in methanol/HEPES buffer (7:3) at pH 7.2.

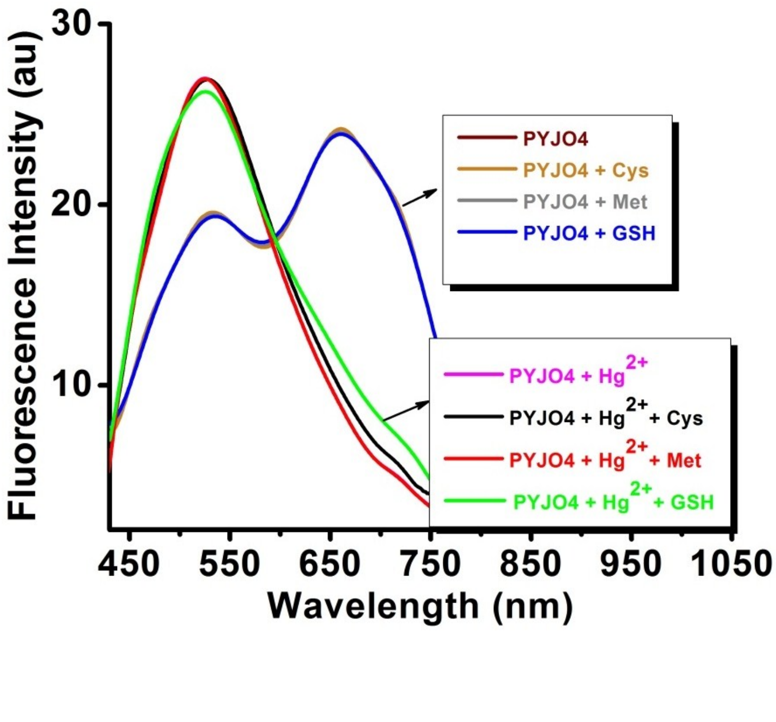


Figure S4 Competitive experiment between **PYJO4**, Cysteine (Cys), Methionine (Met) and Glutathione (GSH). Variation in the fluorescence intensity of **PYJO4** (4.0×10^{-5} M) in presence of Hg^{2+}, Cys (10 mM), Met (10 mM) and GSH (10 mM). λ_{ex}= 405nm. [Hg^{2+}] = 2×10^{-4} M.

Dip-stick experiment

In "Dip-stick" experiment with different metal ions, the TLC plates were saturated with **PYJO4** (4x10^{-5} M) solution in methanol and air-dried. Aqueous solutions of different metal perchlorates (2x10^{-4} M) were prepared. The TLC plates coated with **PYJO4** were subsequently dipped in aqueous solutions of different metal ions and were observed by a naked eye under hand held UV lamp (365 nm).

To check the sensitivity of the **PYJO4** coated test strips, the TLC plates were prepared using more dilute solution of **PYJO4** (2μM) in methanol and air-dried. These plates were then dipped in aqueous solutions of different concentrations of Hg^{2+} (1 μM- 6 μM) ions and observed by a naked eye under hand held UV lamp (365 nm).

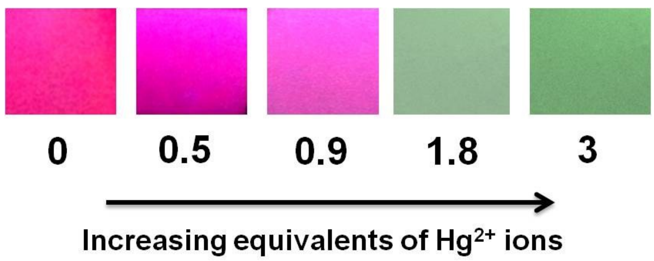


Figure S5 Dip-stick experiment of **PYJO4** (2μM) with different concentrations of Hg^{2+} ions under UV lamp (365 nm).

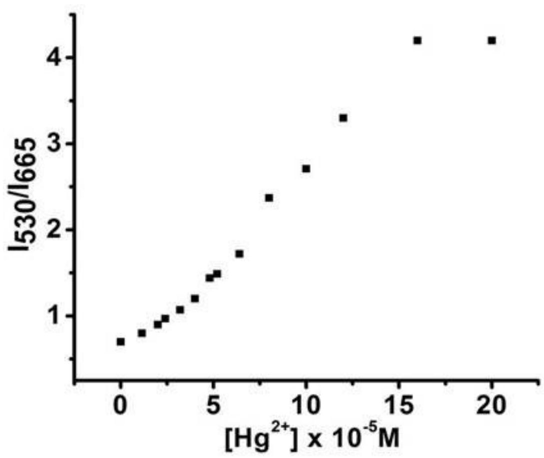


Figure S6 Variation of intensity ratio (I_{530}/I_{665}) of of **PYJO4** (4x10^{-5} M) on increasing concentration of Hg^{2+} .

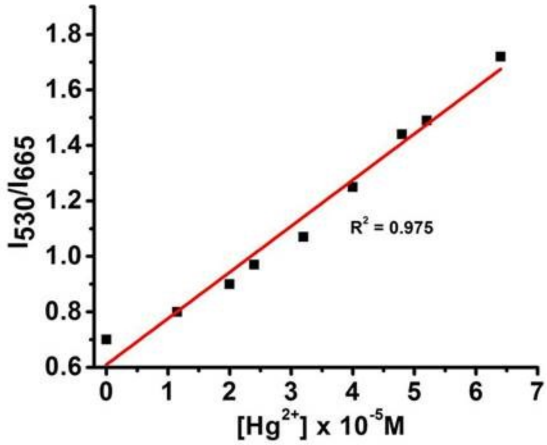


Figure S7 Linear variation of intensity ratio (I_{530}/I_{665}) of of **PYJO4** (4x10^{-5} M) on increasing concentration of Hg^{2+}, λ_{ex}= 405nm

Determination of detection limit

The detection limit was calculated based on the fluorescence titration.[3] The fluorescence emission spectrum of **PYJO4** was measured by seven times and the standard deviation of blank measurement was achieved. To gain the slope, fluorescence titration was carried out between **PYJO4** (8×10^{-7}) and Hg^{2+} the ratio of the fluorescence intensity at 530 nm to the fluorescence intensity at 665 nm ($I_{530/665}$) was plotted against the concentration of Hg^{2+}. So the detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity ratio versus Hg^{2+} concentration.

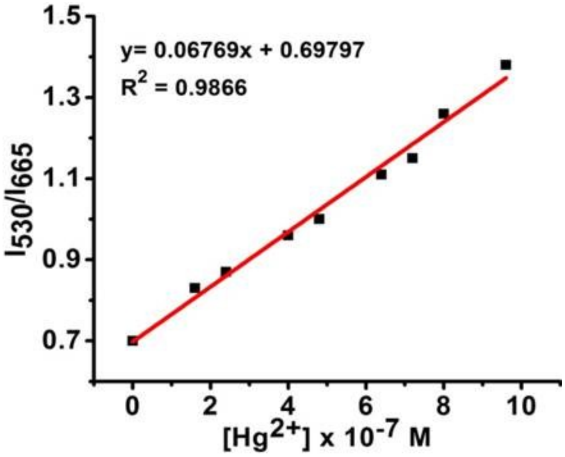


Figure S8 Variation of intensity ratio (I_{530}/I_{665}) of **PYJO4** ($8x10^{-7}$ M) on increasing concentration of Hg^{2+} for the determination of detection limit, λ_{ex}= 405 nm.

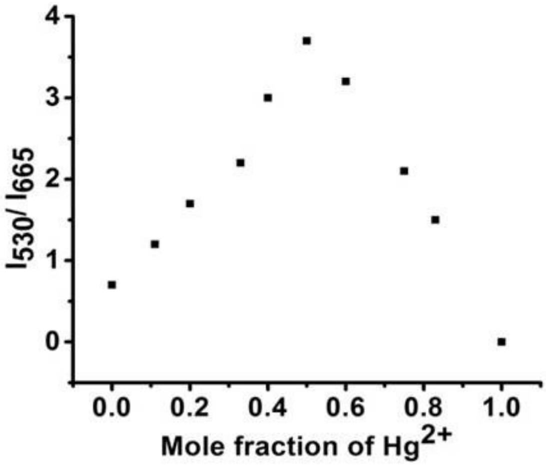


Figure S9 Job's plot showing 1:1 stoichiometry between **PYJO4** and Hg^{2+}.

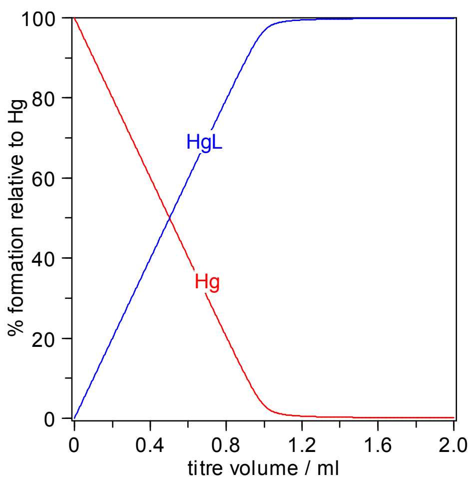


Figure S10 Species distribution curve between **PYJO4** and Hg^{2+}, L:**PYJO4**.

Determination of association constant

The association constant for 1:1 complex was calculated based on the titration curve of **PYJO4** with Hg^{2+} ions. Association constant was determined by a linear fitting of the data with the following equation

$$1/(Io - I) = \frac{1}{[(Io - I^{'}) \times K \times c]} + \frac{1}{(Io - I^{'})}$$

For a 1:1 complex, a straight line would be obtained when $1/(I_o - I)$ is plotted against 1/c, Where F_o is the fluorescence intensity of analyte in the absence of metal ions, F is the observed fluorescence in each concentration tested, F' is the intensity of analyte at infinite concentration of metal ions, c is the concentration of metal ion, and K is the association constant.[4]

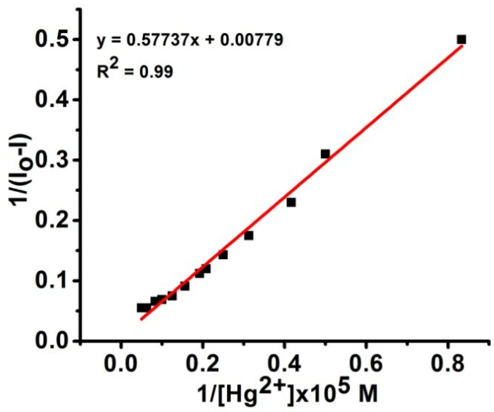


Figure S11 Plot of fluorescence intensity change at 665 nm of **PYJO4** (4×10^{-5} M) with increasing Hg^{2+} ($0–2 \times 10^{-4}$ M).

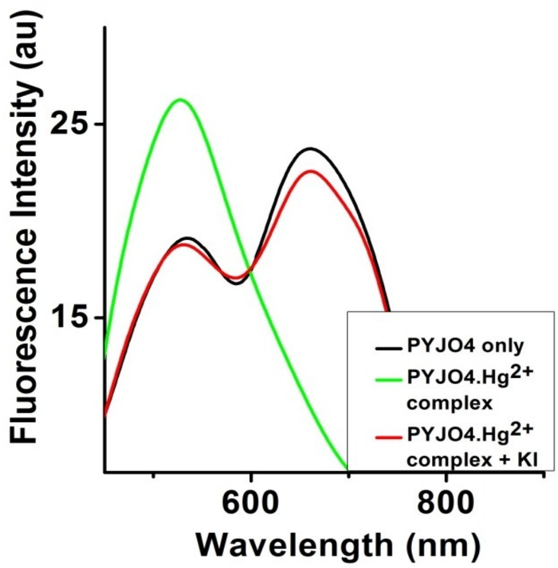


Figure S12 Fluorescence spectra of **PYJO4** (4×10^{-5}M) with Hg^{2+} (3 equiv.) showing reversibility in the presence of KI (7equiv) in methanol/HEPES buffer (7:3) at pH 7.2.

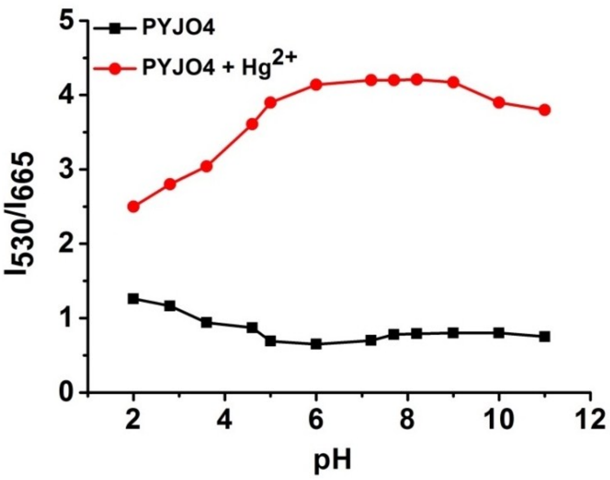


Figure S13 pH study (pH range 2-11) of **PYJO4** (4×10^{-5} M) in the absence and presence of Hg^{2+} (5 equiv.). λ_{ex}= 405 nm.

Table S2. Computed molecular orbital energy diagrams and isodensity surface plots of **PYJO4** as obtained from TDDFT calculation and computed values of vertical excitations, oscillator strength (*f*), assignment, HOMO, LUMO and energy band gap

λ_{max} (nm)	*f*	Assignment	HOMO (eV)	LUMO (eV)	E_g (eV)
513.23	0.2406	HOMO → LUMO (99.4%)	-5.549	-2.546	3.003
349.07	0.3183	HOMO-2 → LUMO (97.5%)			
335.41	0.1451	HOMO-3 → LUMO(98.06%)			

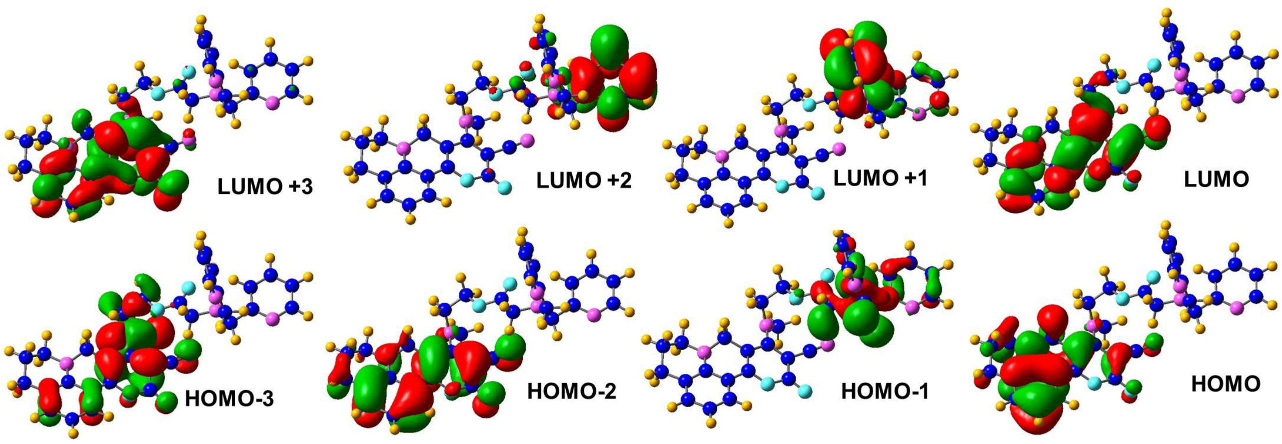


Table S3 Phtophysical data of **PYJO4** in different solvents

solvent	Δf	PYJO4 φ_f (%)[a]
Cyclohexane	0.0014	48
Toluene	0.0134	46
1,4- Dioxan	0.0213	40
Ethyl acetate	0.201	35
THF	0.2096	21
DCM	0.2183	28
DMF	0.2755	11
Acetone	0.2836	14
Ethanol	0.2893	8
Methanol: HEPES (7:3)	-	3

[a]Fluorescence quantum yield relative to harmine in 0.1M H_2SO_4 as a standard (Φ = 45%).

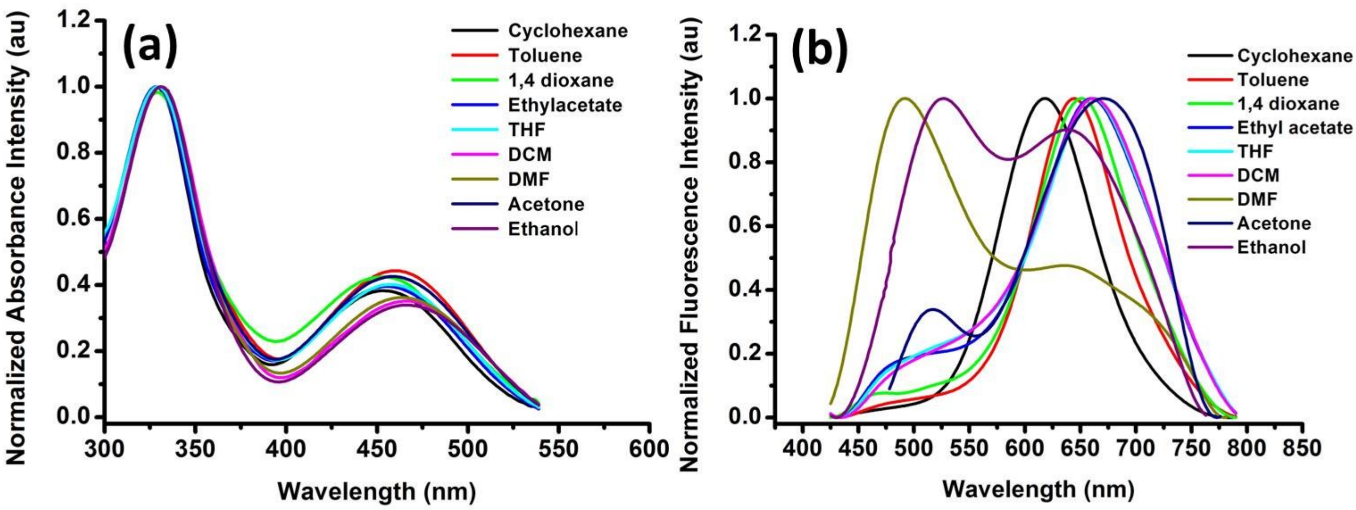


Figure S14 Effect of solvent polarity on absorption and emission spectra of **PYJO4**.

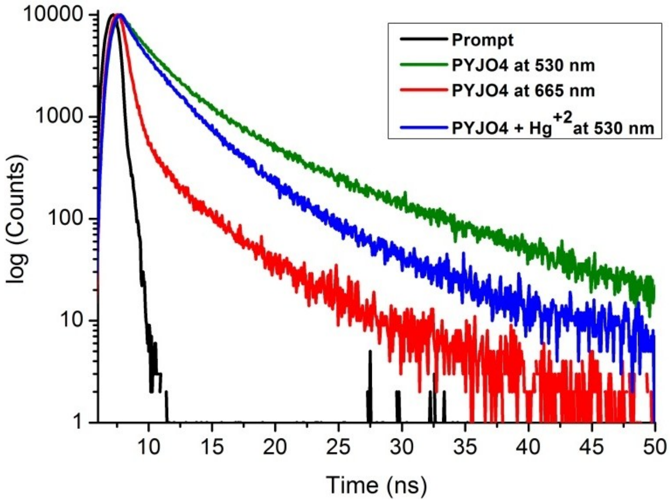


Figure S15. Time-resolved fluorescence decay plot of **PYJO4** ($4x10^{-5}$ M) only and in the presence of added Hg^{+2} ions in methanol/HEPES buffer (7:3) at 25 °C using a nano-LED of 390 nm as the light source.

Table S4 Time resolved fluorescence data of **PYJO4** and **PYJO4.Hg^{+2}** in methanol/HEPES buffer (7:3) at 25 °C

Entry	**λ_{em} (nm)**	**τ_{av} (ns)**	**$\chi 2$**
PYJO4	530	7.08	1.09
PYJO4	665	3.36	1.13
PYJO4 +Hg^{+2}	530	4.52	1.25

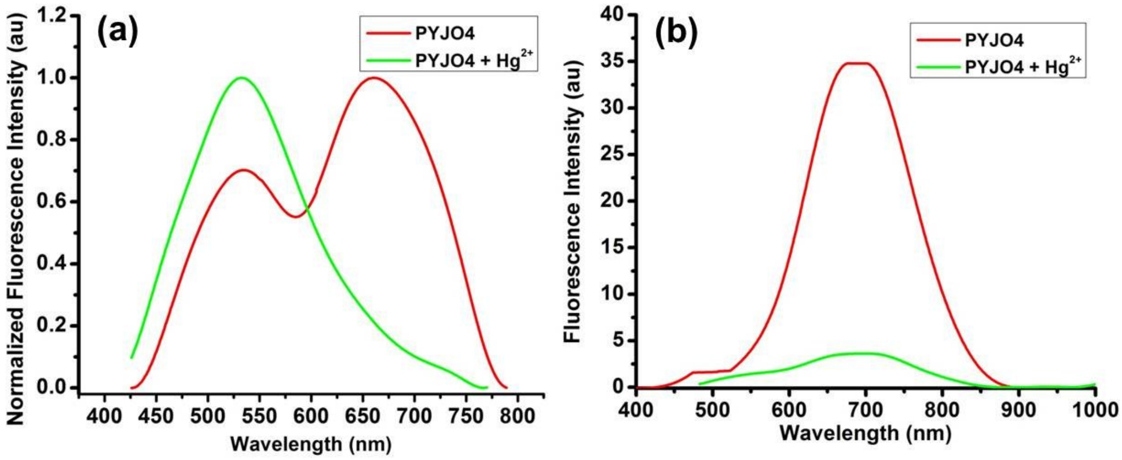
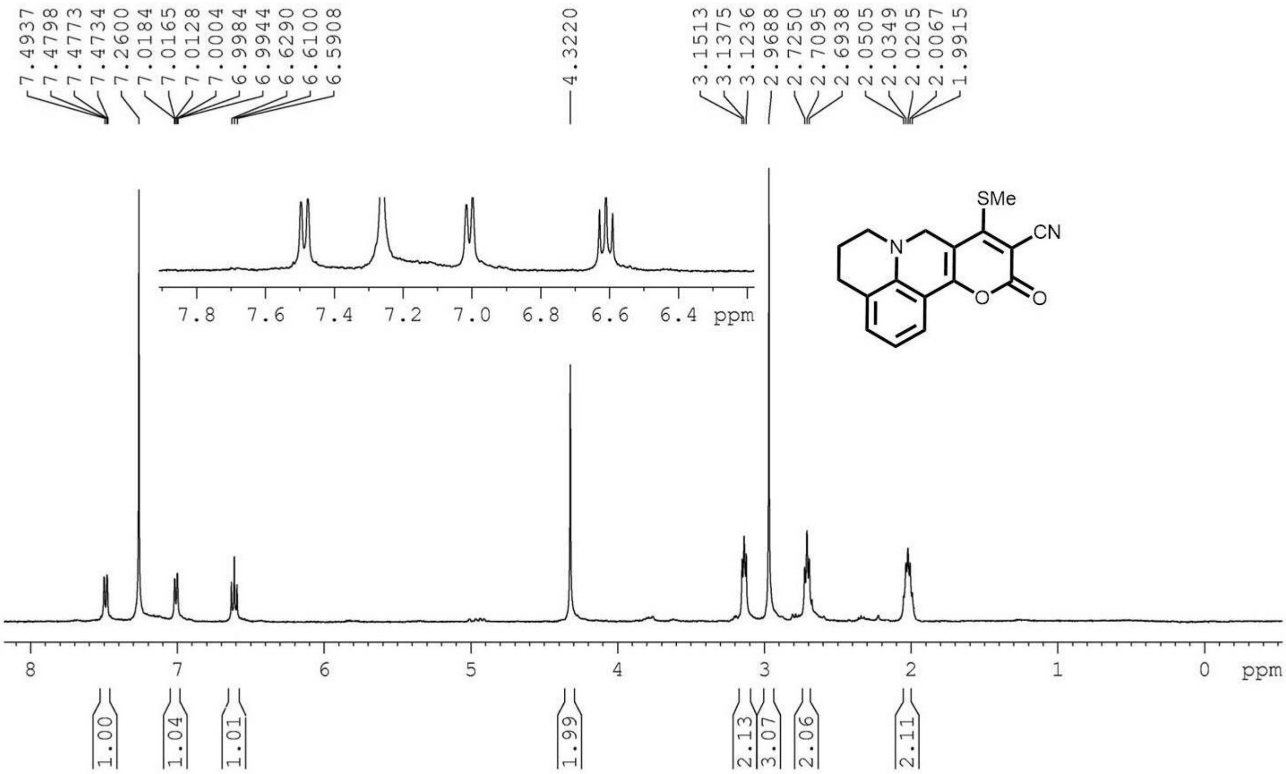
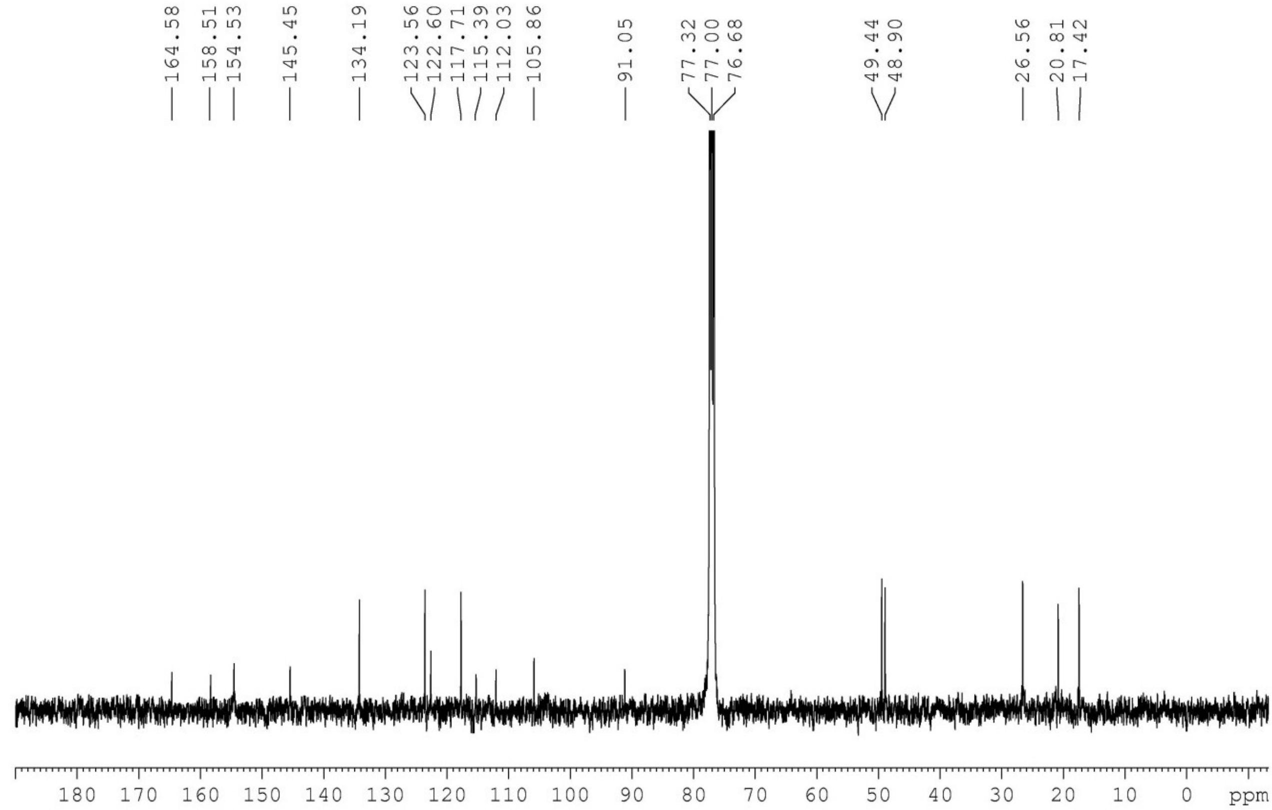


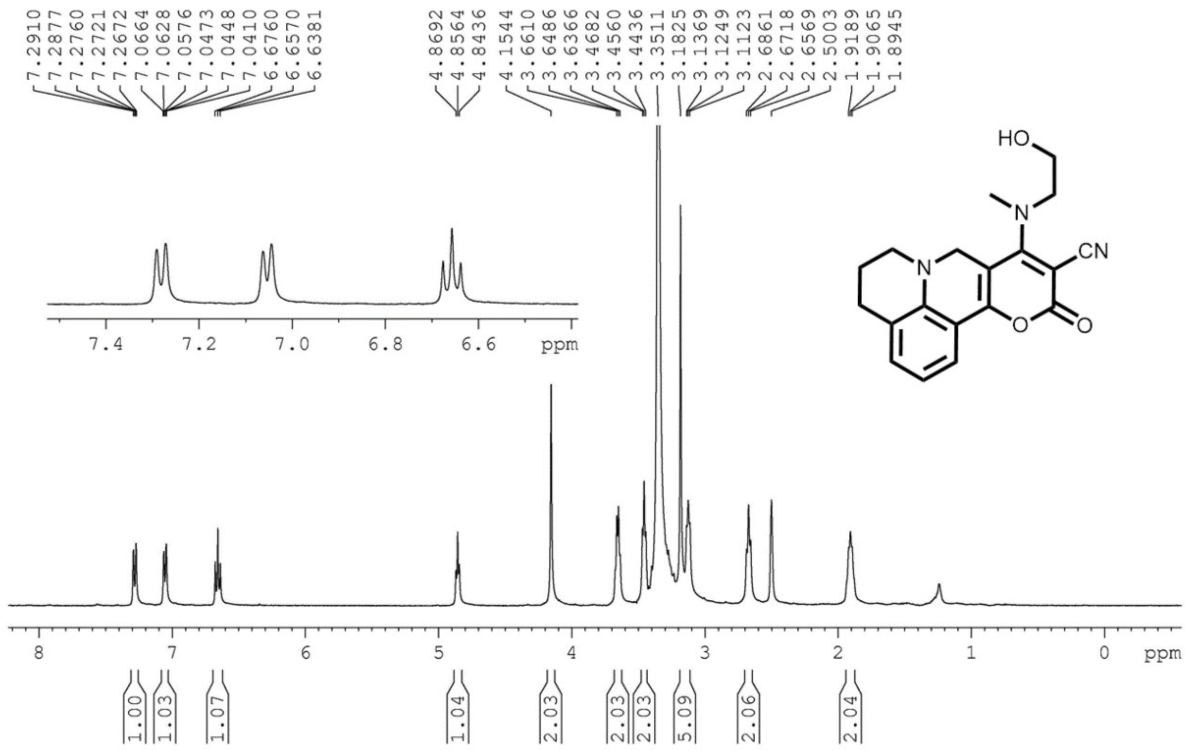
Figure S16 Selection of emission channels for biological studies. Fluorescence spectra of **PYJO4** ($4x10^{-5}$ M) at excitation (a) 405 nm and (b) 488 nm in methanol/HEPES buffer (7:3) at pH 7.2.

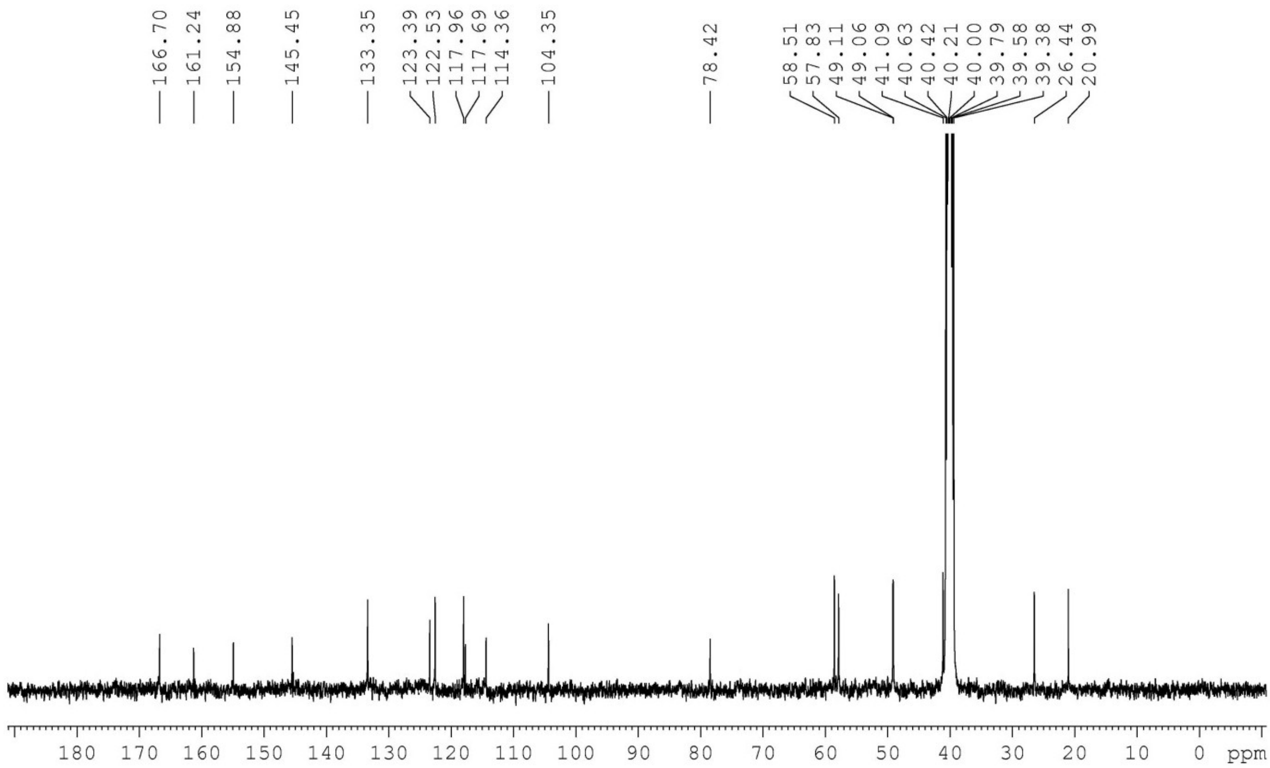
^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) of compound PYJO1



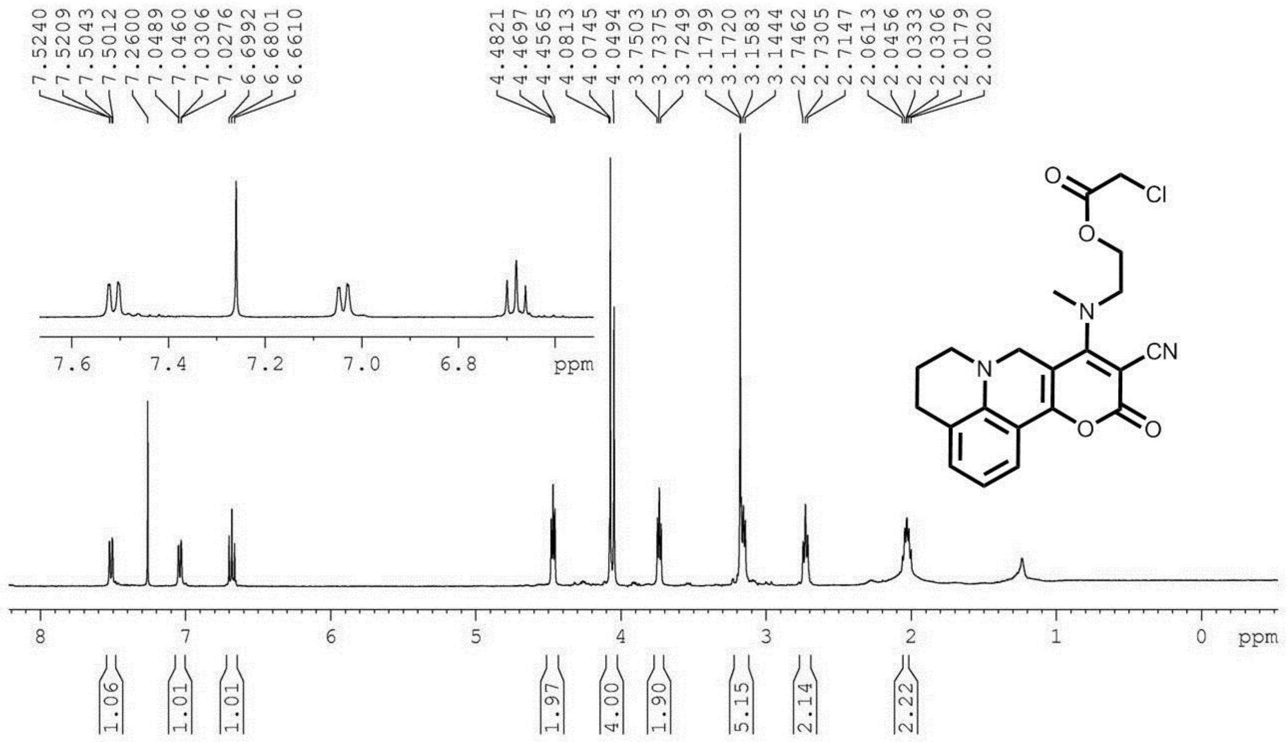


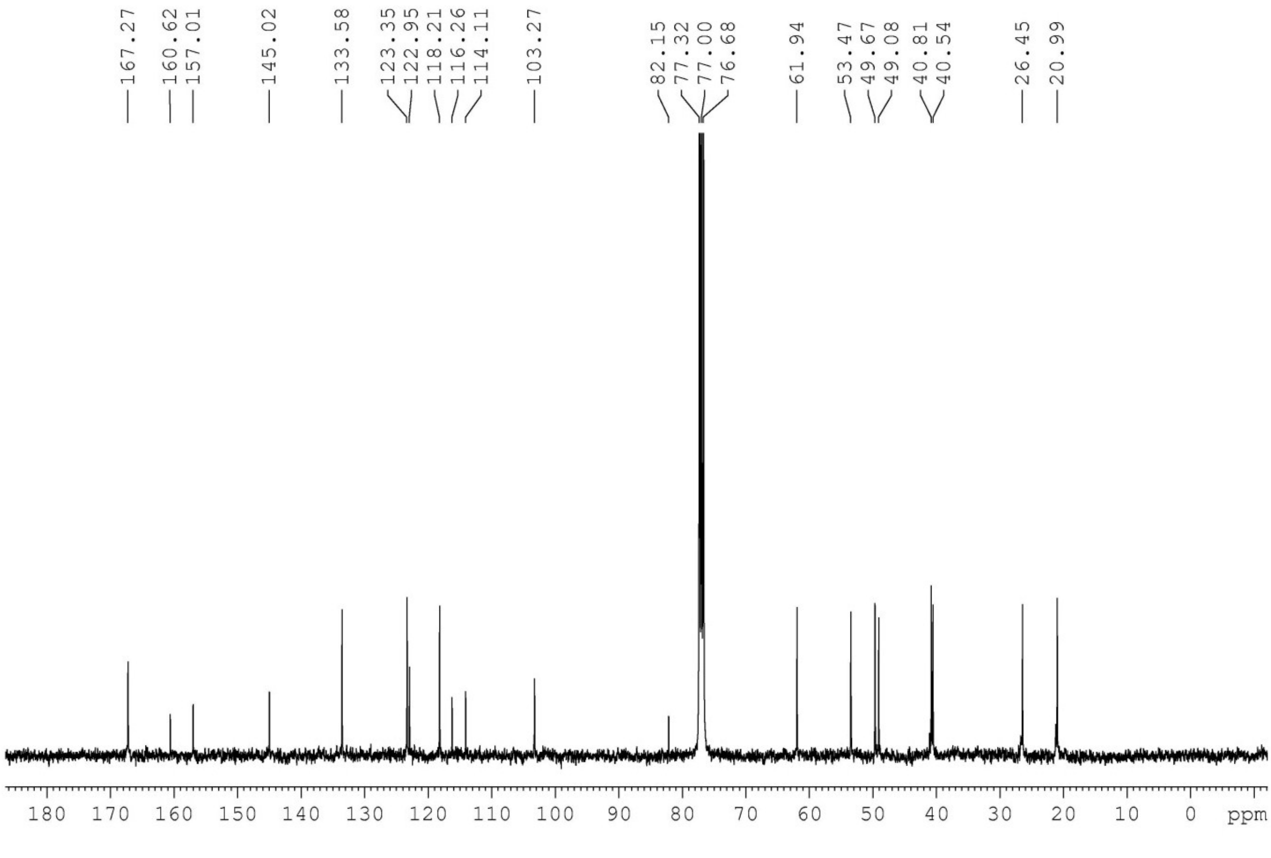
^{1}H NMR ($DMSOd_6$, 400 MHz) and ^{13}C NMR ($DMSOd_6$, 100.6 MHz) of PYJO2



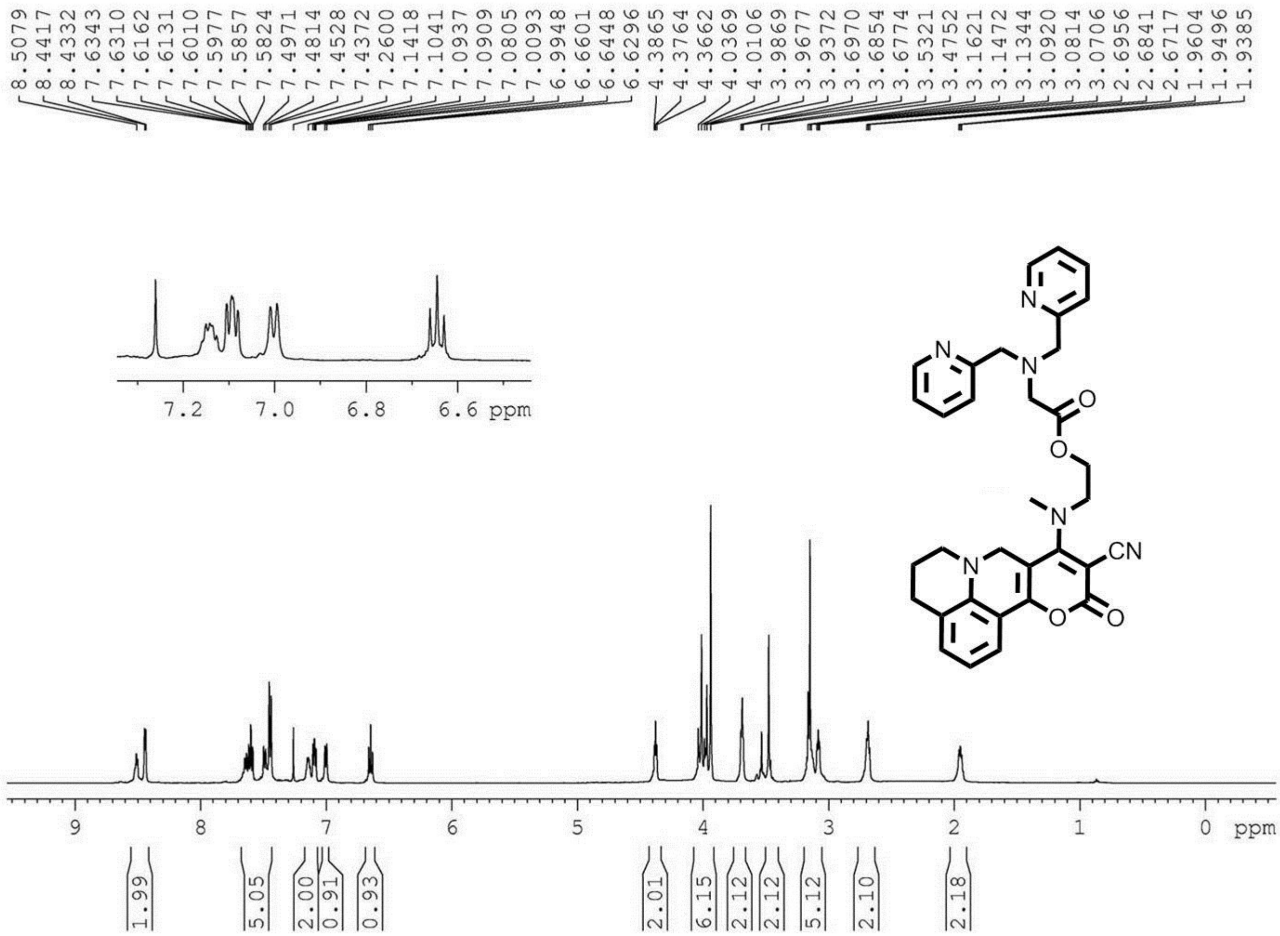


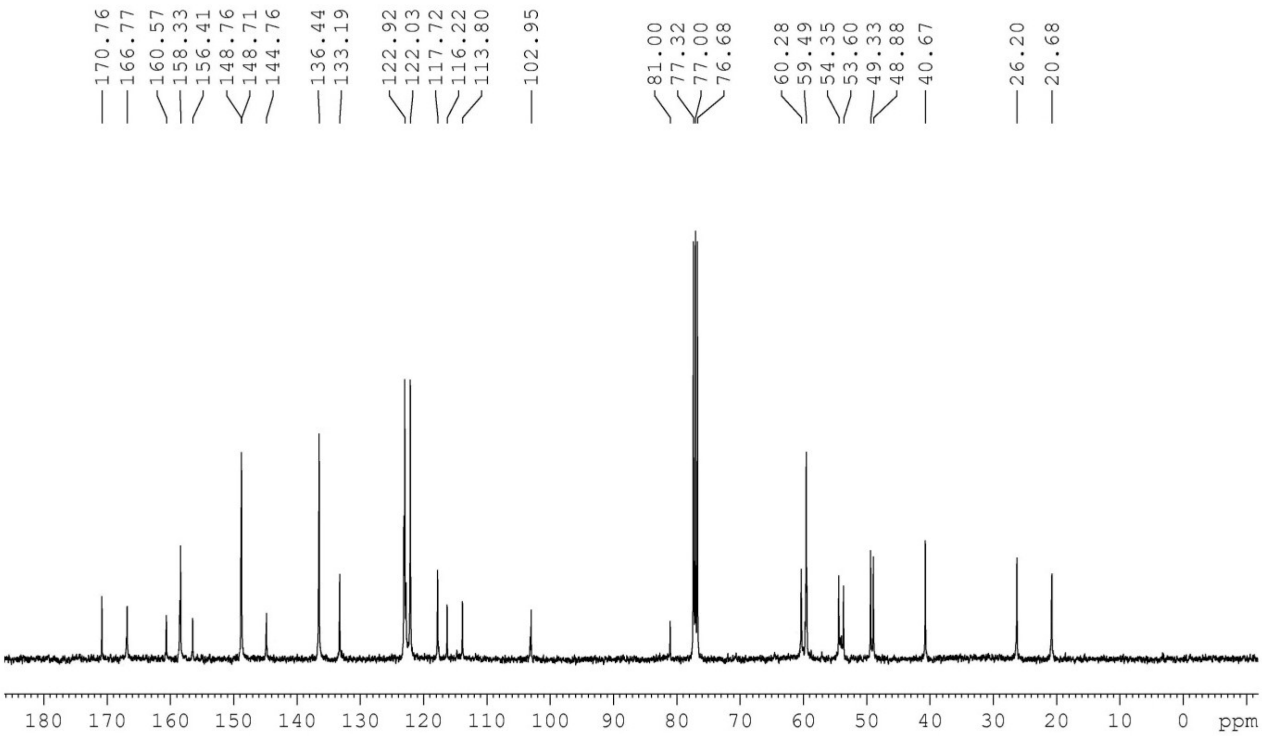
^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100.6 MHz) of PYJO3





[1]H NMR ($CDCl_3$, 500 MHz) and [13]C NMR ($CDCl_3$, 100.6 MHz) of PYJO4





Cartesian coordinates (Å) of **PYJO4** optimized at B3LYP/6-31G (d, p)

Atom	X	Y	Z
C	-6.46273120	1.43005739	2.80689421
C	-5.23407067	0.82754007	2.58911488
C	-4.88168688	0.37307414	1.30766934
C	-5.78625534	0.51478515	0.21878234
C	-7.05533330	1.09848561	0.45763675
C	-7.35759231	1.55420228	1.73798659
N	-5.43571780	0.03045255	-1.02631655
C	-6.34562745	0.15472427	-2.16952120
C	-7.31501587	1.32666994	-2.01818163
C	-8.04601190	1.22774760	-0.67757988
C	-3.64698472	-0.34535713	1.07016108
C	-3.21496780	-0.67890178	-0.18795089
C	-4.03815029	-0.21562929	-1.36723546
O	-3.00362020	-0.74187833	2.18695408
C	-1.84051901	-1.53512331	2.16405955
C	-1.39051261	-1.92484685	0.83998859
C	-2.05797074	-1.52011488	-0.32348962
O	-1.32770419	-1.79306591	3.22418475
N	-1.68166841	-1.96582899	-1.58870580
C	-1.29709981	-1.00427192	-2.62275540
C	0.20959429	-0.77543631	-2.73274903
C	-1.20429590	-3.33460943	-1.79245226
O	0.65764015	-0.18591658	-1.50291965

C	1.99791470	-0.23499242	-1.27110586
C	2.31922459	0.42429382	0.06334728
O	2.78470919	-0.71892099	-2.05197536
N	3.72090361	0.30551633	0.40782414
C	4.21434431	1.41634363	1.21943112
C	4.07824367	-1.01066661	0.95998867
C	5.52867821	-1.35424380	0.68309319
C	4.36688802	2.71043442	0.43928743
N	4.24837555	3.84145487	1.15347524
C	4.42888102	5.00515627	0.51767888
C	4.72151650	5.10888628	-0.84190910
C	4.83325715	3.93034968	-1.57947755
C	4.65479422	2.71036171	-0.93219829
C	5.98847239	-1.45553178	-0.63873257
C	7.32078179	-1.78561299	-0.86010043
C	8.15445292	-1.99809672	0.24028335
C	7.60730814	-1.86743082	1.51414146
N	6.32428065	-1.55163841	1.74462081
C	-0.12526866	-2.57225619	0.79742894
N	0.92824237	-3.06751752	0.73732809
H	-6.73058701	1.79483619	3.79249658
H	-4.52700505	0.69182270	3.39920954
H	-8.32488218	2.02377070	1.90136022
H	-5.73921175	0.29098599	-3.07128778
H	-6.90715230	-0.78247087	-2.29996504
H	-6.75695115	2.26923727	-2.06751776

H	-8.02173145	1.31967952	-2.85415458
H	-8.71572815	0.35577524	-0.69574800
H	-8.68431778	2.10319095	-0.51832841
H	-3.60420310	0.69808734	-1.82123883
H	-4.00438282	-0.99134236	-2.14028738
H	-1.65622869	-1.35868578	-3.59867486
H	-1.77372159	-0.04439953	-2.42000377
H	0.76144834	-1.69967590	-2.91761939
H	0.41751231	-0.09372291	-3.56640439
H	-1.49164363	-3.64073944	-2.80500929
H	-0.12348643	-3.46059286	-1.67863743
H	-1.69916601	-3.99799019	-1.08165577
H	2.06002259	1.48346242	-0.04838924
H	1.62970034	0.01767931	0.82224825
H	5.20162050	1.12763039	1.59973571
H	3.59373847	1.62069516	2.10797128
H	3.90574154	-1.07186113	2.04516179
H	3.43559469	-1.76070504	0.48916763
H	4.32799287	5.90156825	1.12776642
H	4.85247270	6.08230070	-1.30403465
H	5.05783852	3.96110300	-2.64222139
H	4.72413360	1.76417090	-1.45719234
H	5.29548409	-1.27687289	-1.45436722
H	7.70569648	-1.87867473	-1.87199064
H	9.20126638	-2.25703629	0.11553853
H	8.22547909	-2.01998377	2.39733341

References

1. H. L. Clever, S. A. Johnson and M. E. Drrick *J. Phys. Chem. Ref. Data*, 1985, **14**, 631.
2. X. Shi, H. Wang, T. Han, X. Feng, B. Tong, J. Shi, J. Zhi and Y. Dong *J. Mater. Chem.*, 2012, **22**, 19296.
3. B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du and X. Zhang *Chem. Commun*., 2011, **47**, 8656.
4. M. I. Rodriguez-Cacers, R. A. Agbaria and I. M. Warner *J. Fluores.* 2005, **15**, 185.