Supporting Information

for

Perylene diimide-based nanoring architecture for exogenous and endogenous ATP detection: Biochemical assay for monitoring phosphorylation of glucose

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Synthesis of tyrosine-based alkyl amide (**1**)

To the solution of Boc-L-tyrosine (500 mg, 1.77 mmol) in a minimum quantity of DMF, N,N'-diisopropylethylamine (DIEA) (310 µL, 1.77 mmol) was added. Subsequently, 1-Ethyl-3-(3'-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) (375 mg, 1.95 mmol) and hydroxybenzotriazole (HOBt) (264 mg, 1.95 mmol) were slowly added to the reaction mixture. After a time interval of 15 minutes, *tert*-butyl (4-aminobutyl)carbamate (334 mg, 1.77 mmol) was added to the reaction mixture and stirring continued for another 4h. The progress of the reaction was checked by *tlc.* After completion of the reaction, the DMF was removed on a rotary evaporator and residue was extracted with water and ethyl acetate. The ethyl acetate part was dried over Na_2SO_4 and then concentrated under vacuum. The crude product was purified by column chromatography (neutralized SiO_2, 3: 97, EtOAc: $CHCl_3$, v/v) to isolate pure compound **1**, solid, R_f = 0.45; yield = 56%; ^{1}H NMR (500 MHz, $CDCl_3$, 25°C); δ 7.56 (bs, 1H, -OH), 7.05 (d, *J* = 7.5 Hz, 2H), 6.80 (d, *J* = 8.0 Hz, 2H), 5.99 (bs, 1H, -NH), 5.31 (bs, 1H, -NH), 4.79 (bs, 1H, -NH), 4.22 (bt, 1H, tyrosine -CH), 2.86–3.24 (m, tyrosine -CH_2 and -CH_2 of butyl chain, 6H), 1.47 (s, 9H), 1.44 (s, 9H), 1.34–1.21 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$, 25°C) δ 171.40, 156.41, 155.55, 155.48, 130.37, 128.00, 115.90, 80.20, 79.74, 56.51, 40.22, 39.00, 38.28, 28.47, 28.33, 27.63, 26.51; IR spectrum (ATR): ν_{max} [cm^{-1}] = 3339.7, 2937.1, 1759, 1684.8, 1513.3, 1364.2, 1237.5, 1162.9 cm^{-1}.

1. Experimental Section

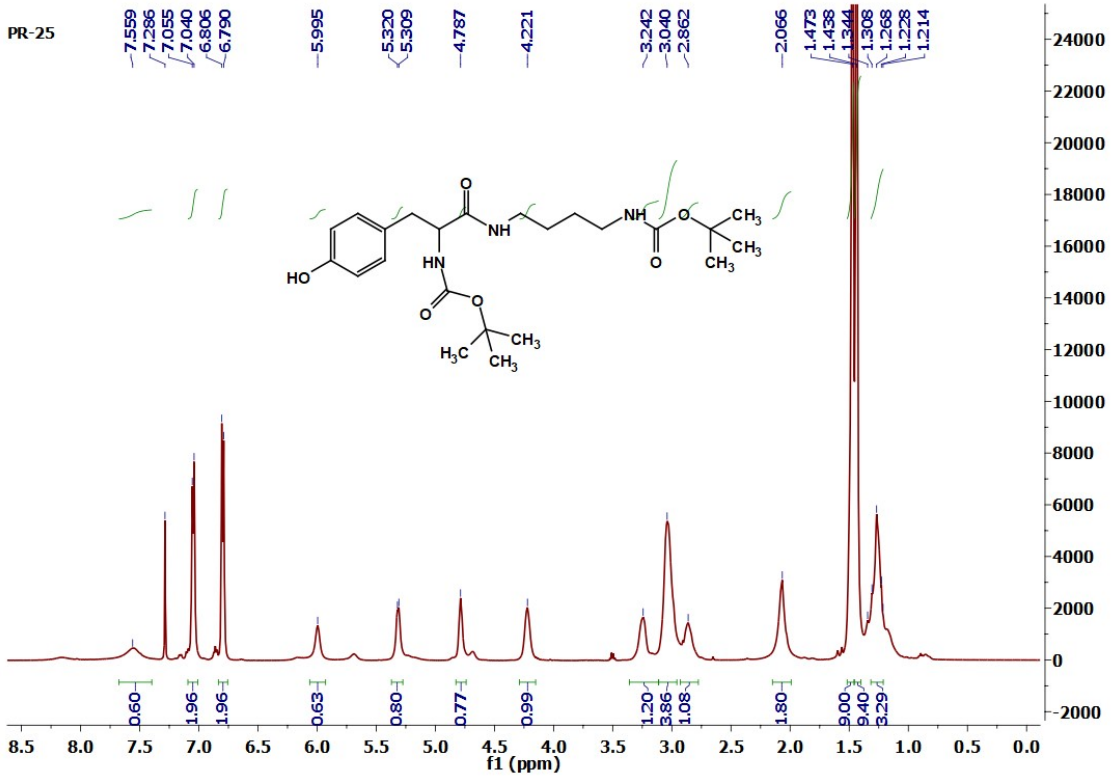


Figure S1a: ^{1}H NMR spectrum of Compound **1** in $CDCl_3$.

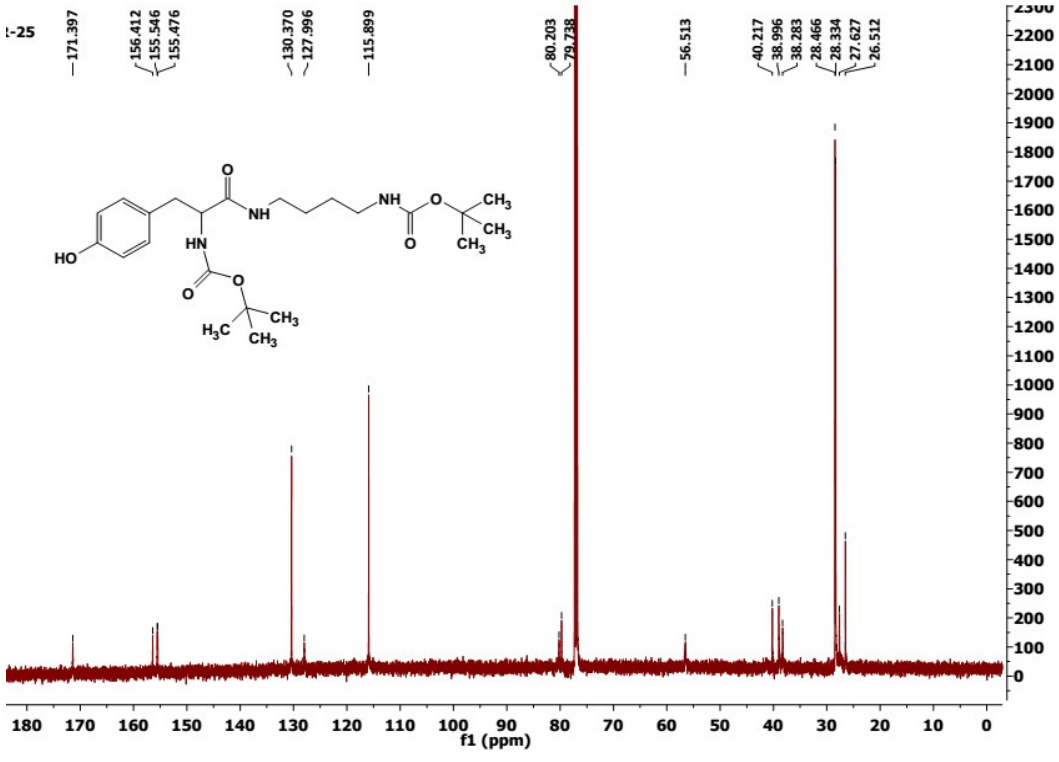


Figure S1b: ^{13}C NMR spectrum of Compound **1** in $CDCl_3$.

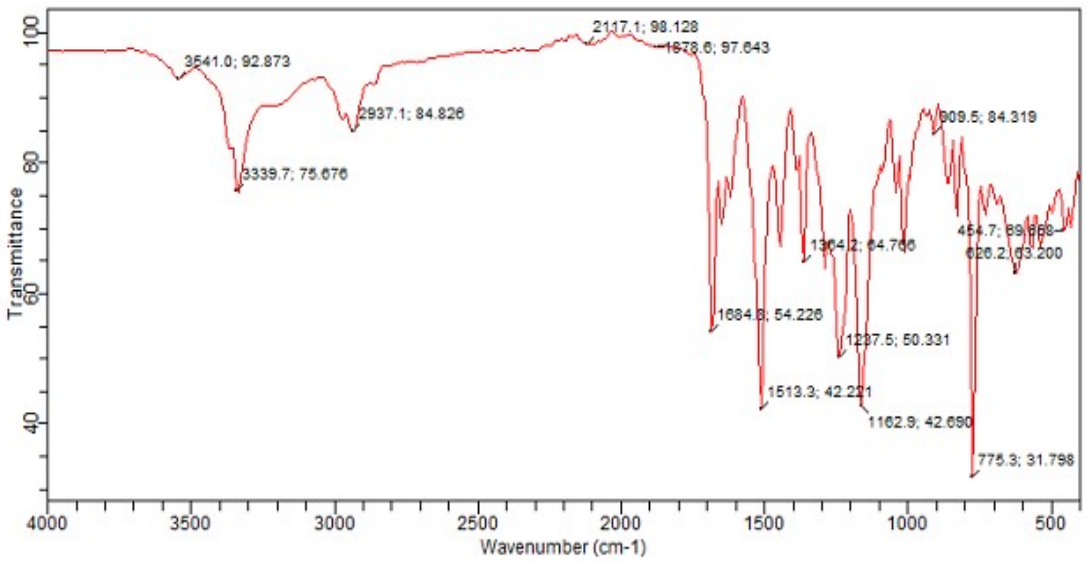


Figure S1c: FTIR (ATR) spectrum of Compound **1**.

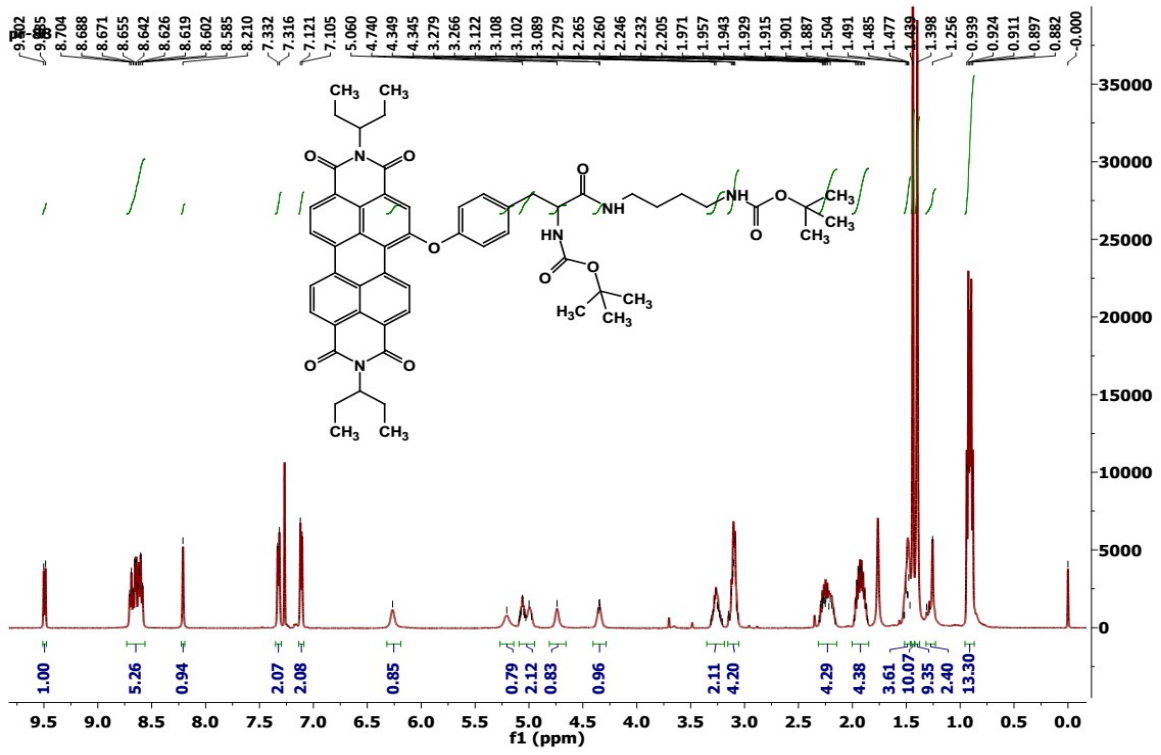


Figure S2a: ^{1}H NMR spectrum of PDI **2** in $CDCl_3$.

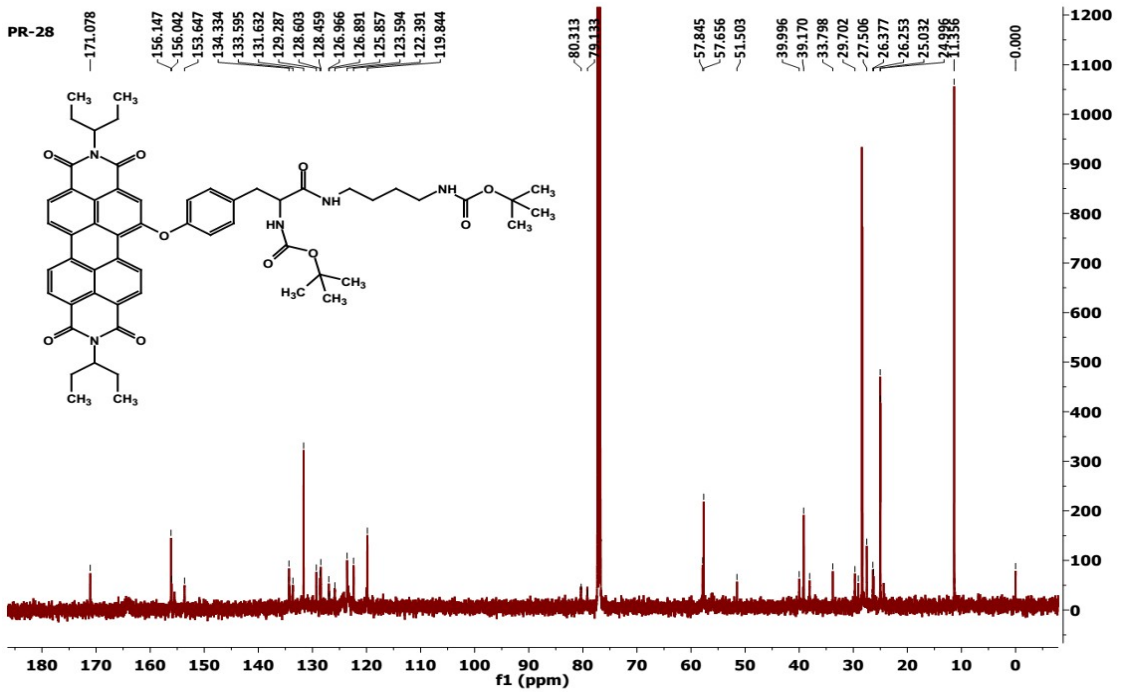


Figure S2b: ^{13}C NMR spectrum of PDI **2** in $CDCl_3$.

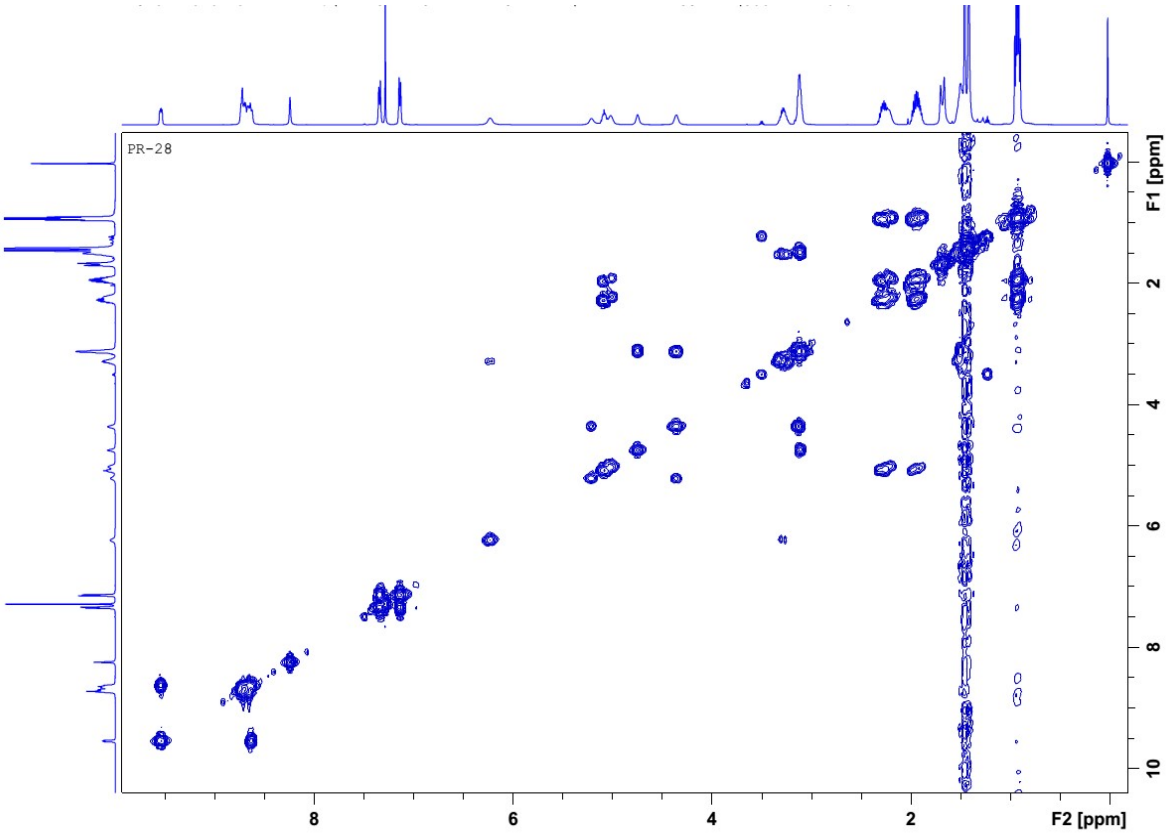


Figure S2c: ^{1}H-^{1}H COSEY NMR spectrum of PDI **2** in $CDCl_3$.

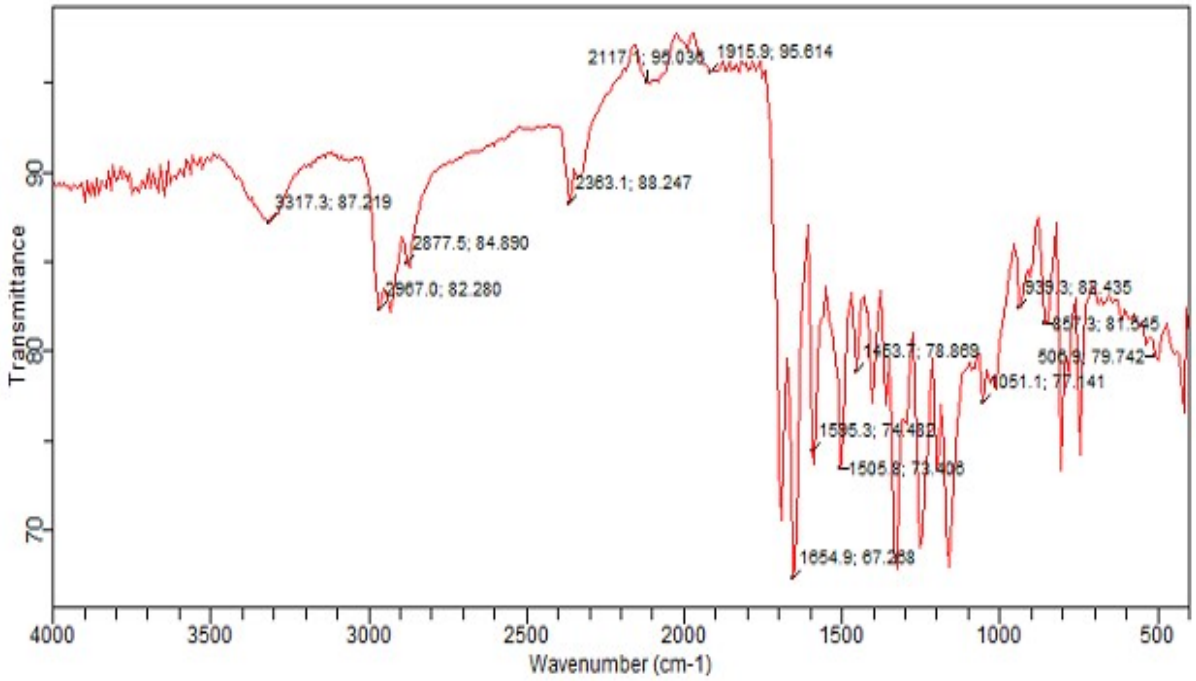


Figure S2d: FTIR spectrum of PDI **2**.

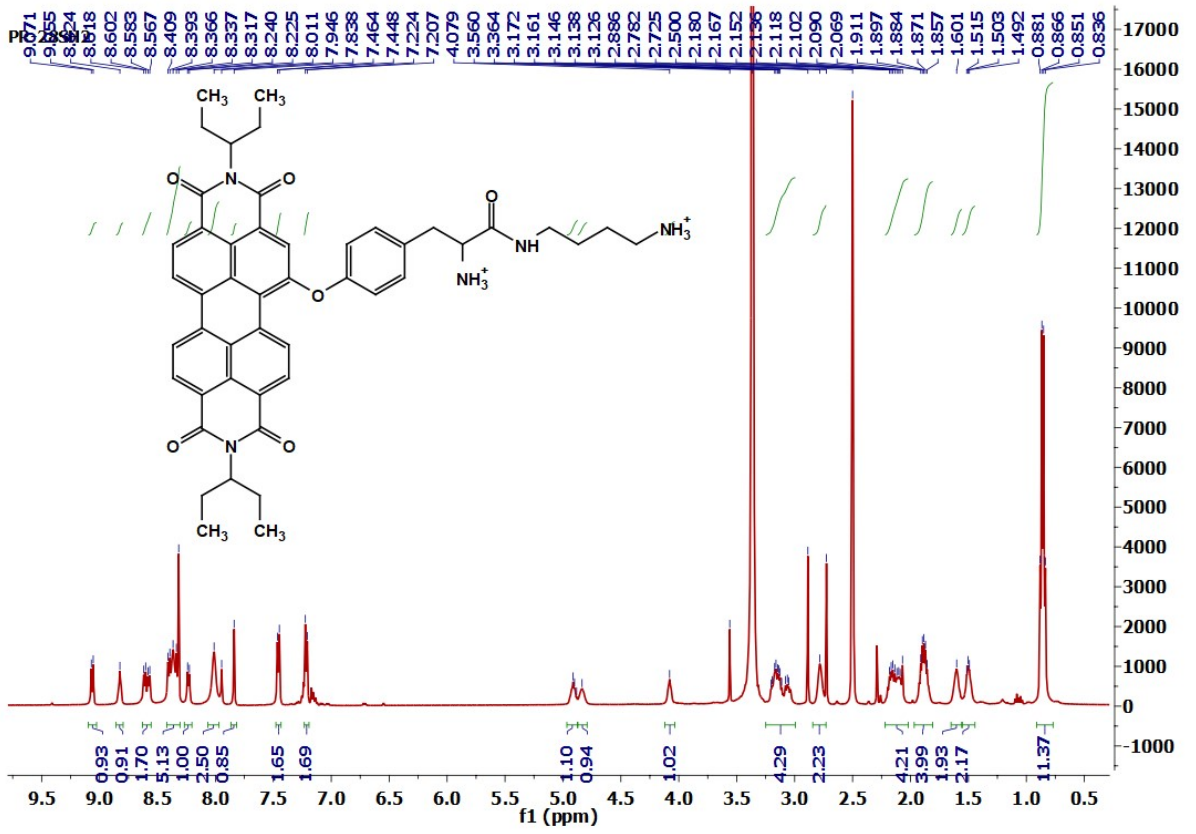


Figure S3a: ^{1}H NMR spectrum of PDI **3** in DMSO-d_6.

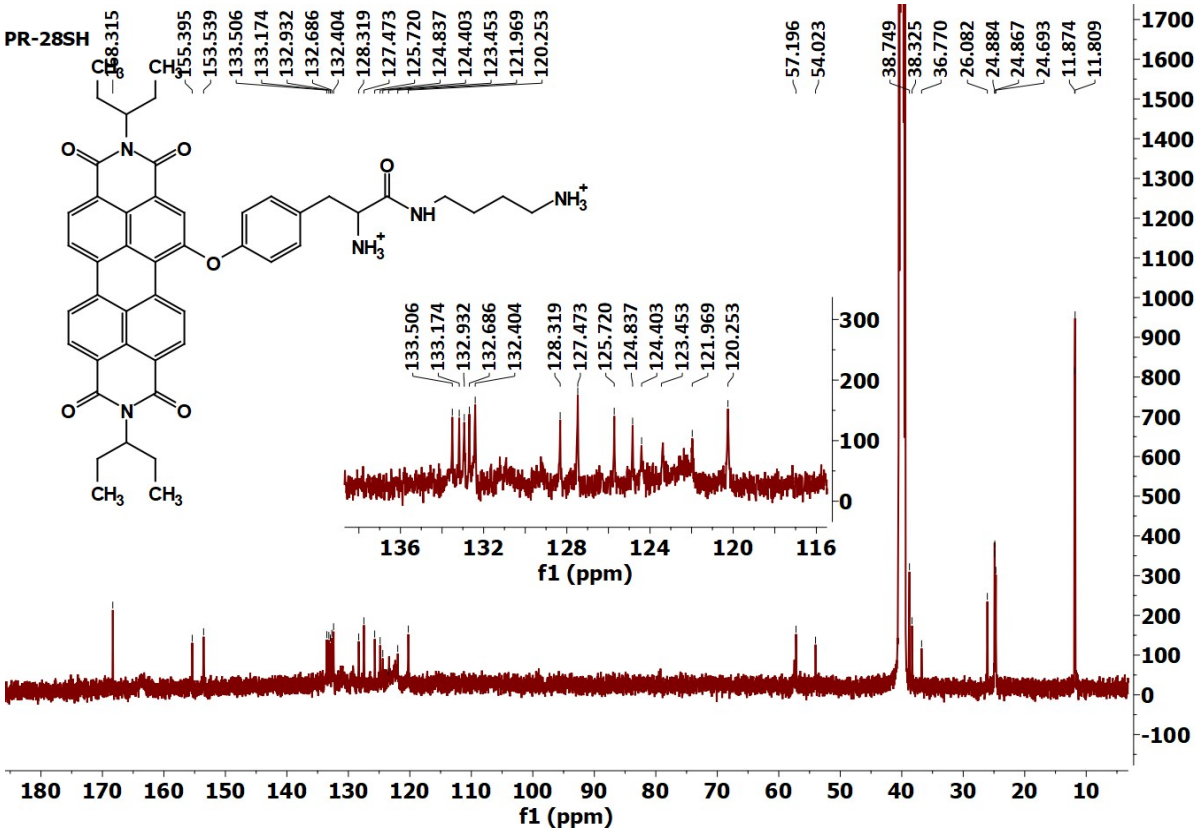


Figure S3b: ^{13}C NMR spectrum of PDI **3** in DMSO-d_6.

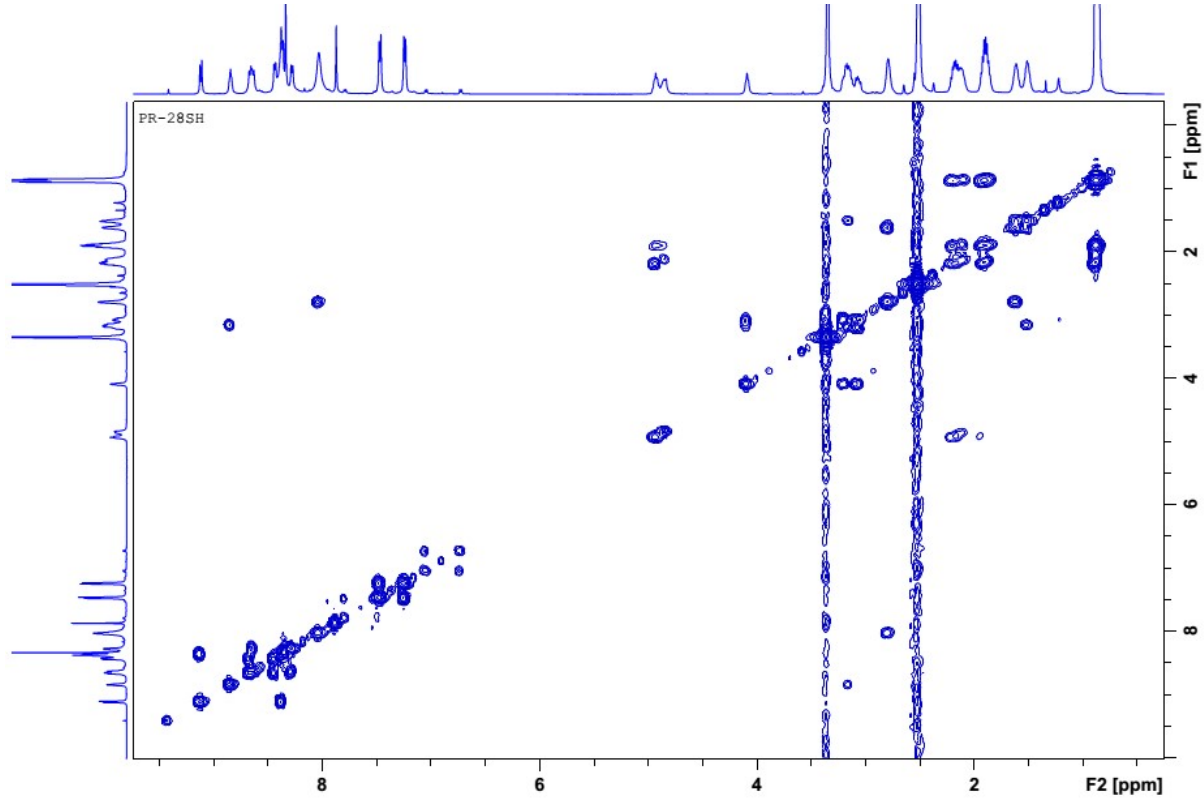


Figure S3c: ^{1}H-^{1}H COY NMR spectrum of PDI **3** in DMSO-d_6.

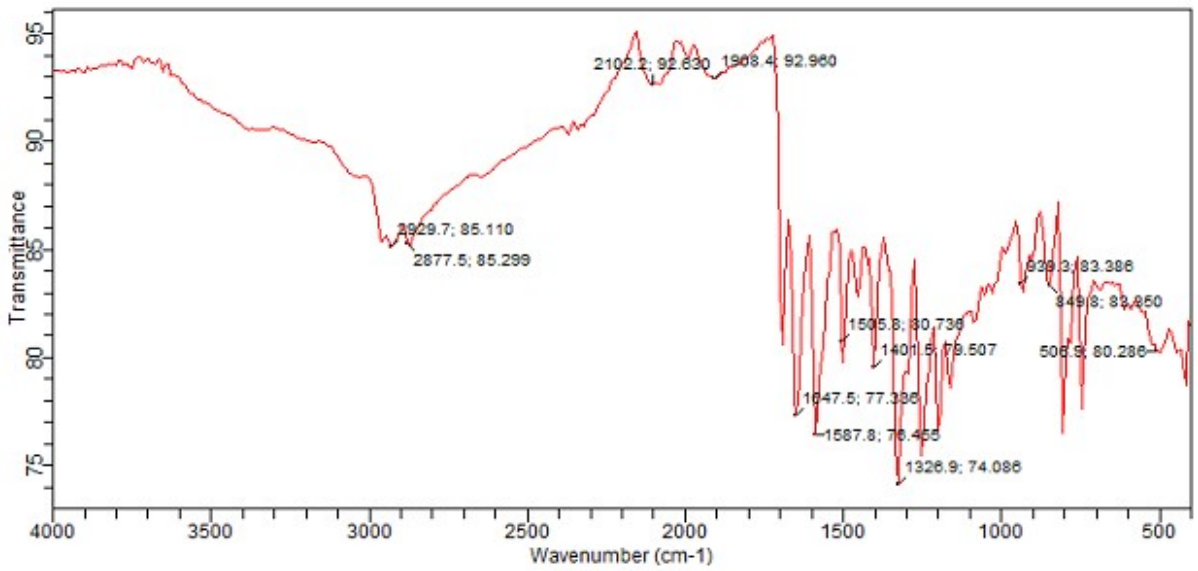
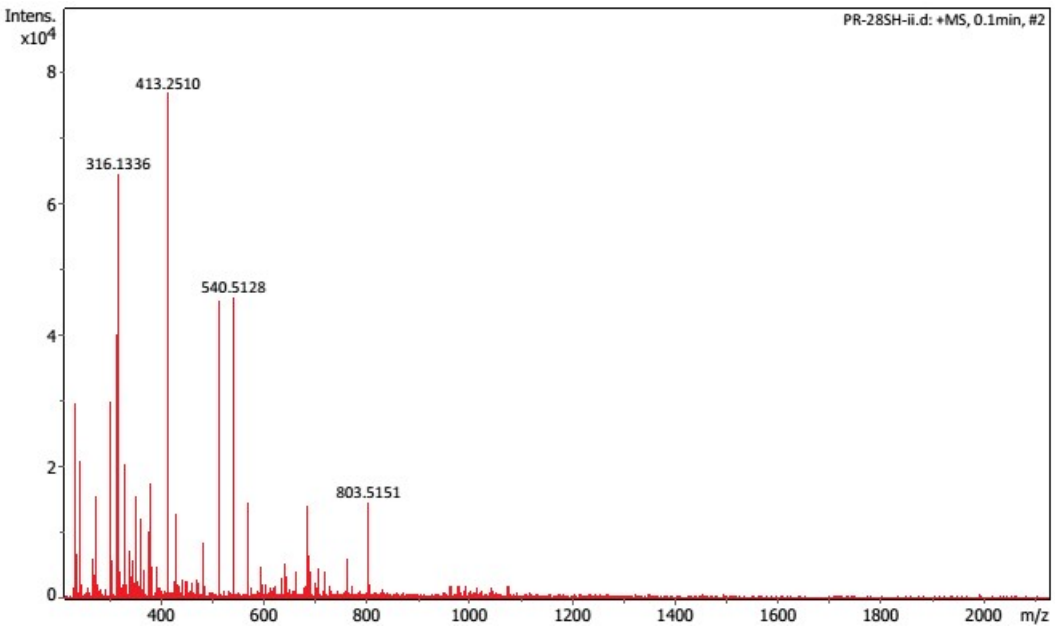


Figure S3d: FTIR spectrum of PDI **3**.



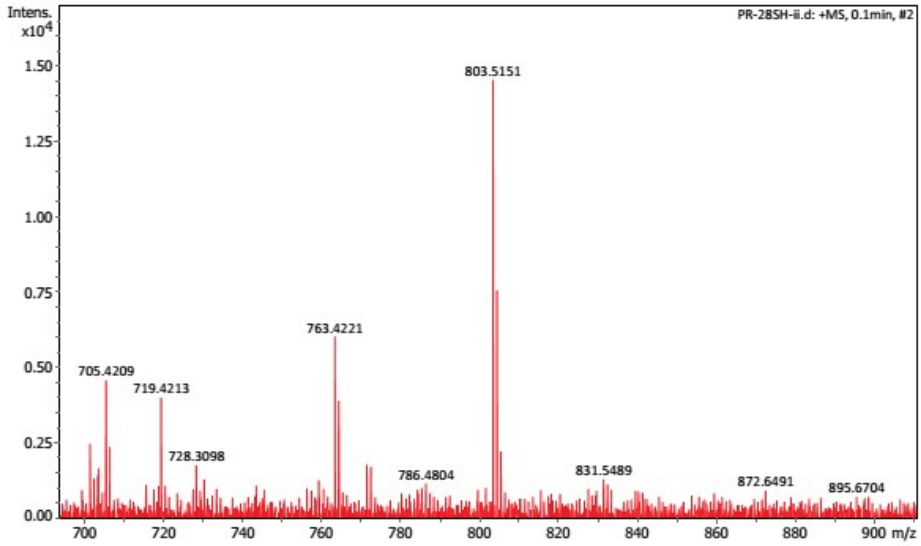


Figure S3e: Mass spectrum (Full and zoomed view) of PDI **3**.

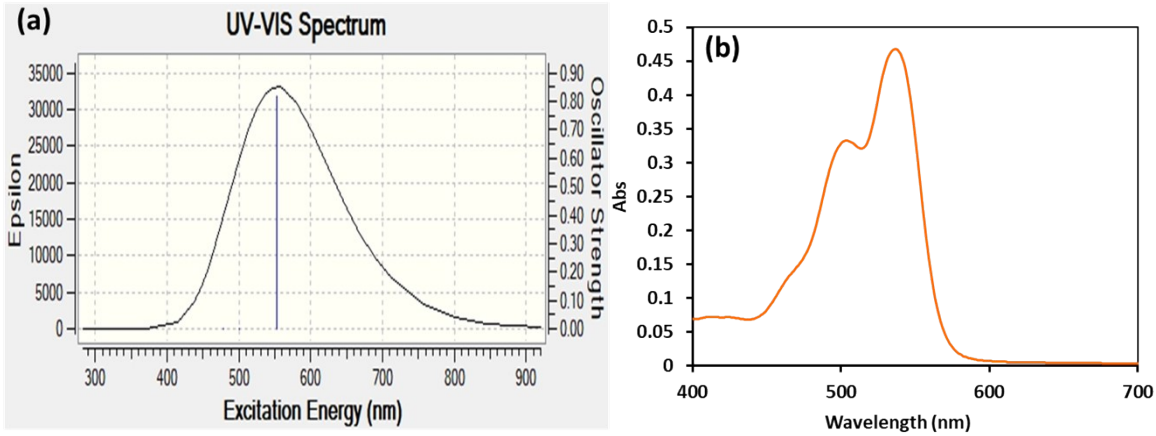


Figure S4a: (a) TD-DFT spectrum of PDI **3**; (b) experimentally determined UV-Vis spectrum of PDI **3**.

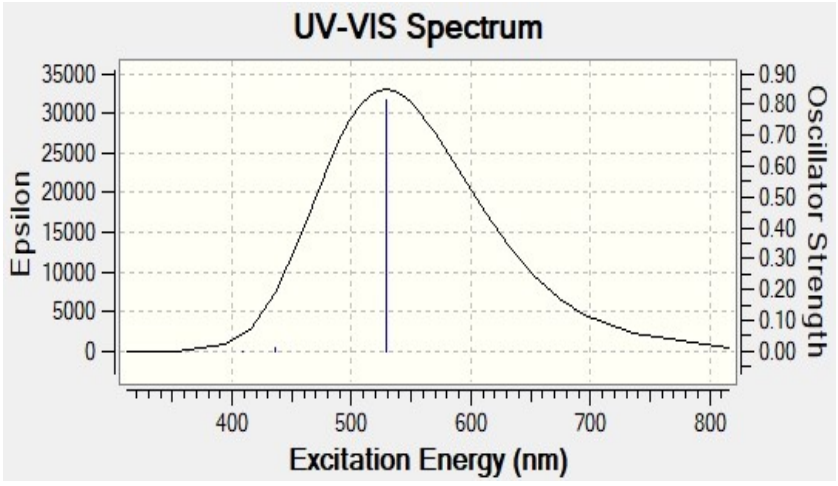


Figure S4b: TD-DFT spectrum of PDI **2.**

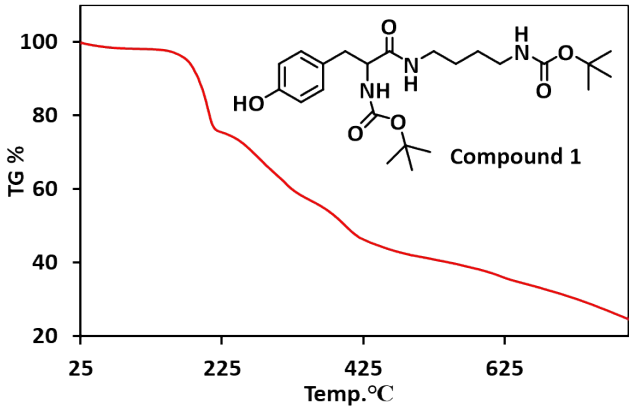


Figure S5: TGA data of compound **1**.

Anton Paar Polarimeter - Measurement(s)
MCP 150
Software Version: 1.50.4074.82
Serial Number: 82104498

Unique Id 413

Sample Name:	Compound 1 ($CHCl_3$)
Date:	03/12/2020 - 05:00 PM
Username:	Administrator
Sample State:	Ok
Measurement Mode:	Specific Rotation
Measurement Result:	+5.000 °
Concentration:	+0.400 g/100ml
Optical Rotation:	+0.002 °
Set Temperature:	+25.0 °C
Temperature:	+25.0 °C

Anton Paar Polarimeter - Measurement(s)
MCP 150
Software Version: 1.50.4074.82
Serial Number: 82104498

Unique Id 539

Sample Name:	PDI 2 ($CHCl_3$)
Date:	10/31/2020 - 11:36 AM
Username:	Administrator
Sample State:	Ok
Measurement Mode:	Specific Rotation
Measurement Result:	+27.500 °
Concentration:	+0.400 g/100ml
Optical Rotation:	+0.011 °
Set Temperature:	+25.0 °C
Temperature:	+25.0 °C

Anton Paar Polarimeter - Measurement(s)
MCP 150
Software Version: 1.50.4074.82
Serial Number: 82104498

Unique Id 544

Sample Name:	PDI 3 ($CHCl_3$)
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Username:	Administrator
Sample State:	Ok
Measurement Mode:	Specific Rotation
Measurement Result:	+25.000 °
Concentration:	+0.400 g/100ml
Optical Rotation:	+0.010 °
Set Temperature:	+25.0 °C
Temperature:	+25.0 °C

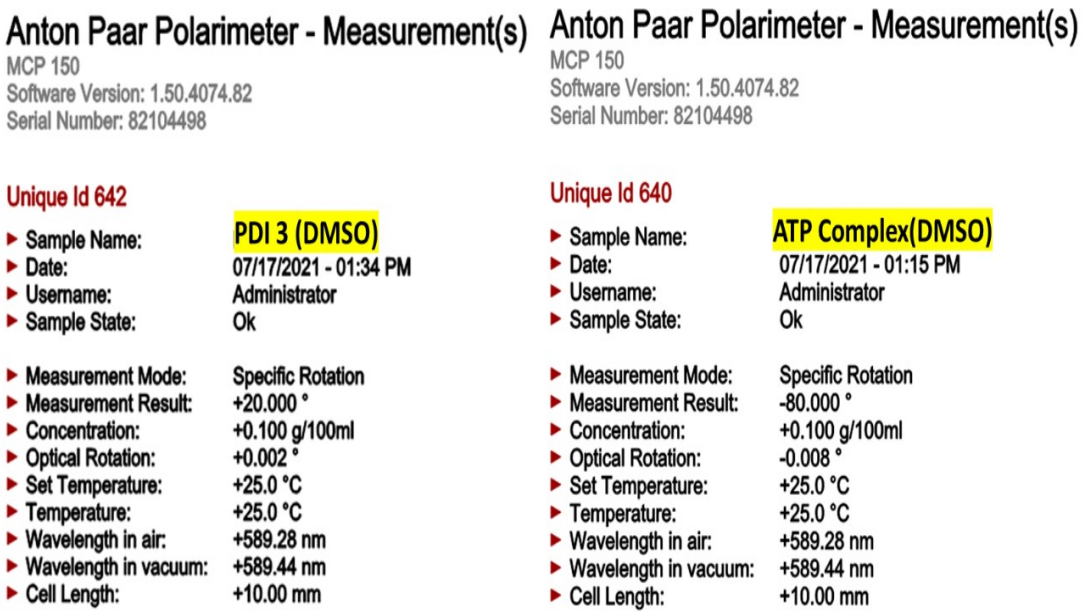


Figure S6: Specific optical rotations of all synthesized derivatives.

Table for Specific optical rotation values

Compound	Solvent	Optical Rotation	Concentration	Specific Rotation
Compound 1	$CHCl_3$	+0.002°	+0.400 g/100ml	+5.000°
PDI 2	$CHCl_3$	+0.011°	+0.400 g/100ml	+27.500°
PDI 3	$CHCl_3$	+0.010°	+0.400 g/100ml	+25.000°

Table for Specific optical rotation values

Compound	Solvent	Optical Rotation	Concentration	Specific Rotation
Compound 1	$CHCl_3$	+0.002°	+0.400 g/100ml	+5.000°
PDI 2	$CHCl_3$	+0.011°	+0.400 g/100ml	+27.500°
PDI 3	$CHCl_3$	+0.010°	+0.400 g/100ml	+25.000°
PDI 3	DMSO	+0.002°	+0.100 g/100ml	+20.000°
PDI 3 + ATP	DMSO	-0.008°	+0.100 g/100ml	-80.000°

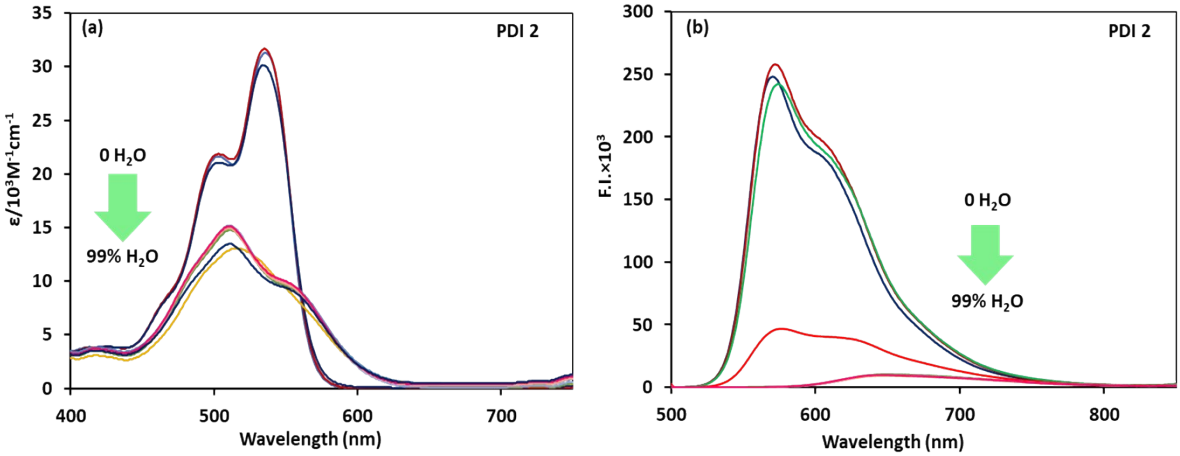
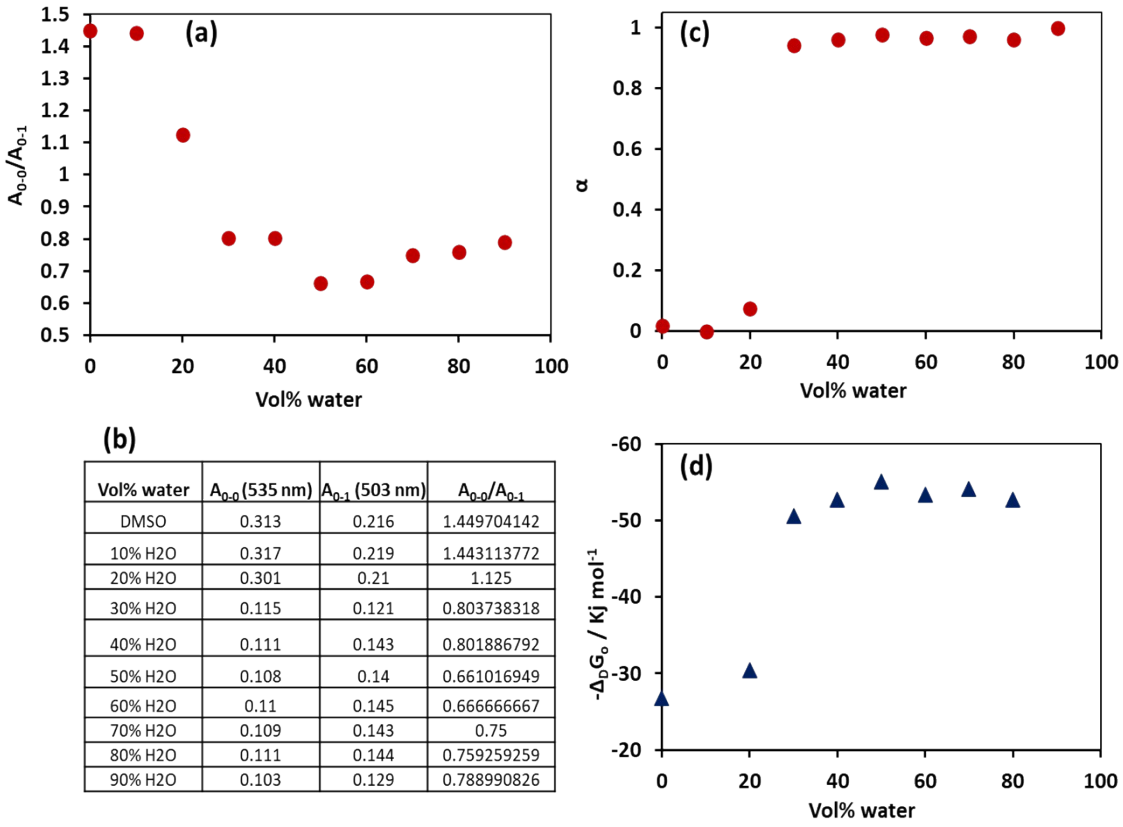


Figure S7: The UV–Vis (a) and fluorescence (b) spectra of **PDI 2** (10 μM) in different DMSO-water ratios. λ_{ex} = 490 nm, slit width (ex/em) =5/5.



Vol% water	A_{0-0} (535 nm)	A_{0-1} (503 nm)	A_{0-0}/A_{0-1}
DMSO	0.313	0.216	1.449704142
10% H2O	0.317	0.219	1.443113772
20% H2O	0.301	0.21	1.125
30% H2O	0.115	0.121	0.803738318
40% H2O	0.111	0.143	0.801886792
50% H2O	0.108	0.14	0.661016949
60% H2O	0.11	0.145	0.666666667
70% H2O	0.109	0.143	0.75
80% H2O	0.111	0.144	0.759259259
90% H2O	0.103	0.129	0.788990826

Figure S8: (a,b) Plot and tabulated value of Franck–Condon (A_{0-0}/A_{0-1}) values of PDI **2** versus H_2O fraction in DMSO; (c) Plot of the degree of aggregation (α_{agg}) versus H_2O fraction in DMSO and (d) plot of ΔG* versus H_2O fraction in DMSO.

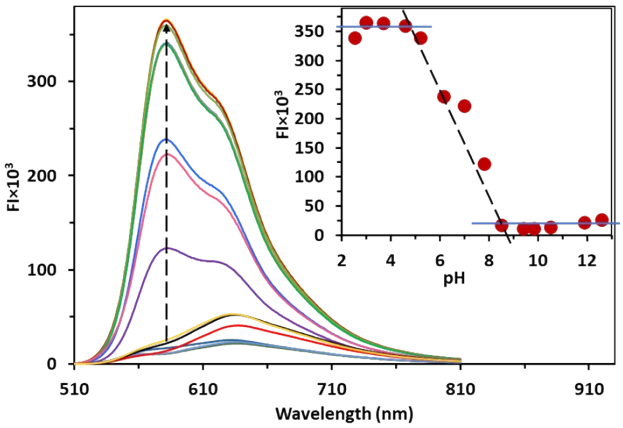


Figure S9. The fluorescence pH titration of PDI **3** (5 µM) in 50% H_2O-DMSO solution.

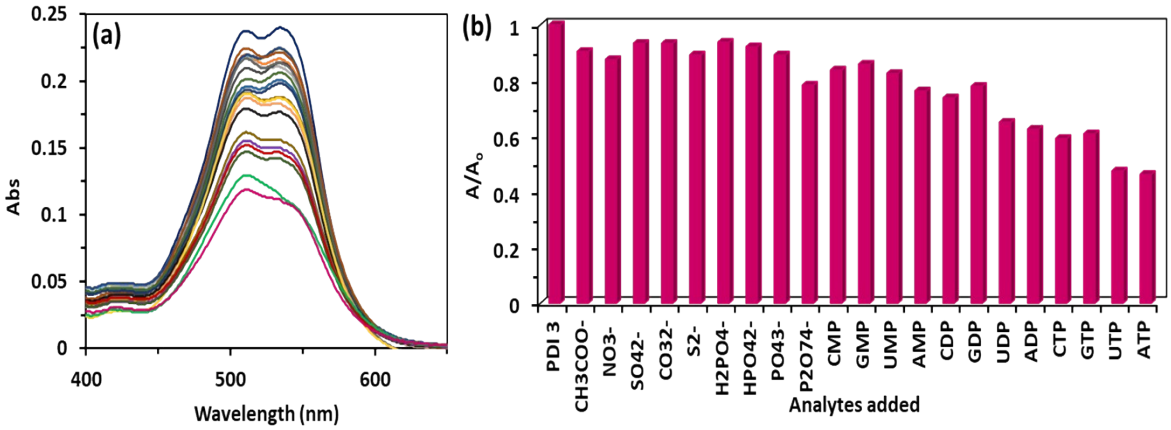


Figure S10: (a) UV-Vis response of **PDI 3** toward various anionic analytes; (b) bar graph of I/I_o plots of absorbance and emission data showing response towards anions and NPPs.

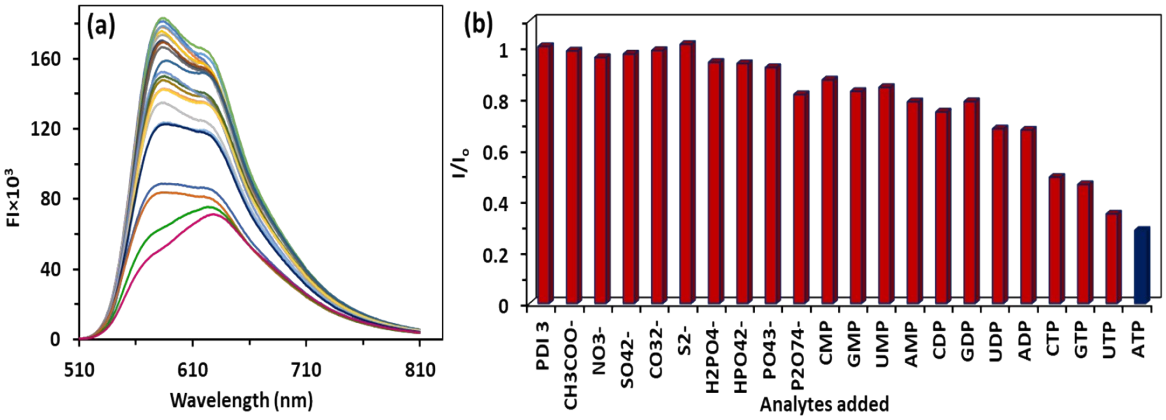


Figure S11: (a) Fluorescence response of **PDI 3** toward various anionic analytes; (b) bar graph of I/I_o plots of absorbance and emission data showing response towards anions and NPPs. (Not complete Image).

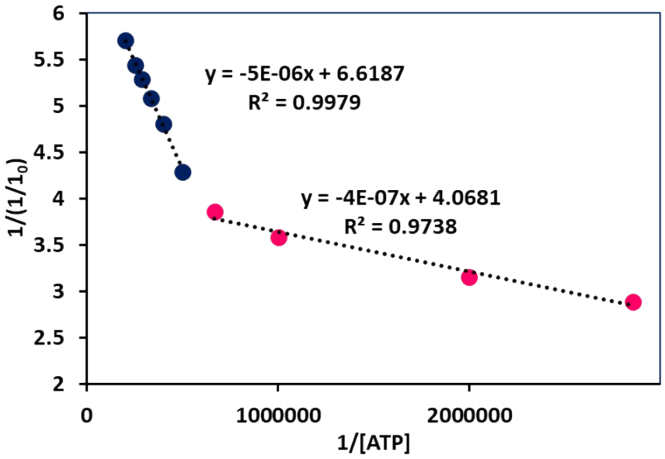


Figure S12a: B-H Plot of **PDI 3** with ATP.

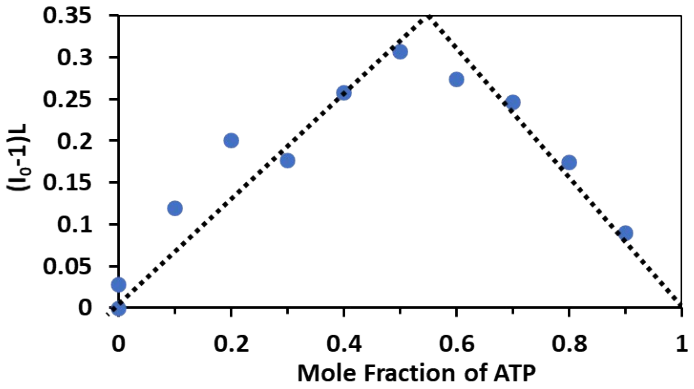


Figure S12b: Job plot of **PDI 3** with ATP.

Table S2 Changes in the Franck-Codon factor ($A_{0\text{-}0}/A_{0\text{-}1}$) on interaction with ATP:

ATP Equivalents	$A_{0\text{-}1}$ (512 nm)	$A_{0\text{-}0}$ (535 nm)	$A_{0\text{-}0}/A_{0\text{-}1}$
PDI 3	0.268	0.274	1.022388
0.001 ATP	0.254	0.26	1.023622
0.005 ATP	0.23	0.238	1.034783
0.01 ATP	0.219	0.227	1.03653
0.015 ATP	0.215	0.223	1.037209
0.02 ATP	0.209	0.216	1.033493
0.07 ATP	0.192	0.197	1.026042
0.1 ATP	0.188	0.193	1.026596
0.15 ATP	0.184	0.187	1.016304
0.2 ATP	0.178	0.178	1
0.3 ATP	0.169	0.166	0.982249
0.4 ATP	0.167	0.159	0.952096
0.5 ATP	0.162	0.15	0.925926
0.8 ATP	0.156	0.142	0.910256
1 ATP	0.154	0.136	0.883117

1.5 ATP	0.151	0.132	0.874172
2 ATP	0.147	0.123	0.836735
3 ATP	0.145	0.12	0.827586
4 ATP	0.14	0.114	0.814286
5 ATP	0.14	0.114	0.814286
6 ATP	0.139	0.112	0.805755

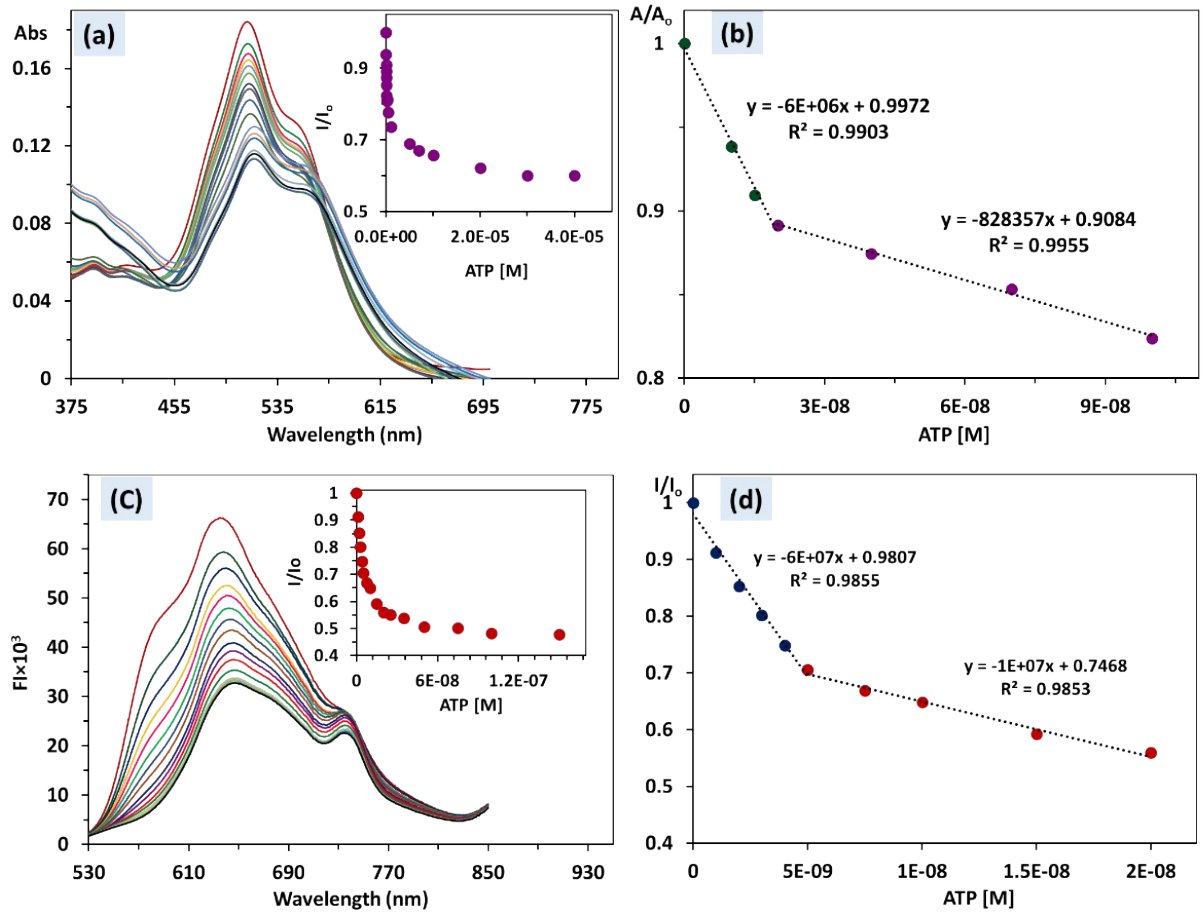


Fig. S13 Changes in (a) absorbance (10 μM) and (c) emission (5 μM) spectra of PDI **3** with gradual increase of ATP concentrations in 9:1 HEPES buffer: DMSO medium; (λ_{ex} = 490 nm; slit width (E_x/E_m) = 10/10 nm; [Inset of (a,c)] plot of absorbance (A/A_o) and emission (I/I_o) changes with change in ATP concentration, respectively; Linear correlation plot of (b) A/A_o and (d) I/I_o versus the concentration of ATP to determine the lowest limit of detection.

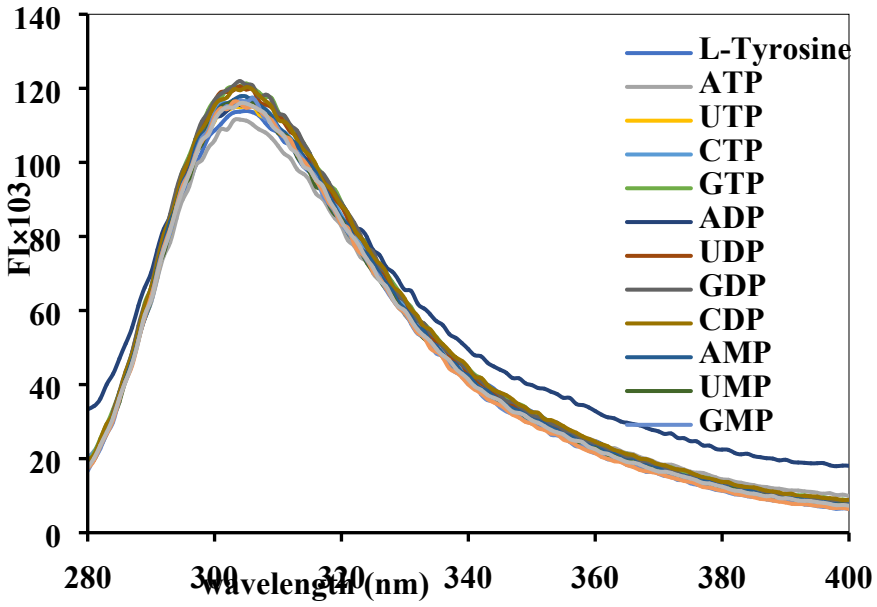


Fig. S13a Changes in emission (5 μM) spectra of tyrosine with gradual increase of nucleoside polyphosphates (NPPs) concentrations in 9:1 HEPES buffer: DMSO medium; (λ_{ex} = 260 nm; slit width (E_x/E_m) = 10/10 nm.

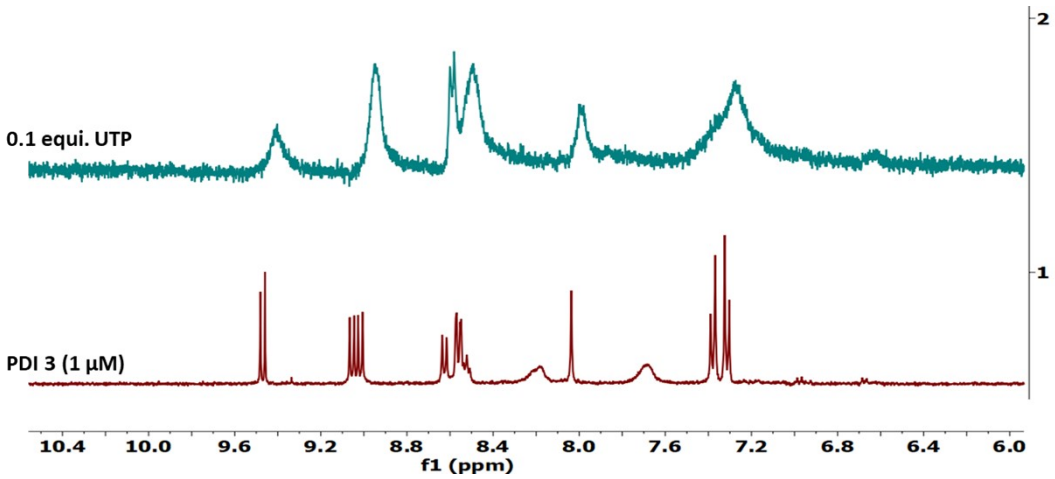


Fig. **S14** Partial ^{1}H NMR spectra (stacked) of PDI **3** (1 mM) after incremental addition of ATP in DMSO-d_6 solution.

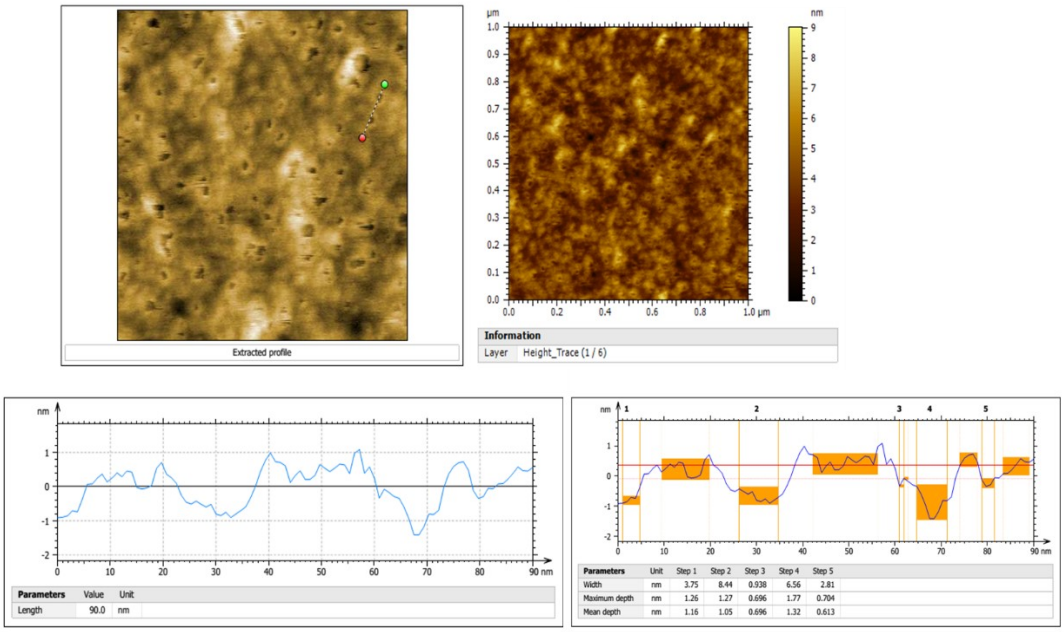
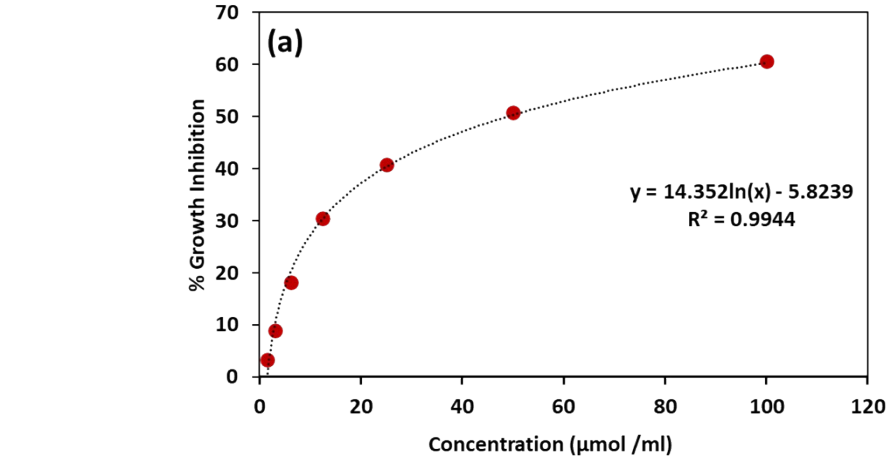
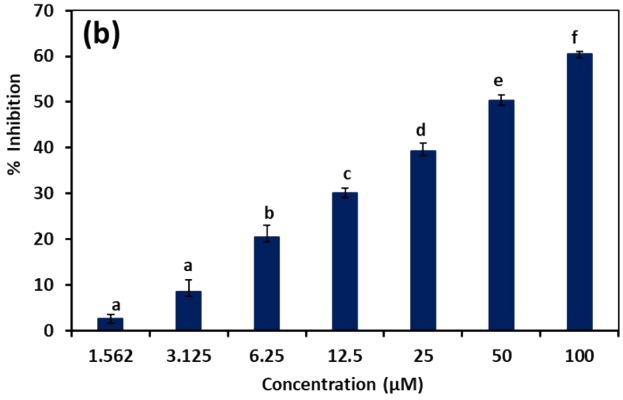


Fig. S15. AFM images of PDI **3** in 50% H_2O-DMSO solution.





Conc. (in µM)	% Inhibition (Mean±SE)
1.562	2.625±0.908
3.125	8.558±2.508
6.25	20.460±2.524
12.5	30.133±0.951
25	39.302±1.615
50	50.342±1.157
100	60.590±0.400
IC_{50}	49.917
One-way ANOVA	
F-Ratio	172.55

Fig. S16 MTT assay to check the cytotoxicity of **PDI 3** with statistical distribution.

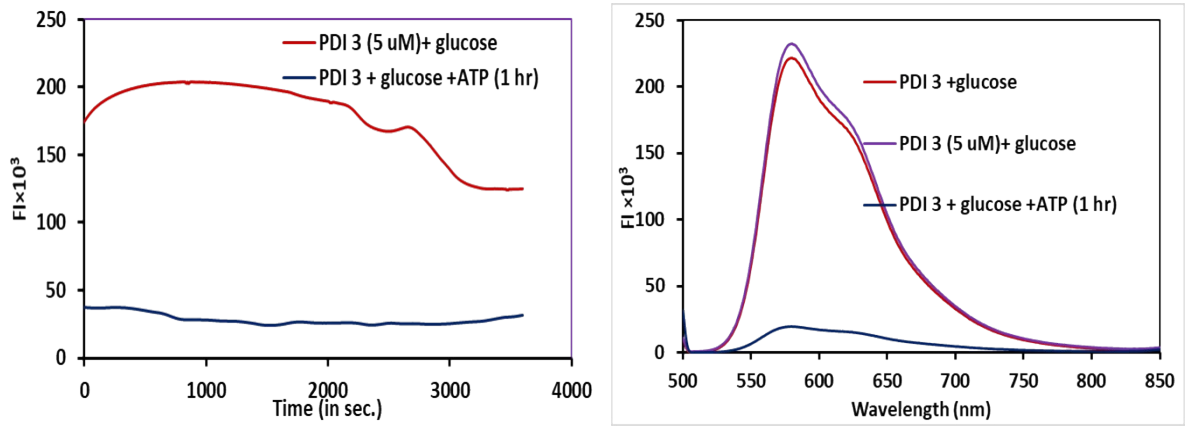


Fig. S17: Fluorescence spectrum of PDI **3**+ATP after regular interval of time.