Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

ESIPT based Hg^{2+} and fluoride chemosensor for sensitive and selective 'turn on' red signal and cell imaging

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General: The chemicals and solvents were purchased from Sigma-Aldrich Chemicals Private Limited and were used without further purification. Melting points were determined on a hot-plate melting point apparatus in an open-mouth capillary and were uncorrected. ^{1}H NMR spectra were measured with Bruker AVANCE III 400 MHz spectrometer with TMS as an internal standard. Chemical shifts are expressed in δ units and $^{1}H–^{1}H$ coupling constants in Hz. UV-vis data was collected on a JASCO UV-V530 spectrophotometer and fluorescence experiment was done using Perkin Elmer LS 55 fluorescence spectrophotometer with a fluorescence cell of 10 mm path. Electrospray ionization mass (ESI MS) spectra were run on a Thermo Finnigan (San Jose, CA, USA) LCQTM Advantage MAX quadrupole ion trap instrument, by infusing samples directly into the source at 20 µL/min with a syringe pump. The spray voltage was set at 3.7 kV and the capillary temperature at $80^{0}C$. Elemental analyses were performed on a CE Instrument EA1110 instrument. For bio-imaging studies the photographs were taken in DeWinter Victory-FL fluorescence inverted microscope using DeWinter Biowizard software v 4.4 using 40X objective.

1. **(a) General procedure for drawing Job plot by Fluorescence method:**

Stock solutions of same concentration of **PDP** and Hg^{2+} were prepared in the order of 2.0×10^{-5} (M) by using HEPES buffer solution. The intensity of emission in each case with different host–guest ratio but equal in volume was recorded. Job plots were drawn by plotting $\Delta I.X_{host}$ vs X_{host} (ΔI = change of intensity of the fluorescent spectrum at 635 nm during titration and X_{host} is the mole fraction of the host).

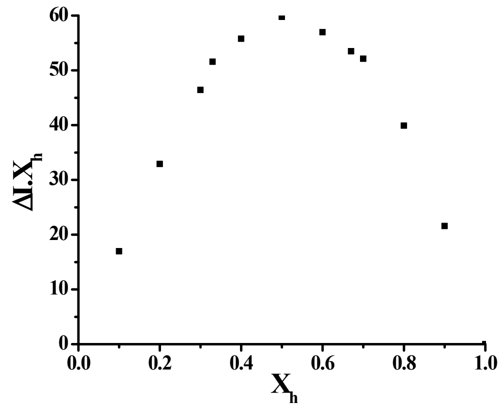


Fig-S$_1$: Jobs plot diagram of **PDP** for Hg^{2+}.

(b) General procedure for drawing Job plot by UV–vis method:

Stock solution of same concentration of **PDP** and F^- were prepared in the order of 2.0×10^{-5} (M) in pure CH_3CN. The absorbance in each case with different *host–guest* ratio but equal in volume was recorded. Job plots were drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of intensity of the absorbance spectrum during titration and X_{host} is the mole fraction of the host in each case, respectively).

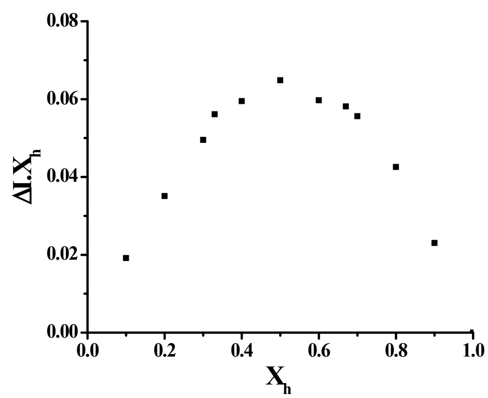


Fig-S$_2$: Jobs plot diagram of **PDP** for TBAF.

2. Association constant determination using fluorescence spectra for Hg^{2+} ion:

The binding constant value of Hg^{2+} with **PDP** has been determined from the emission intensity data following the modified Benesi–Hildebrand equation, $1/\Delta I = 1/\Delta I\ max + (1/K[C])\ (1/\Delta I\ max)$. Here ΔI = I-Imin and $\Delta I\ max = I_{max} - I_{min}$, where I_{min}, I and I_{max} are the emission intensities of sensor considered in the absence of Hg^{2+}, at an intermediate Hg^{2+} concentration and at a concentration of complete saturation where K is the binding constant and [C] is the Hg^{2+} concentration respectively. From the plot of $1/(I-I_{min})$ against $[C]^{-1}$ for sensor, the value of K has been determined from the slope. The association constant (K_a) as determined by fluorescence titration method for sensor with Hg^{2+} is found to be 7.5 × 10^3 M^{-1} (error < 10%).

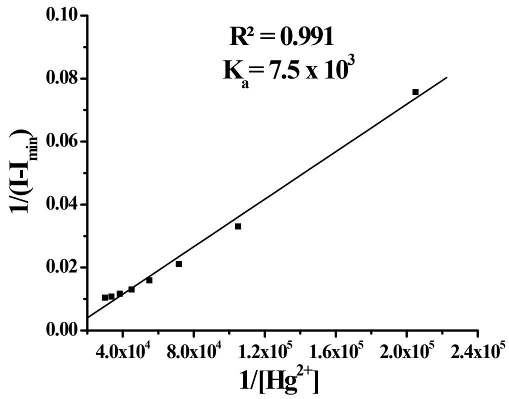


Figure S_3: Benesi–Hildebrand plot from fluorescence titration data of PDP (C =2×10^{-5}M) with Hg^{2+} (C = 2×10^{-4}M).

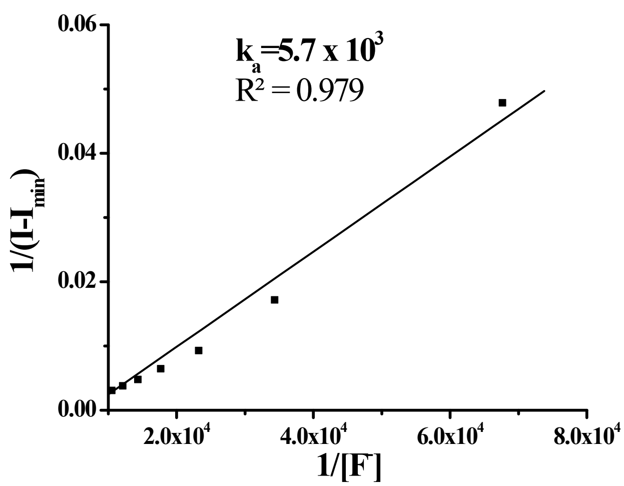


Figure S_4: Benesi–Hildebrand plot from fluorescence titration data of PDP (C =2×10^{-5}M) with F^- (C = 1×10^{-3}M).

The association constant (K_a) as determined by fluorescence titration method for PDP with F^- is found to be 5.7 × 10^3 M^{-1}

3. Calculation of the detection limit:

The detection limit (DL) of PDP sensor in emission spectra for Hg^{2+} was determined from the following equation[1]:

DL = K* Sb1/S

Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

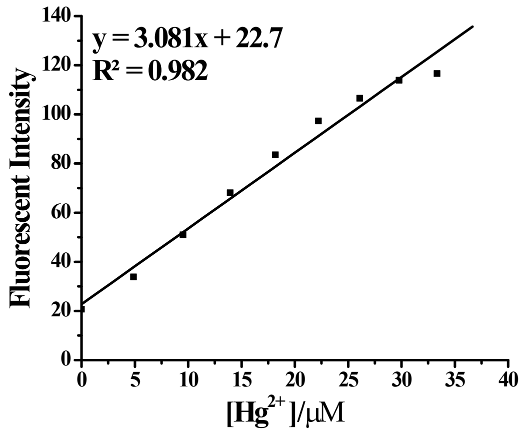


Figure S_5: Changes of Fluorescence intensity of PDP (C= 2×10^{-5}M) as a function of [Hg^{2+}] (C = 2×10^{-4} M) at 635 nm.

From the graph Fig.S_5, we get slope = 3.081, and Sb1 value is 5.0414.

Thus using the formula we get the Detection Limit for Hg^{2+} = 4.9 μM in Fluorescence spectra.

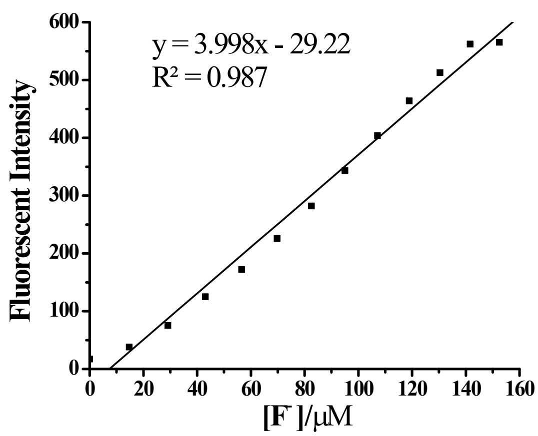


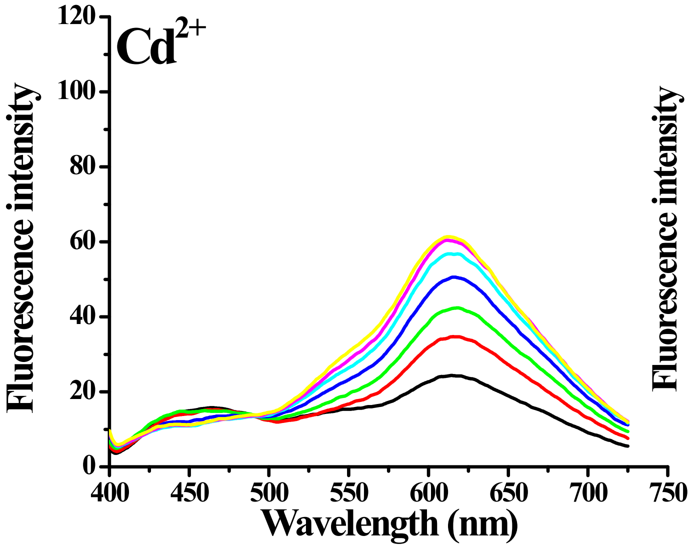
Figure S_6: Changes of Fluorescence intensity of PDP (C= 2×10^{-5}M) as a function of [F^-] (C = 1×10^{-3} M) at 653 nm.

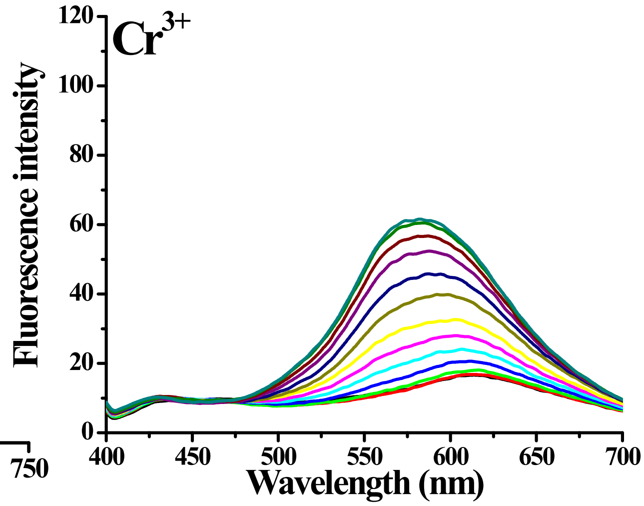
From the graph Fig.S_6, we get slope = 3.998, and Sb1 value is 16.989.

Thus using the formula we get the Detection Limit for F^- = 12.7 μM in Fluorescence spectra.

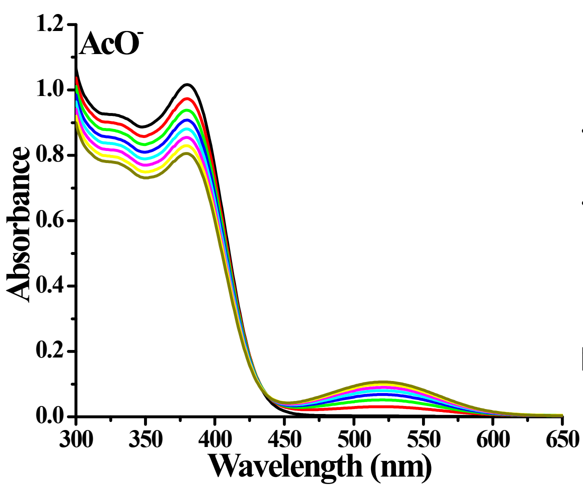
Ref.1: Zhu, M.; Yuan, M.; Liu, X.; Xu, J.; Lv, J.; Huang, C.; Liu, H.; Li, Y.; Wang, S.; Zhu, D. *Org. Lett.* **2008,** *10,* 1481-1484.

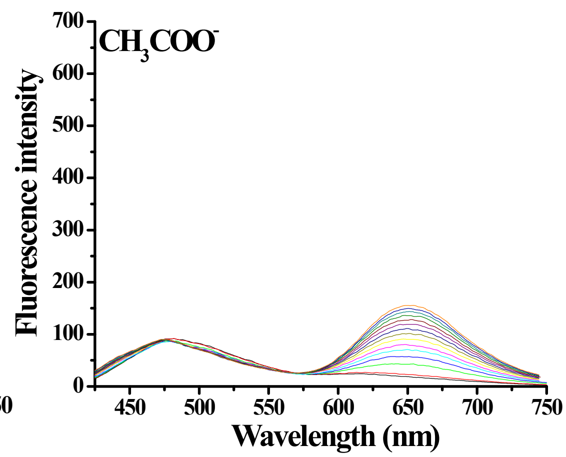
4. Fluorescence spectra of PDP on titration with different interfering metal ions:





5. Fluorescence as well as UV-vis spectra of PDP with interfering acetate ion.





6. **Synthesis of PDP:**

5-amino-1,10-phenanthroline (0.97 g, 5 mmol.) was added to a solution of the 2-hydroxy-5-methyl-benzene-1,3-dicarbaldehyde (0.41 g, 2.5 mmol) in ethanol (50 mL) at reflux, and heating was continued for 2 day. A yellow precipitate was filtered and washed with ethanol. The product was purified by recrystallization from ethanol Yield: 790 mg, 62%.

^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ = 13.56 (s, 1H), 9.26 (d, 2H, *J* = 5.92 Hz), 9.16 (d, 2H, J = 5.60 Hz), 9.15(s, 1H), 8.76 (d, 2H, *J* = 7.88 Hz), 8.28 (d, 2H, J = 9.44 Hz), 7.71-7.68 (m, 4H), 7.65-7.63 (m, 3H), 7.56 (s, 2H), 2.49 (s, 3H).

ESI (m/z, %): M+ Calculated for $C_{33}H_{22}N_6O$ is 518.19; Found: 519.27 $(M+H)^+$

Anal calcd for $C_{33}H_{22}N_6O$: 76.43% C, 4.28% H, 16.21% N, 3.09% O; found: 76.41% C, 4.31% H, 16.25% N, 3.16% O.

7. **a) ^{1}H NMR spectra of PDP in $CDCl_3$:**

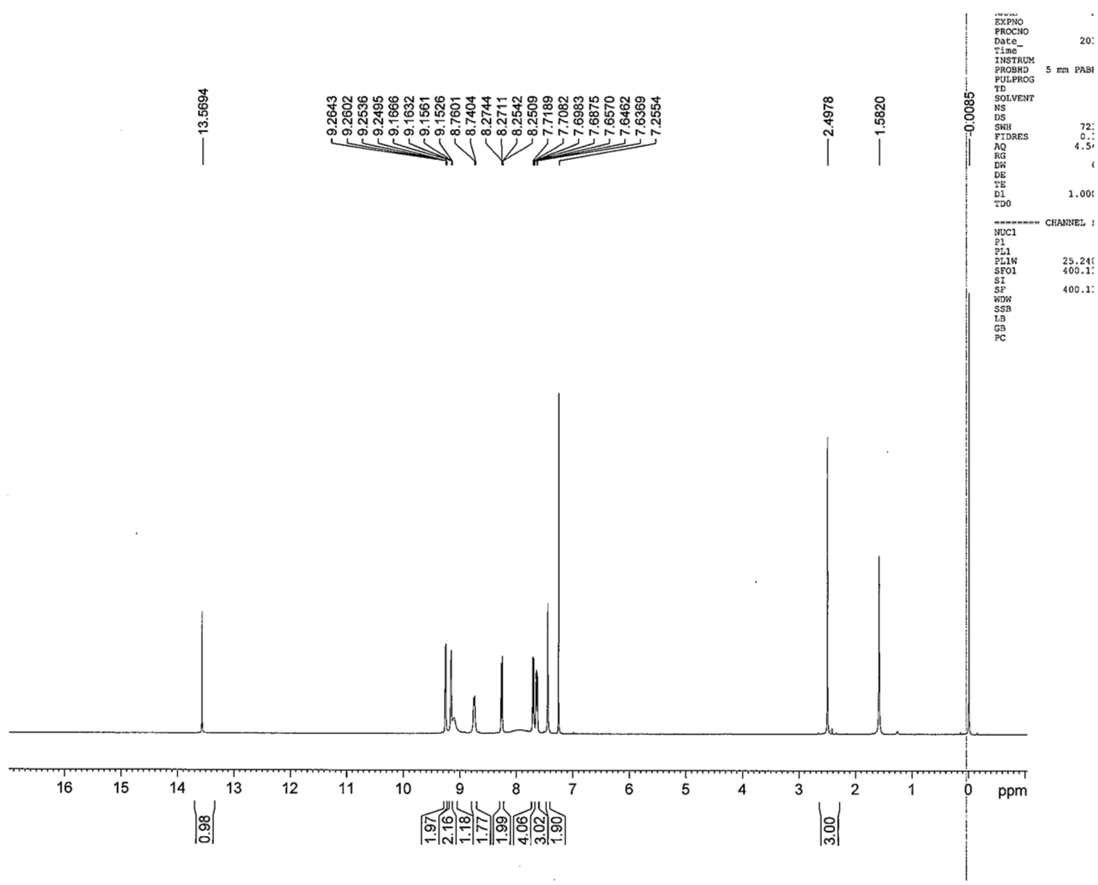


Figure: S_7

b) Mass (ESI) spectra of PDP

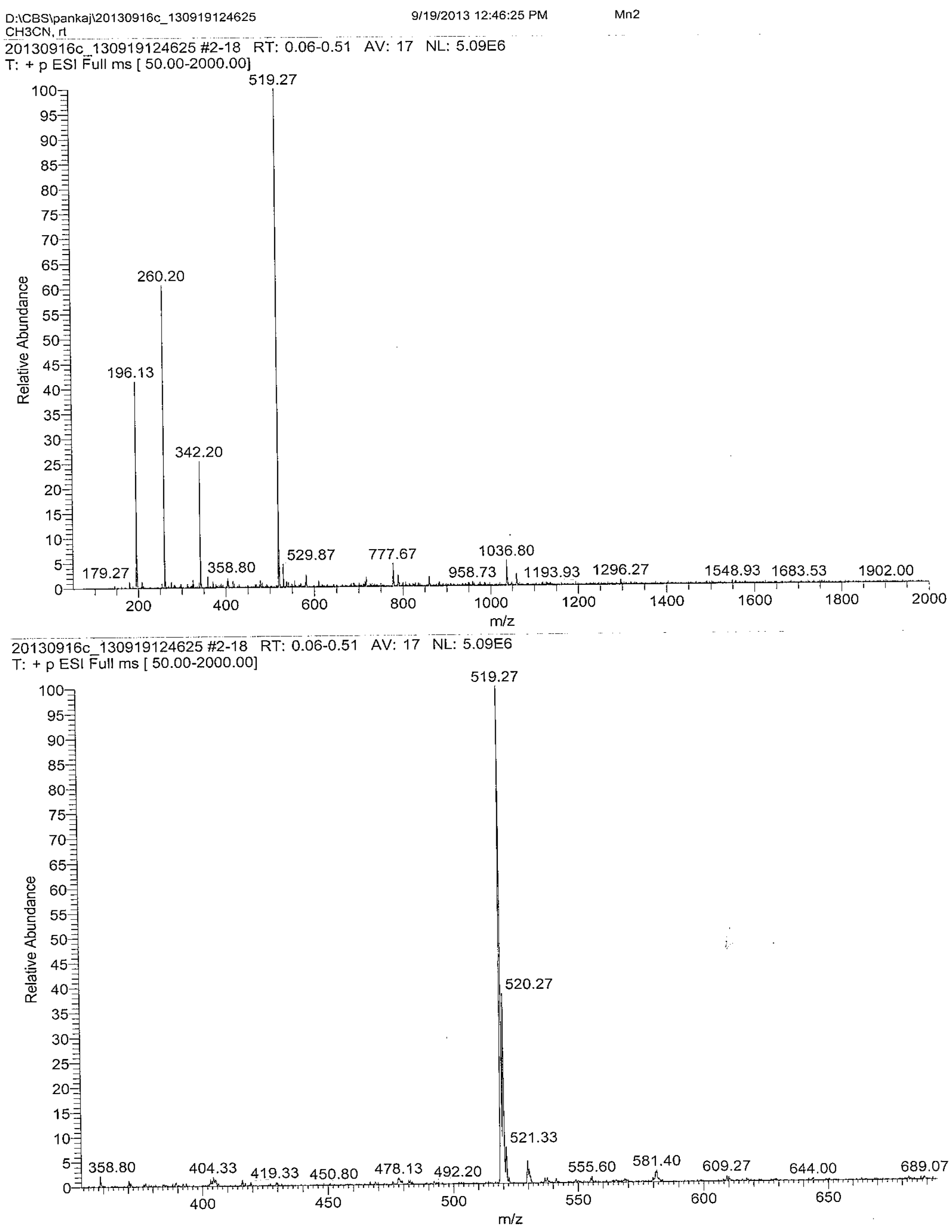


Figure: S_8

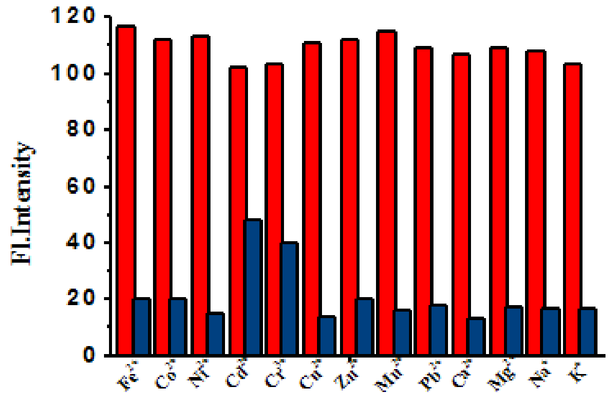


Figure S_9: (b) Mercury selectivity profile of the sensor PDP (20 μM): (blue bars) change of emission intensity of PDP + 3.0 equiv. of different metal ions; (red bars) change of emission intensity of PDP + 3.0 equiv. of different metal ions, followed by 3.0 equiv Hg^{2+} at 635 nm.

(a) **(b)** **(c)**

Figure S_{10}. NCI-H460 cells showed red fluorescence. (a) NCI-H460 cells without any treatment. (b) NCI-H460 cells in presence of $HgCl_2$ (1 x10^{-5} M) (c) NCI-H460 cells in presence of both PDP (2x10^{-5} M) and $HgCl_2$ (1 x10^{-5} M). The photographs were taken in DeWinter Victory-FL fluorescence inverted microscope using DeWinter Biowizard software v 4.4 using 40X objective.

The study of the fluorescence appearance with increasing concentrations of Hg to the probe incubated cell:

For this experiment this time we treated the cells with PDP (c=4x10^{-6} M). Then we cells with increasing concentrations of $HgCl_2$ (c=2x10^{-5} M, 4x10^{-5} M, 6x10^{-5} M, 8x10^{-5} M, and 1.2x10^{-4} M). The photographs were taken in DeWinter Victory-FL fluorescence inverted microscope using DeWinter Biowizard software v 4.4 using10X objective. In this connection, we like to emphasize that the cells which are showing the fluorescence are definitely not dead cells as they were still attached to the surface whereas the dead cells were floating.

In this new figure a) and b), cells were treated with 2x10^{-5} M $HgCl_2$ and 4x10^{-6} M PDP. Figure a) shows bright field fluorescence image of the cells whereas Figure b) shows the dark field fluorescence image of the cells. In figure c) and d), cells were treated with 4x10^{-5} M $HgCl_2$ keeping the PDP concentration fixed. Figure c) shows bright field fluorescence image of the cells whereas Figure d) shows the dark field fluorescence image of the cells. In figure e) and f), cells were treated with 6x10^{-5} M $HgCl_2$ keeping the PDP concentration fixed. Figure e) shows bright field fluorescence image of the cells whereas Figure f) shows the dark field fluorescence image of the cells.

The data shown above indicate that the fluorescence intensity of the cells increases with increasing concentrations of $HgCl_2$.

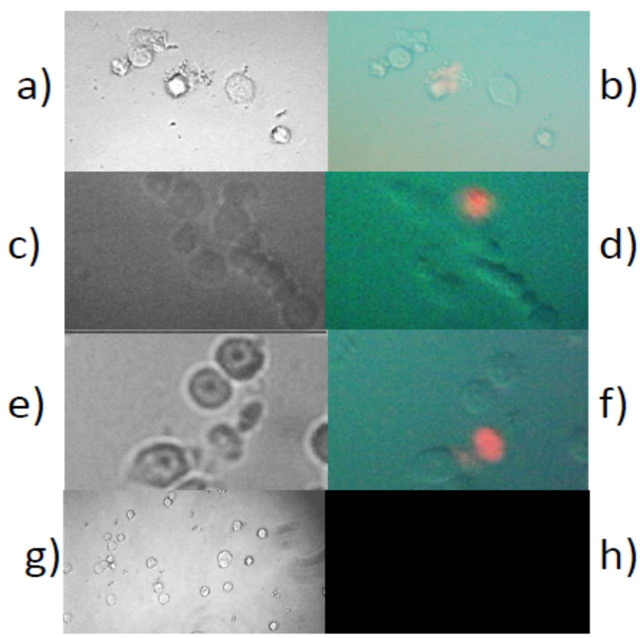
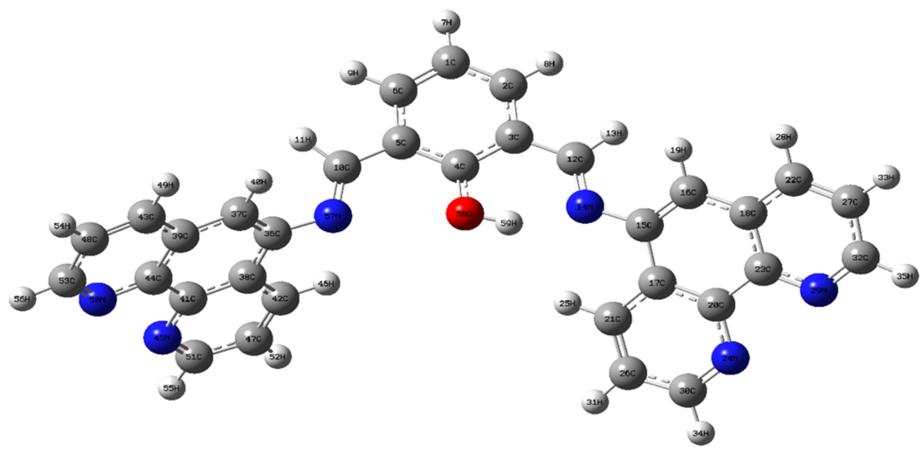


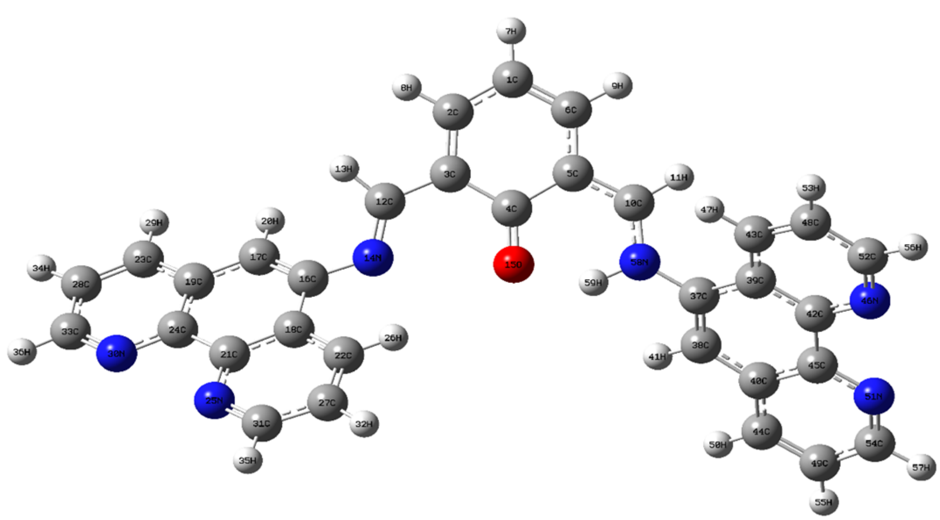
Figure S$_{11}$: NCI-H460 cells showed red fluorescence. Figure a) and b): cells were treated with 2x10^{-5} M $HgCl_2$ and 4x10^{-6} M PDP. Figure a) shows bright field fluorescence image of the cells whereas Figure b) shows the dark field fluorescence image of the cells. Figure c) and d): cells were treated with 4x10^{-5} M $HgCl_2$ keeping the PDP concentration fixed (4x10^{-6} M). Figure c) shows bright field fluorescence image of the cells whereas Figure d) shows the dark field fluorescence image of the cells. Figure e) and f), cells were treated with 6x10^{-5} M $HgCl_2$ keeping the PDP concentration fixed. Figure e) shows bright field fluorescence image of the cells whereas Figure f) shows the dark field fluorescence image of the cells. Figure g) and h): NCI-H460 cells were treated with PDP alone. g) shows the bright field fluorescence image of cells, (h) shows the dark field image of cells. The photographs were taken in DeWinter Victory-FL fluorescence inverted microscope using DeWinter Biowizard software v 4.4 using 10X objective.

Computational study:

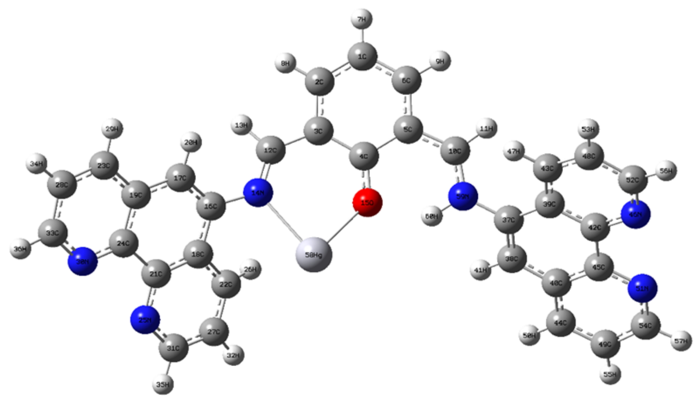
DFT calculations were performed using the Gaussian 03 (Revision B.04) package while Gauss View used for visualization of Molecular orbital. The Keto and enol form of PDP were optimized with B3LYP functional and 6-311+G (d, p) basis set with no symmetry constrain. For Hg^{2+} bound species LANL2DZ of Hg and for rest of the atoms 6-31g*+ were used for optimization as well as single point calculation gas phase. Molecular orbitals were analysed using the AOMix program



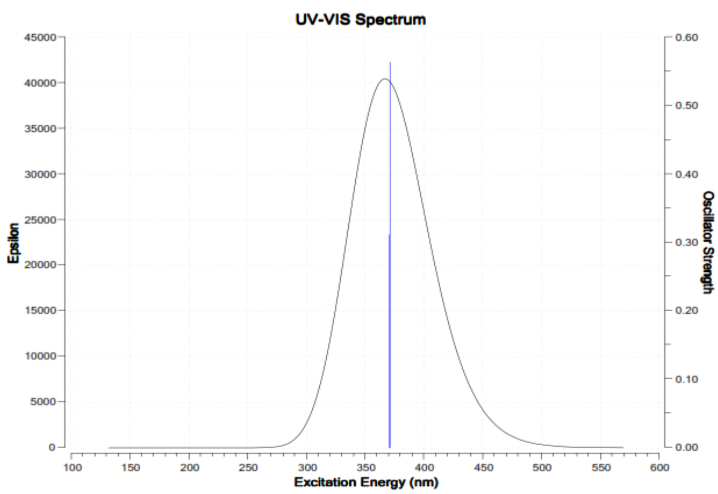
Optimized structure of Enol form of PDP



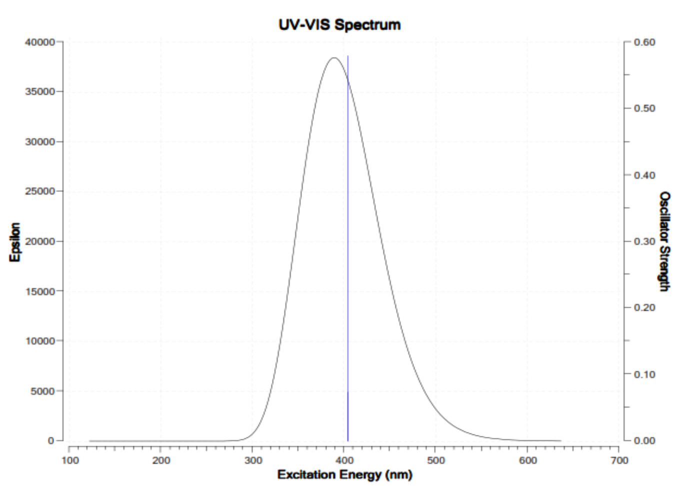
Optimized structure of keto form of PDP



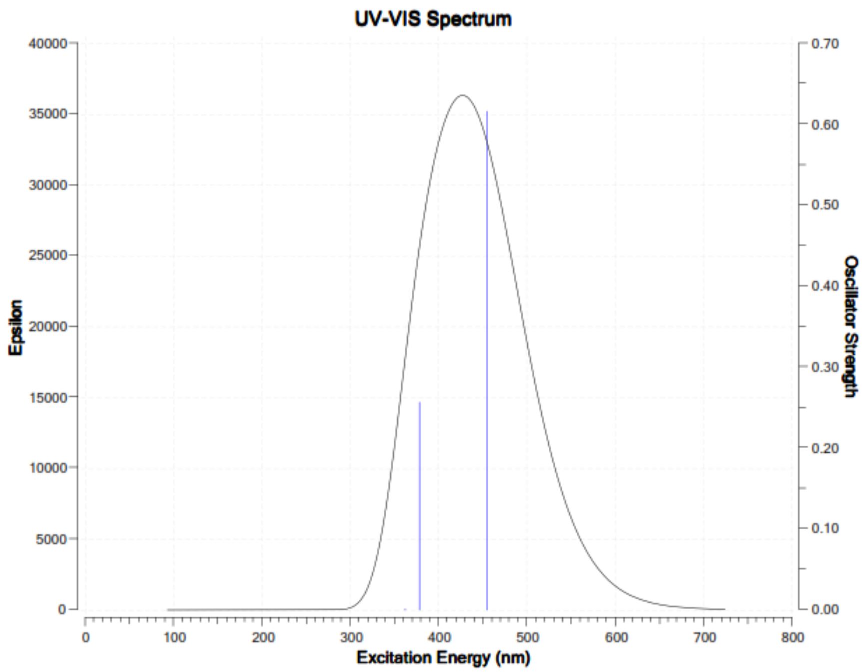
Optimized structure of Hg^{2+} bound Complex



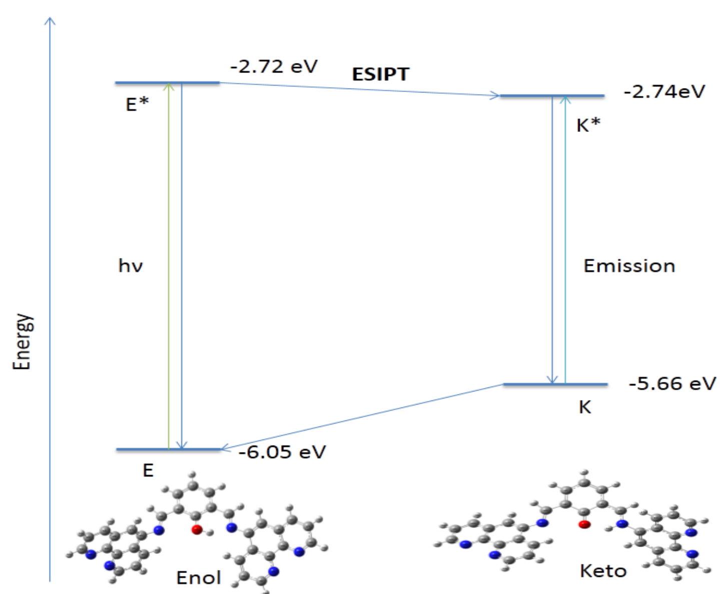
UV-vis spectrum of Enol form of PDP.



UV-vis spectrum of Keto form of PDP.



UV-vis spectrum of PDP-Hg^{2+} complex



Probable mode of intramolecular proton transfer.

Optimized parameter:

Enol(PDP) form:

C	-0.02052200	4.34726600	-1.89403100
C	1.21563900	3.74836500	-1.66682600
C	1.31221700	2.49139800	-1.05796900
C	0.12023200	1.80962700	-0.66044700
C	-1.14367400	2.42009700	-0.87701100
C	-1.17234000	3.68571900	-1.49834000
H	-0.08140800	5.32234800	-2.36033800
H	2.12815000	4.25690700	-1.95999800
H	-2.14063800	4.14639500	-1.66699300
C	-2.44452300	1.84887200	-0.51491500
H	-3.27870500	2.54873800	-0.66916800
C	2.62688100	1.92155100	-0.81677500
H	3.48355400	2.53019500	-1.12844100
N	2.80732600	0.75347400	-0.30348200
C	4.08517000	0.27058500	0.00411300
C	5.06742600	1.04480300	0.55786600
C	4.32794600	-1.14259000	-0.22006700
C	6.34505800	0.50405900	0.89719700
H	4.87131700	2.08440800	0.79872200
C	5.59280500	-1.71215300	0.09086200
C	3.34020300	-1.99449200	-0.75846400

C	7.35629600	1.29524600	1.48452100
C	6.63619000	-0.86812300	0.66195100
N	5.86548900	-3.01751900	-0.11756500
H	2.36726500	-1.59055100	-1.00372300
C	3.63256300	-3.32501200	-0.95652600
C	8.57013000	0.72334400	1.79026900
H	7.16622300	2.34468300	1.68635800
N	7.83191300	-1.41336900	0.96078900
C	4.91826500	-3.78566100	-0.62118000
H	2.89645200	-4.00979200	-1.36071900
C	8.75738700	-0.64174800	1.50086700
H	9.36901200	1.29994500	2.24133700
H	5.17868500	-4.82991300	-0.77602900
H	9.70863400	-1.11842800	1.72434500
C	-3.99553600	0.27849300	0.19548800
C	-4.70134600	0.77473800	1.25476000
C	-4.56798600	-0.76494600	-0.62029600
C	-5.99902700	0.27356800	1.58593400
H	-4.26311100	1.54994400	1.87478600
C	-5.87475000	-1.25963300	-0.34924100
C	-3.86646300	-1.31033600	-1.71441800
C	-6.72231800	0.75705500	2.69695700
C	-6.61050000	-0.73901300	0.79693000
N	-6.45387100	-2.19858400	-1.12515000
H	-2.85944400	-0.96873100	-1.92207600
C	-4.47034700	-2.27823600	-2.48728000

C	-7.97057900	0.24326600	2.96797900
H	-6.28486300	1.52569700	3.32591300
N	-7.83577600	-1.23161400	1.07067300
C	-5.77505500	-2.68020700	-2.15149900
H	-3.96115900	-2.72488000	-3.33327100
C	-8.48442300	-0.75405500	2.11715300
H	-8.55157800	0.58880300	3.81510600
H	-6.28440100	-3.43097800	-2.75122600
H	-9.46867700	-1.17571500	2.30621000
N	-2.67334000	0.67007100	-0.08426700
O	0.19482700	0.65317600	-0.01271300
H	1.14377400	0.39722700	0.02855400

Keto(PDP) form:

C	-0.04102300	4.72873100	-0.49467900
C	-1.22677100	3.96916000	-0.37170000
C	-1.24910700	2.59265400	-0.23780500
C	0.01661100	1.85690100	-0.18888100
C	1.23657000	2.67325500	-0.31797300
C	1.16630100	4.08392100	-0.47382300
H	-0.09704900	5.80392100	-0.60645200
H	-2.17844800	4.49348400	-0.39128000
H	2.09158100	4.64440300	-0.57282800

C	2.48775900	2.04728800	-0.31354400
H	3.38644300	2.64168000	-0.45366000
C	-2.55525300	1.94864700	-0.14522400
H	-3.39938400	2.65575800	-0.11196700
N	-2.76359500	0.68934100	-0.09679000
O	0.08848400	0.61664900	-0.04486500
C	-4.06592900	0.18934200	-0.08420500
C	-5.06789500	0.65255800	-0.89511900
C	-4.31969500	-0.94262600	0.78592500
C	-6.37257700	0.07011100	-0.87868500
H	-4.86765500	1.45565900	-1.59631400
C	-5.61127200	-1.52931200	0.85109800
C	-3.30125600	-1.49254900	1.59013900
C	-7.40908800	0.53688100	-1.71541900
C	-6.67404400	-1.00912700	-0.00181400
N	-5.88954300	-2.56613500	1.67028100
H	-2.30508300	-1.07251000	1.52654700
C	-3.59961400	-2.55453900	2.41439200
C	-8.65199300	-0.05019400	-1.64719400
H	-7.21329700	1.35547300	-2.40065600
N	-7.89669100	-1.57481900	0.05657100
C	-4.91627500	-3.04842100	2.42079900
H	-2.84374000	-3.00885000	3.04447700
C	-8.84297800	-1.10616300	-0.73678400
H	-9.47049200	0.28445500	-2.27384300
H	-5.18094400	-3.88181000	3.06808100

H	-9.81569000	-1.58626500	-0.65837900
C	3.85974200	0.01575400	-0.22442300
C	3.92426600	-1.07478900	-1.03939700
C	4.99375700	0.39583800	0.58829400
C	5.12358600	-1.83767200	-1.16735000
H	3.05688700	-1.35344000	-1.62808300
C	6.21979400	-0.31916800	0.45115700
C	4.94536100	1.41293400	1.56659500
C	5.20704200	-2.96177200	-2.01706200
C	6.29198500	-1.45854100	-0.45491000
N	7.32212000	0.00005000	1.15979500
H	4.02058700	1.94006200	1.76181300
C	6.07460400	1.71072700	2.29459800
C	6.40128200	-3.63519800	-2.12370000
H	4.33172100	-3.27834500	-2.57425600
N	7.45885000	-2.12420700	-0.57255600
C	7.24995500	0.98455300	2.03419100
H	6.06333600	2.47970300	3.05754800
C	7.50186800	-3.16919300	-1.37800300
H	6.50556200	-4.50287200	-2.76397200
H	8.16379400	1.21221700	2.57739800
H	8.45911100	-3.67978400	-1.44857400
N	2.63991800	0.73689300	-0.16525000
H	1.71319100	0.25605700	-0.09798400

PDP-Hg^{2+} bound complex:

C	0.35563900	4.63426200	-1.71814500
C	-0.83168100	3.89769200	-1.65581100
C	-0.87626000	2.53496100	-1.23606800
C	0.37217400	1.86590900	-0.92285100
C	1.59518400	2.64991100	-0.96910800
C	1.55875200	4.01188800	-1.36199500
H	0.34353100	5.67067400	-2.03598200
H	-1.76503900	4.38771600	-1.92564700
H	2.48845700	4.57538400	-1.40656100
C	2.88840500	2.08577000	-0.68461600
H	3.74975100	2.74698200	-0.71319800
C	-2.21282000	1.97992900	-1.15087700
H	-2.98983600	2.63272700	-1.56232600
N	-2.60243100	0.82224700	-0.65086600
O	0.47835200	0.55877400	-0.65707200
C	-3.94606900	0.45360500	-0.41974500
C	-4.90885000	1.35206000	0.04994600
C	-4.24704400	-0.95684600	-0.48111800
C	-6.19927500	0.91712500	0.46236700
H	-4.66435200	2.40830200	0.14763100
C	-5.48814300	-1.44750300	0.05117800
C	-3.34061800	-1.91956900	-1.04127000

C	-7.20373300	1.83589700	0.90255300
C	-6.50795500	-0.49175500	0.50263800
N	-5.76186500	-2.77807300	0.12651300
H	-2.56155500	-1.59579000	-1.73221700
C	-3.64902600	-3.29243200	-0.95186100
C	-8.42711700	1.34114900	1.33825900
H	-7.00647600	2.90459900	0.89070900
N	-7.70519800	-0.96060500	0.93968700
C	-4.86196200	-3.67340900	-0.33401400
H	-3.00510600	-4.04490900	-1.39592600
C	-8.63518300	-0.06895500	1.33798300
H	-9.21929800	2.00088400	1.67524900
H	-5.13685000	-4.71961000	-0.23918300
H	-9.58212700	-0.48614400	1.66933000
C	4.38052600	0.17970800	-0.15534500
C	4.60493300	-1.04723900	-0.75185600
C	5.37329800	0.80021400	0.70535000
C	5.85776500	-1.72086200	-0.61487300
H	3.83665100	-1.49676000	-1.37971000
C	6.65127100	0.15272900	0.83743700
C	5.15454400	1.97346800	1.48754900
C	6.11752500	-2.97999600	-1.23527400
C	6.90659400	-1.11620500	0.15671600
N	7.64928400	0.67313200	1.61258200
H	4.18801500	2.46773900	1.50109900
C	6.17714900	2.47472200	2.28733000

C	7.36515400	-3.57055400	-1.07408900
H	5.34658600	-3.46187800	-1.83134900
N	8.12942900	-1.70579400	0.29451400
C	7.42747900	1.79767700	2.30334000
H	6.03293200	3.35882800	2.89957600
C	8.35155100	-2.88468800	-0.30555000
H	7.60711300	-4.52478600	-1.52983100
H	8.25468500	2.17009800	2.90124800
H	9.34698300	-3.30427100	-0.18618900
Hg	-1.12442100	-0.81219300	0.30776000
N	3.11618000	0.80487100	-0.43126200
H	2.27174300	0.21309500	-0.49818000