Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

A new turn on Pd^{2+}-specific fluorescence probe and its use as an Imaging reagent for cellular uptake in Hct116 cells

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Materials.

Rhodamine B, Ethylenediamine, 4-Chloro-3-nitrocoumarin and Fe powder all metal perchlorate salts such as $NaClO_4$, $KClO_4$, $Mg(ClO_4)_2$, $Ca(ClO_4)_2$, $Cu(ClO_4)_2$, $Zn(ClO_4)_2$, $Co(ClO_4)_2$, $Ni(ClO_4)_2$, $Cr(ClO_4)_3$, $Fe(ClO_4)_2$, $Cd(ClO_4)_2$, $Hg(ClO4)_2$, $Pb(ClO_4)_2$, $Pd(PPh_3)_4$, K_2PtCl_4, K_2PdCl_6 and $PdCl_2$ were obtained from Sigma-Aldrich and were used as received. Solvents used for synthesis of intermediates and final compounds were of AR grade and HPLC grade solvents for spectroscopic studies from S.D. Fine Chemicals in India.

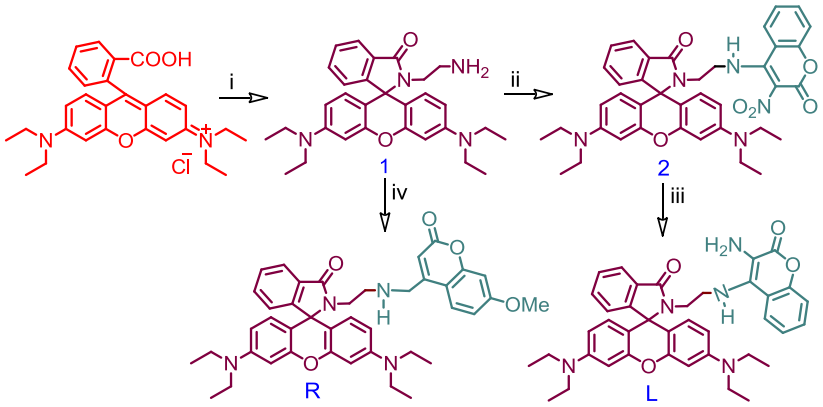
Analytical Methods:

^1H NMR spectra were recorded AV 400 MHz or AV-500 MHz Bruker NMR spectrometers using $CDCl_3$-d_3 and CD_3CN-d_3 as the solvent and tetra methyl silane (TMS) as an internal standard. ESI-Ms measurements were carried out on a Waters QTof-Micro instrument. Electronic spectra were recorded with a Shimadzu UV-3101 PC spectrophotometer; while fluorescence spectra were recorded using Quanta Master 400, PTI spectrofluorometer.

General experimental procedure for UV-Vis and Fluorescence studies:

$5x10^{-2}$ M solution of the perchlorate salts of the respective ion (Na^+, K^+, Fe^{3+}, Na^+, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{3+}, Pd^0, Pd^{4+}, $Pt^{2+}$and Hg^{2+}) were prepared in pure aqueous medium, while $PdCl_2$ in brine solution for all studies. A stock solution of the receptor **L** (1 x 10^{-4} M) was prepared in 10 mM HEPES buffer (pH 7. 2): acetonitrile (1: 1, v/v). Solution of the compound **L** was further diluted for spectroscopic titrations, and the effective final concentration of the solution of compound **L** used for the fluorescence study was 10 μM, while the final analyte concentration during emission spectral scanning was 1 x 10^{-4} M. For all luminescence measurements, λ_{Ext} = 530 nm with an emission slit width of 2/2 nm. The relative fluorescence quantum yields (ϕ_f) were estimated using Rhodamine B (ϕ_f = 0.3 in aqueous medium at RT) as a reference.

Synthesis:



i. Ethylene diamine, EtOH, D 24 hr; **ii.** 4-Chloro-3-nitrocoumarin, CH_2Cl_2, K_2CO_3, 2hr; **iii.** Fe-HCl, MeOH-H_2O, D 1hr; **iv.** 4-Bromomethyl-7-methoxy coumarin Et_3N, Dry THF/N_2, D 10 hr.

Scheme 1: Methodologies that were adopted for synthesis of **1, 2, L and R.**

Synthesis of 2: A mixture of **1** (300 mg, 0.62 mmol), 4-Chloro-3-nitrocoumarin (140 mg, 0.62 mmol) were dissolved in dichloromethane (10 mL) stirred it room temperature for 2 hr under inert atmosphere. By monitoring TLC reaction was stopped. The crude product was subjected to silica gel chromatography using DCM: Methanol (99: 1, v/v) as eluent. Major fraction was collected and dried under vacuum, which afforded a red solid 400 mg, 95.92%. ESI- Ms (m/z) calculated for $C_{39}H_{39}N_5O_6$: 673, observed: 696 [M +Na]. ^{1}H NMR [500 MHz, $CDCl_3$-d_3: δ (ppm)]: 8.49 (1H, s, -NH); 8.22 (1H, d, 8Hz, ArH); 7.96 (1H, d, *J* = 6.5 Hz, ArH); 7.64 (1H, t, *J* = 8 Hz, ArH); 7.53 - 7.49 (3H, m, ArH); 7.33 (1H, d, *J* = 8 Hz, ArH); 7.15 (1H, d, *J* = 6.5Hz, ArH); 6.42 (2H, s, ArH); 6.37 (2H, d, *J* = 9Hz, ArH); 6.28 (2H, d, *J* = 8.5 Hz, ArH); 3.55 (2H, t, *J* = 4 Hz, CH_2); 3.38 (8H, t, *J* = 7 Hz, CH_2); 2.90 (2H, t, *J* = 4 Hz, CH_2); 1.21 (12H, t, *J* = 7 Hz, CH_3). ^{13}C NMR (500 MHz, $CDCl_3$-d_3, δ (ppm)): 133.34, 129.92, 12844, 127.92, 124.94, 124.11, 123.47, 122.95, 117.79, 114.29, 108.39, 103.57, 97.82, 66.12, 45.99, 44.44, 39.83 and 12.57.

Synthesis of L:[1] Mixture of **2** (220 mg, 0.326 mmol) and Fe powder (116 mg, 2.071 mmol) were dissolved in methanol (15 mL) to this water 6 mL was added. Start the reflux by the time 6M HCl (4mL) was added. By monitoring TLC, after 1.5 hr reaction was stopped. The crude product was subjected to silica gel chromatography using DCM: Methanol (99: 3, v/v) as eluent. Major fraction was collected and dried under vacuum, which afforded a sticky red solid. Yield: 190 mg, 90.47 %. ESI- Ms (m/z) calculated for $C_{39}H_{41}N_5O_4$: 643, observed: 666.37 [L+ Na]. ^{1}H NMR [400 MHz, $CDCl_3$-d_3: δ (ppm)]: 7.98 (1H, s, -ArH); 7.62 (1H, d, *J* = 6.4 Hz, ArH); 7.50 (1H, d, *J* = 6.8 Hz, ArH); 7.33 (1H, t, *J* = 6 Hz, ArH); 7.28-7.22(3H, m, ArH); 7.13(1H, t, *J* = 6.4 Hz, ArH); 6.42 (1H, s, ArH); 6.39 (3H, t, *J* = 5.2 Hz, ArH); 6.18 (2H, d, *J* = 5.6 Hz, ArH); 5.27 (1H, s, -NH); 3.55 (2H, t, *J* = 4.4 Hz, CH_2); 3.32 (8H, q, *J* = 5.6 Hz, CH_2); 3.23 (2H, t, *J* = 4.4 Hz, CH_2); 1.16 (12H, t, *J* = 5.6 Hz, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$-d_3, δ (ppm)): 170.06, 161.23, 153.33, 148.92, 136.75, 132.92, 130.61, 128.61, 128.35, 128.00, 123.93, 128.83, 122.90, 121.61, 117.48, 116.71, 111.96, 108.04, 104.81, 97.65, 65.43, 60.41 46.59, 44.34, 40.32 and 12.55.

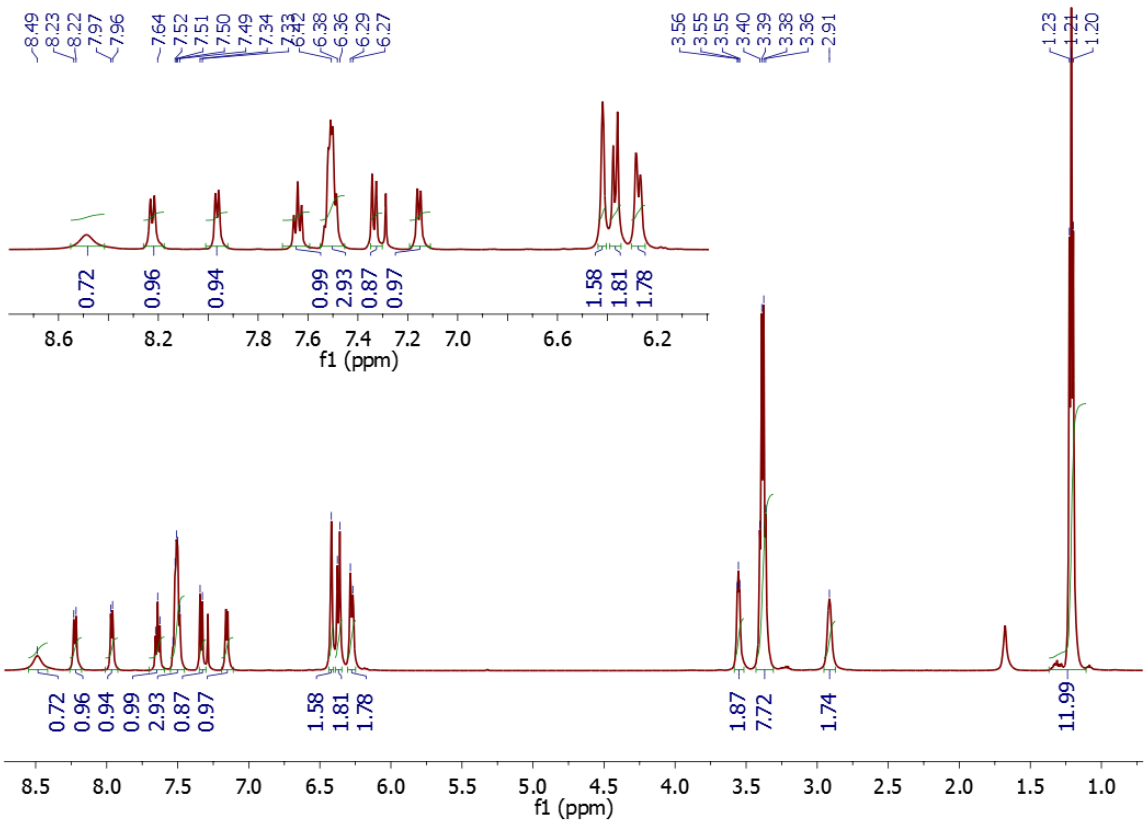
Synthesis of R: Synthetic procedure that was adopted for synthesis **R** from our previous literature.[2]

References

(1) H. Lu, L. Xiong, H. Liu, M. Yu, Z. Shen, F. Li and X. You, *Org. Biomol. Chem*., 2009, **7**, 2554.

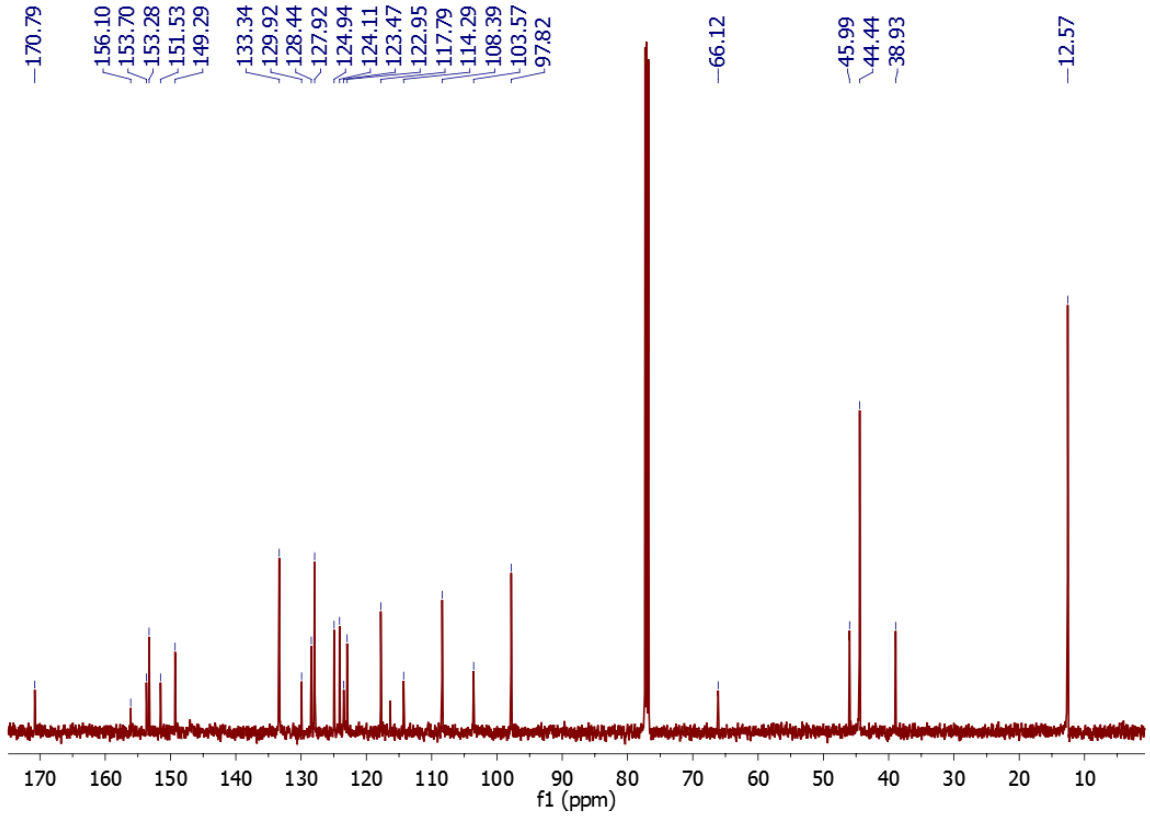
(2) S. Saha, P. Mahato, M. Baidya, S. K Ghosh and A. Das, *Chem. Commun*., 2012, **48**, 9293.

[1]HNMR spectra of 2



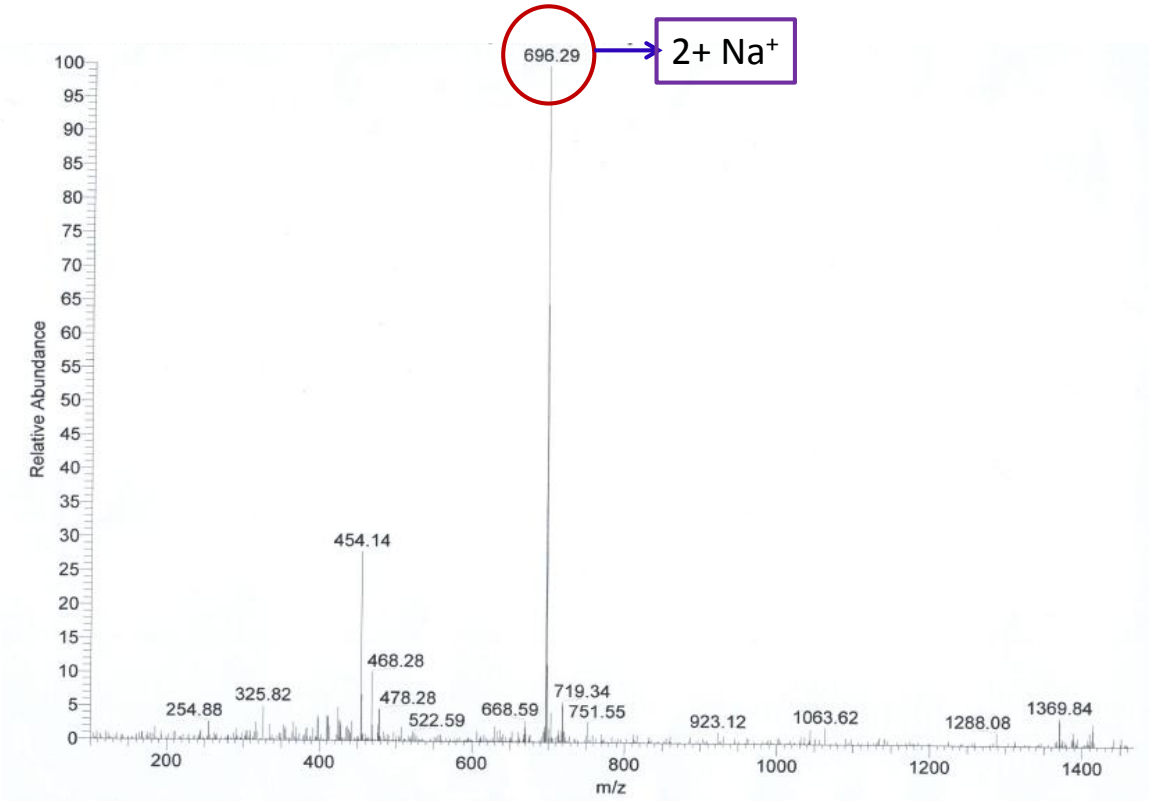
SI Figure 1: ^{1}H NMR spectra of **2** in $CDCl_3$-d_3 medium.

^{13}C NMR spectra of 2



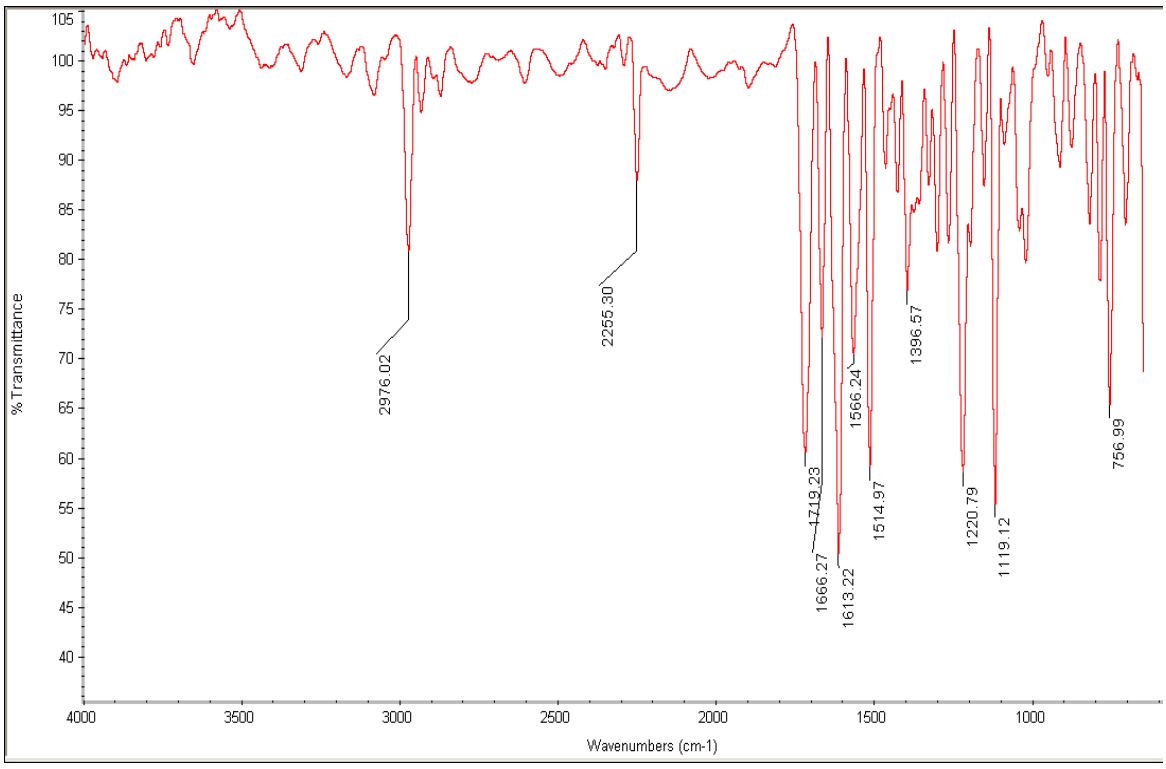
SI Figure 2: ^{13}C NMR spectra of 2 in $CDCl_3$-d_3 medium.

Mass spectra of 2



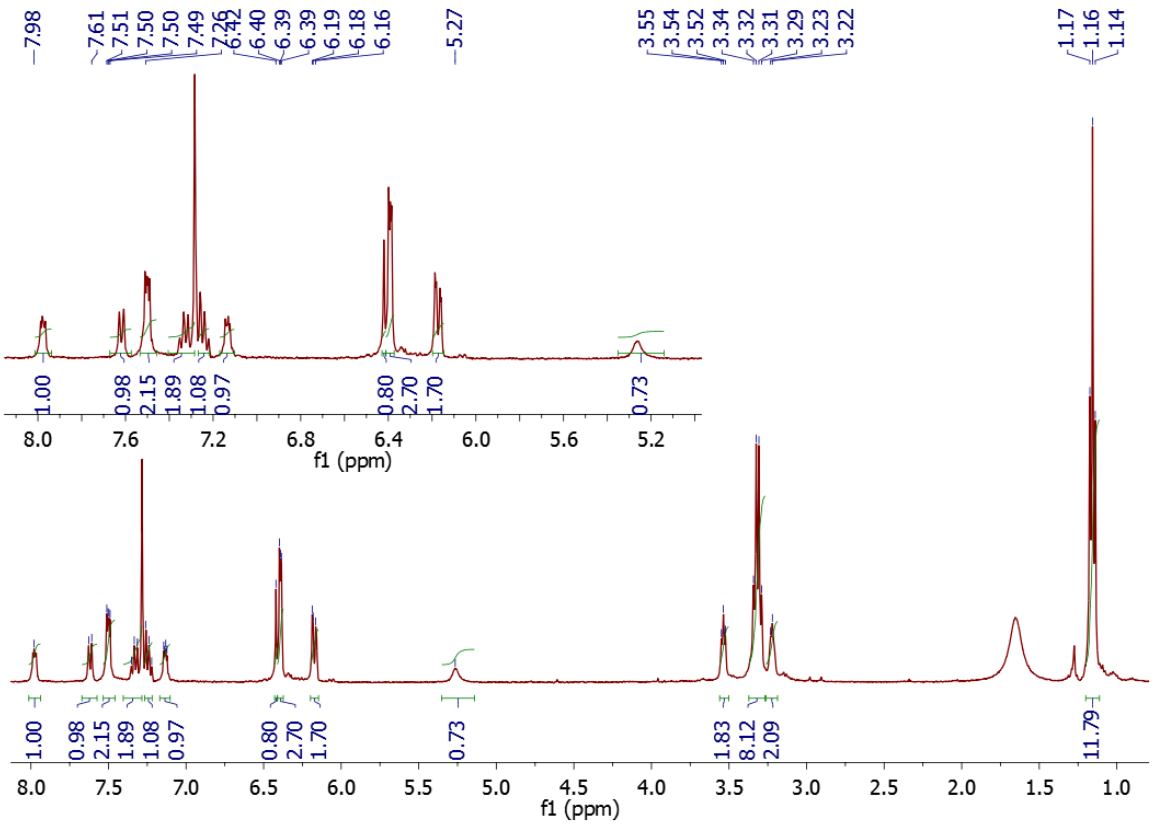
SI Figure 3: ESI- Ms Spectrum of **2** in CH_3OH.

IR spectra of 2 in Acetonitrile



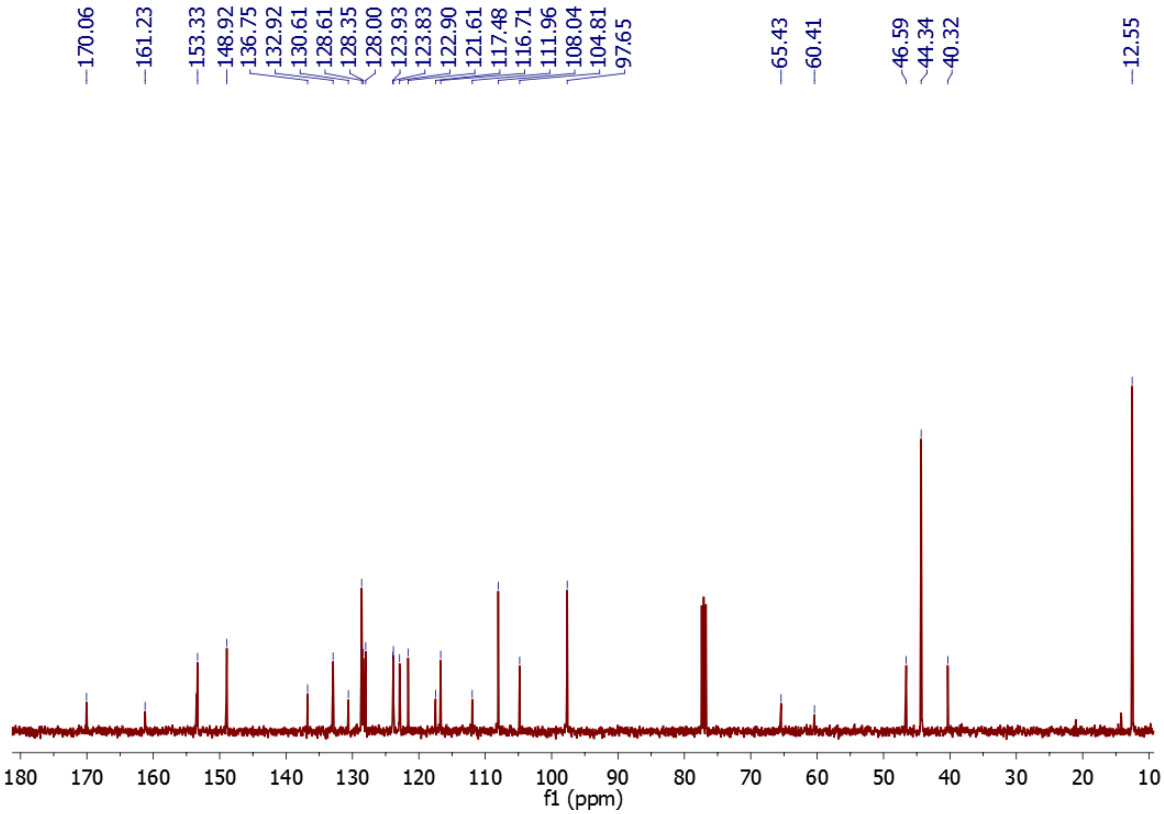
SI Figure 4: IR Spectra of 2 in Acetonitrile.

^{1}H NMR spectra of L



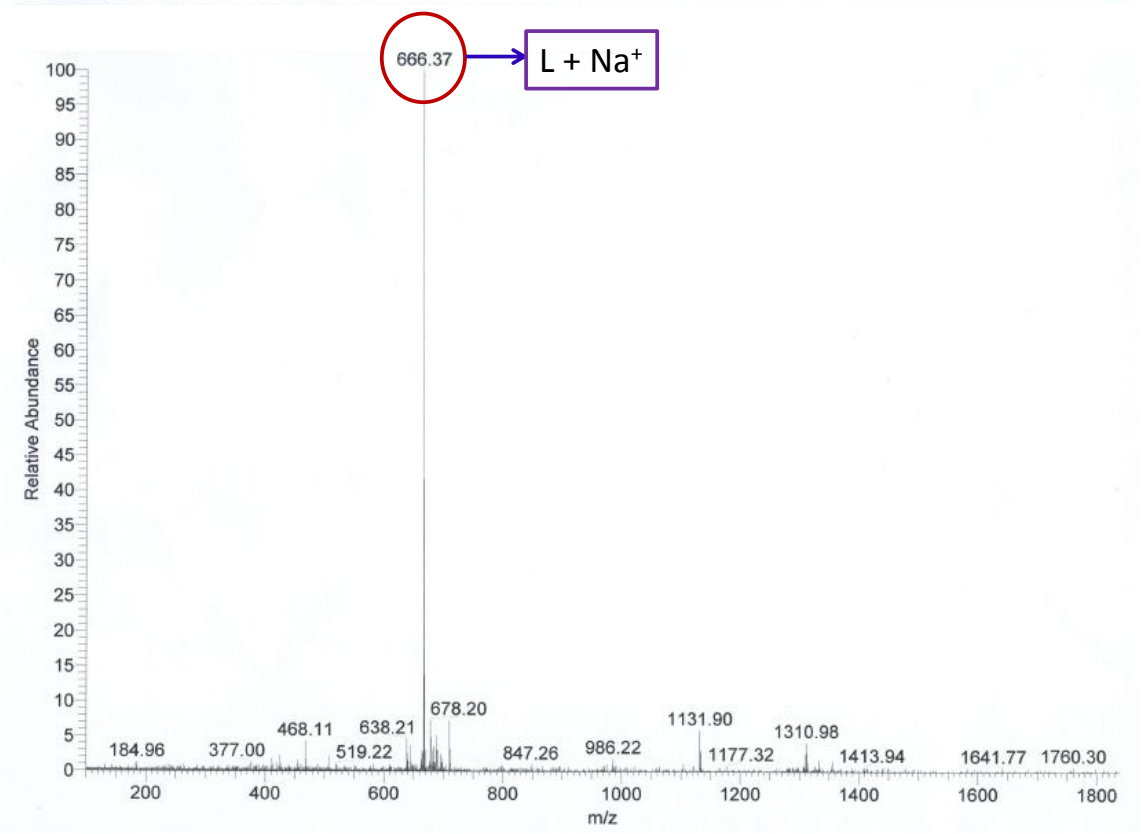
SI Figure 5: ^{1}H NMR spectra of **L** in $CDCl_3$-d_3 medium.

^{13}C NMR spectra of L



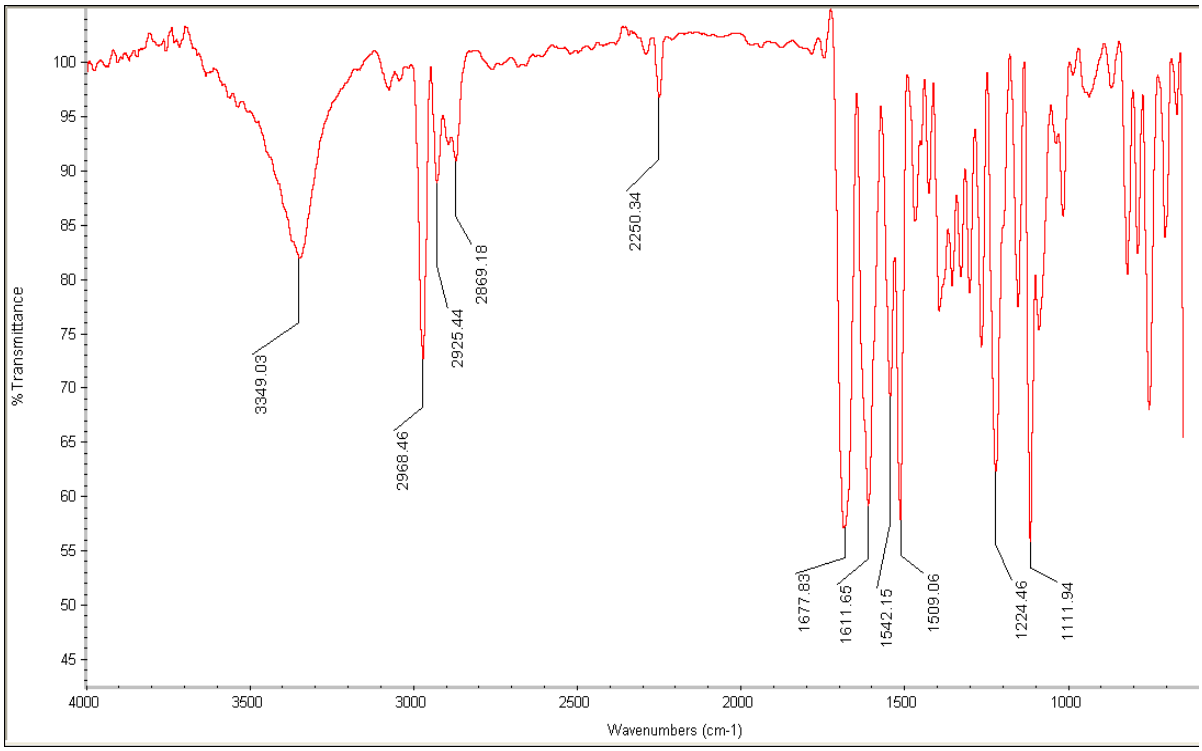
SI Figure 6: ^{13}C NMR spectra of 2 in $CDCl_3$-d_3 medium.

Mass spectra of L



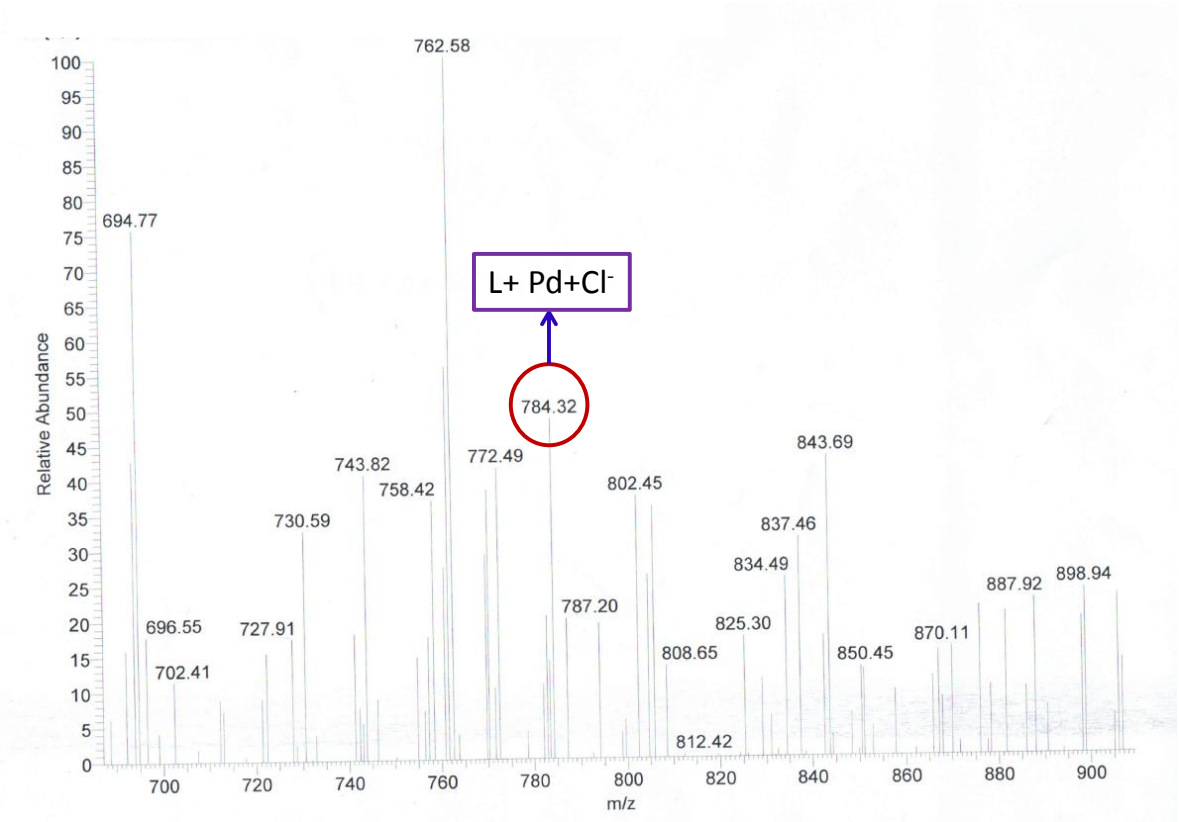
SI Figure 7: ESI- Ms Spectrum of **L** in Methanol.

IR spectra of L in Acetonitrile



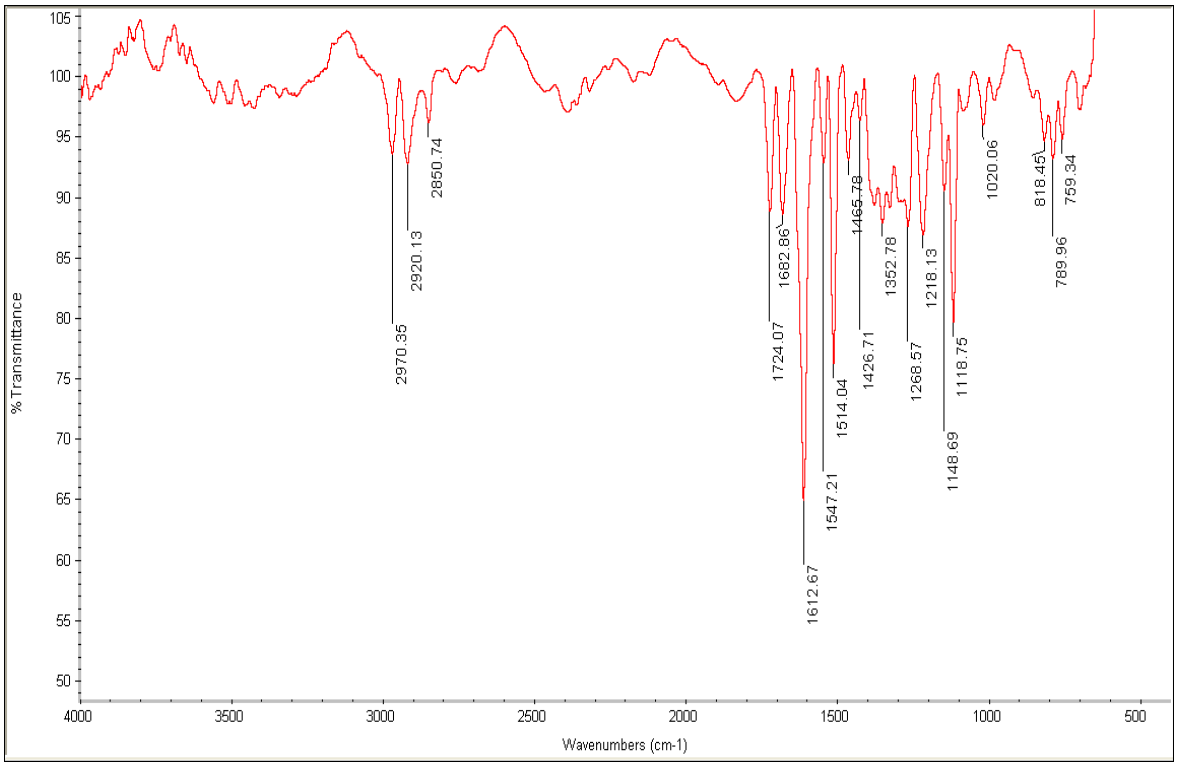
SI Figure 8: IR Spectra of L in Acetonitrile.

Mass spectra of L +Pd^{2+} in Acetonitrile



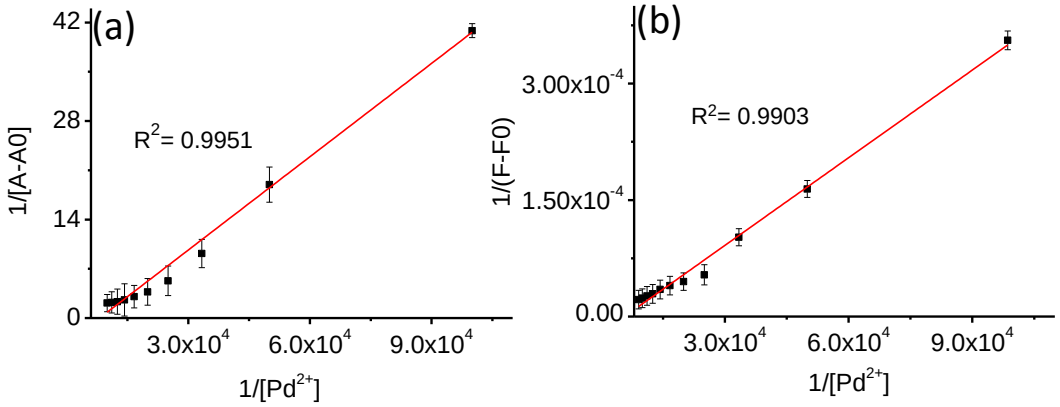
SI Figure 9: Mass Spectra of L+Pd^{2+} in Methanol.

IR spectra of R in Acetonitrile



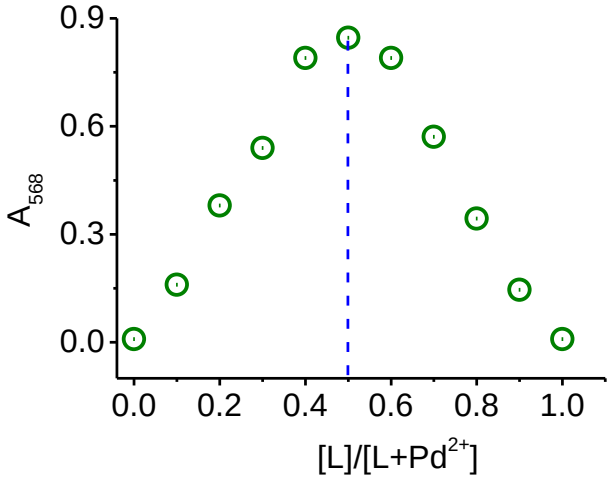
SI Figure 10: IR Spectra of **R** in Acetonitrile.

Benesi-Hildebrand plot for binding studies of [Pd^{2+}] towards L



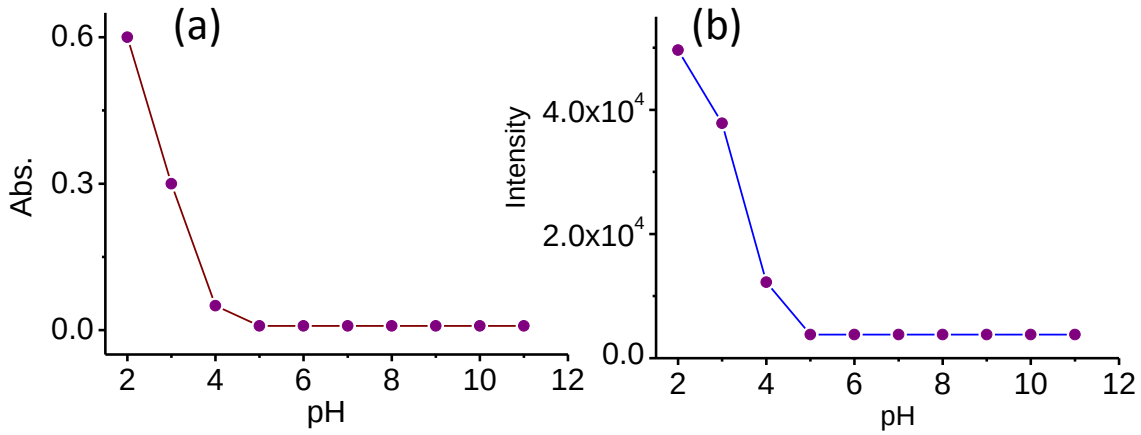
SI Figure 11: Benesi-Hildebrand plot of **L** (10 μM) for varying [Pd^{2+}] (0 to 20 μM) (a) from UV-Visible titration; (b) Fluorescence titration by using λ_{Ext} = 530 and λ_{Mon} = 594 nm. Good linear fit confirms the 1: 1 binding stoichiometry in in aq. HEPES buffer-acetonitrile (1: 1, v/v; pH 7.2) medium.

Job's plot for L with Pd^{2+} showing 1:1 stoichiometry:



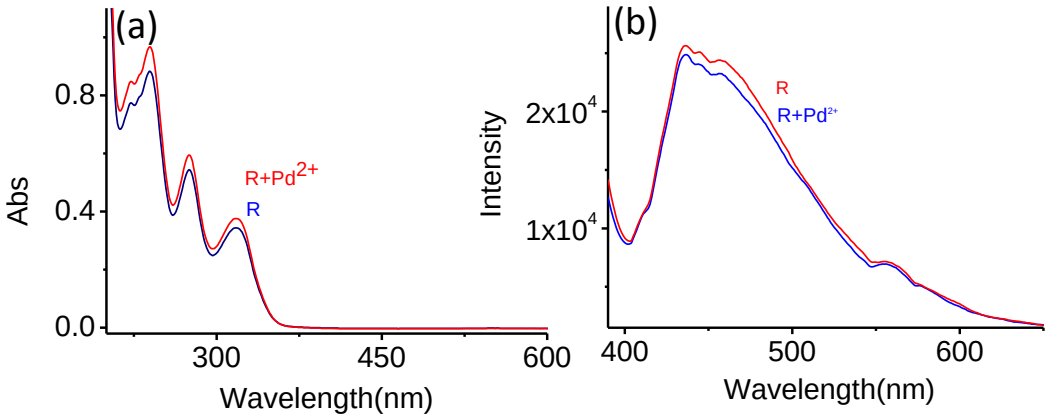
SI Figure 12: Job's plot between **L** and Pd^{2+} confirmed 1:1 adducts.

Change in Uv and Fluorescence of L as a function of the solution pH:



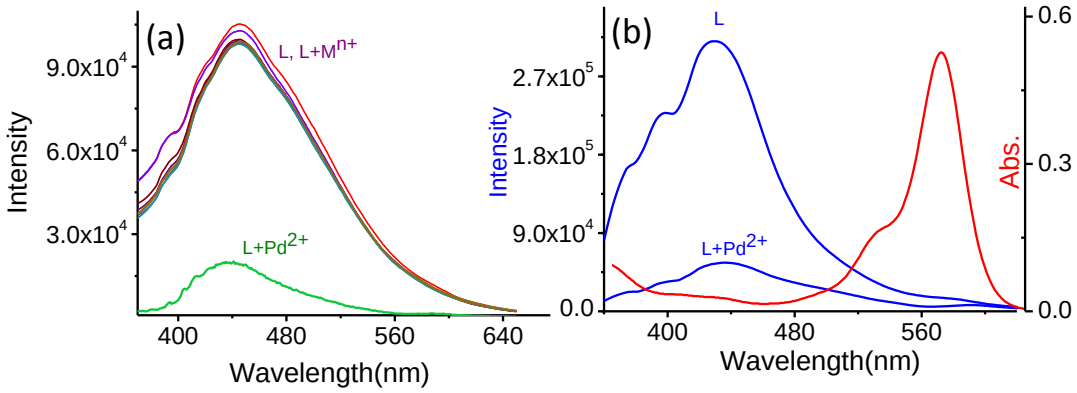
SI Figure 13: (a) UV; (b) Fluorescence response of **L** (10 μM) as a function of pH in Aacetonitrile-Universal buffer (1: 1, v/v), pH is adjusted by using aqueous solutions of 1 M HCl or 1 M NaOH.

Uv-Vis and Fluorescence response of R towards Pd^{2+}



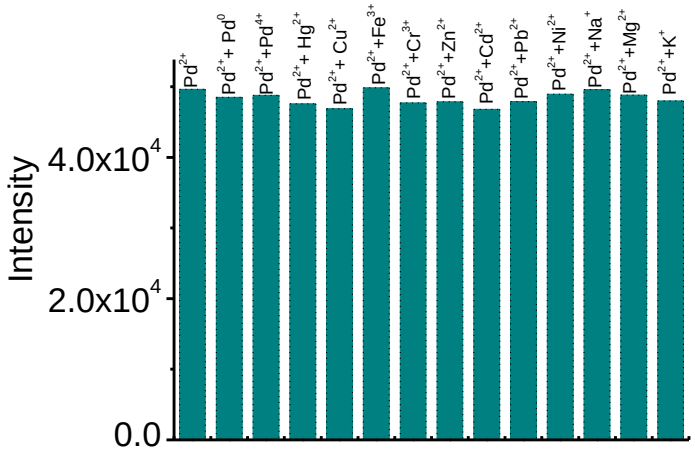
SI Figure 14: Changes in (a) Absorption and (b) Emission spectra (λ_{Ext} of 530 nm; slit = 2/2 nm) of the receptor **R** (10 μM) in absence and presence of Pd^{2+}; Studies were performed in aq. HEPES buffer-acetonitrile (1: 1, v/v; pH 7.2) medium.

Fluorescence response of L at λ_{Ext} = 360 nm



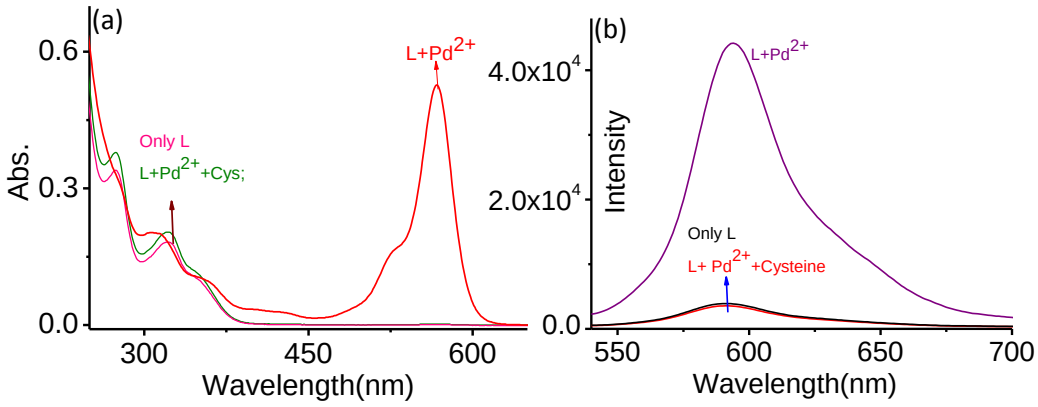
SI Figure 15: (a) Changes in Emission spectra (λ_{Ext} of 360 nm; slit = 2/2 nm) of the receptor **L** (10 μM) in absence and presence of different metal ions (M^{n+} = Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Al^{3+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Pb^{2+}, Pd^{2+} ,Pt^{2+}, Pd^{0}, Pd^{4+}); All studies were performed in aq. solution of Acetonitrile: HEPES buffer (1:1(v/v); 10 mM; pH 7.2).(b) Overlap spectra of L at λ_{Ext} = 360 nm.

Spectrophotometric interference study of L with Pd^{2+} in presence of various metal ions



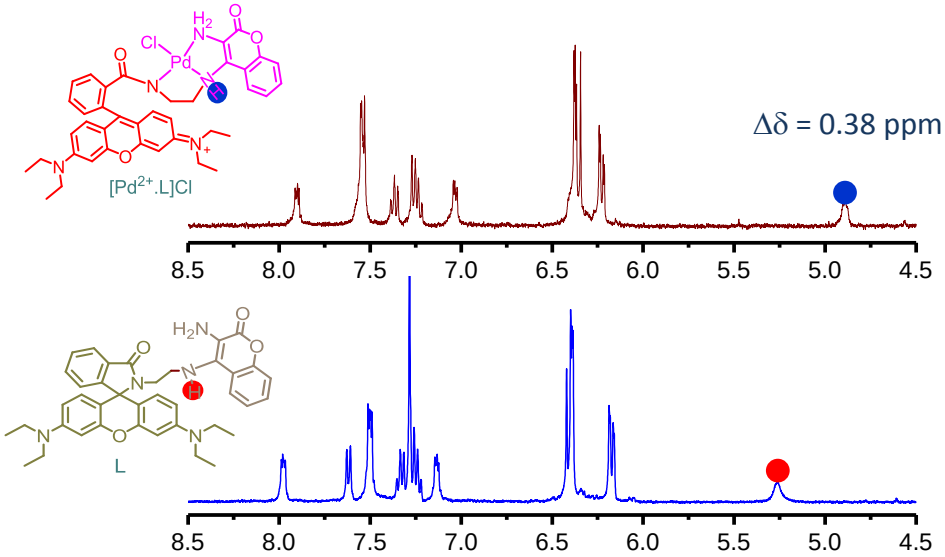
SI Figure 16: Spectrophotometric interference study of **L** (10 μM) with Pd^{2+}(10 μM) in presence of various metal ions (20 μM) in HEPES buffer by using λ_{Ext} = 530 and λ_{Mon} = 594nm.

Uv-Vis and Fluorescence spectral studies for establishing the reversible binding of Pd^{2+} to the L:



SI Figure 17: (a) UV and (b) Fluorescence studies for establishing the reversible binding of Pd^{2+}(2eq) to **L** (10 μM) in presence of Cysteine (4 eq) and using λ_{Ext} = 530 nm; and slit width 2/2 nm, in aq. solution of Acetonitrile and HEPES buffer (1:1; 10 mM; pH 7.2).

^{1}H NMR of L in absence and in presence of Pd^{2+} in CD_3CN-d_3

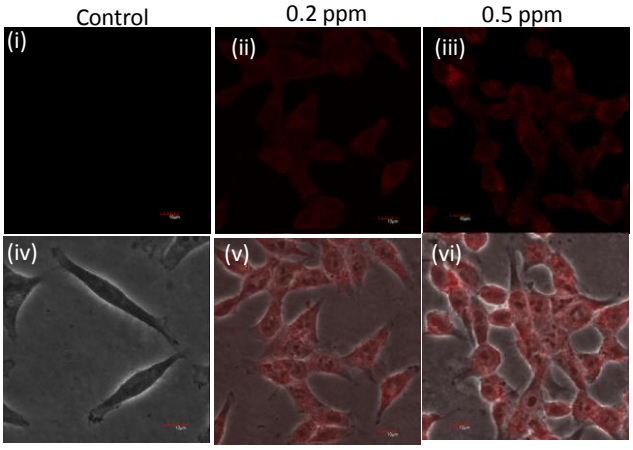


SI Figure 18: Partial ^{1}H NMR spectra of **L** (3 mM) in absence and in presence of Pd^{2+} were recorded in CD_3CN-d_3.

Cell culture and fluorescence imaging[1]

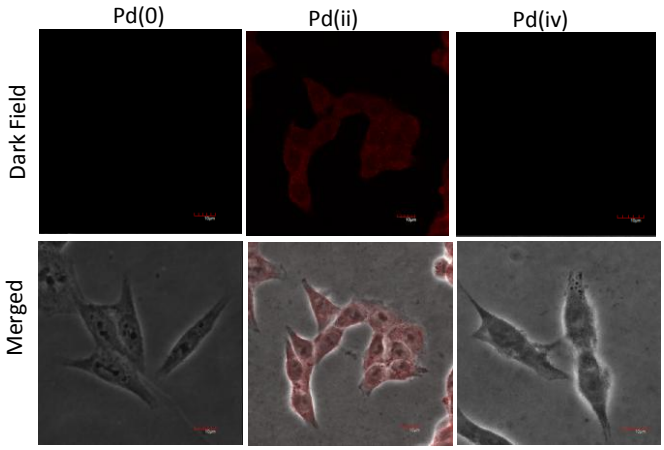
Hct116 cells were seeded on coverslips placed in 6 well plates. After 24 hours cells were treated with **L** (10μM) for 20 minutes. Cells were then washed thrice with Phosphate Buffer Saline (1X PBS) and fixed with 4% PFA for10 minutes and washed again with Phosphate Buffer Saline (1X PBS). Permeabilization of the cells was done using 0.2% Triton X 100 for 5 minutes. The **L**-stained colon cancer cells Hct116 incubated with Pd^{2+} (0.1 ppm) for 30 min. Again three washes were given and then cover slips mounted using mounting medium. Nail paints was used to seal the coverslips mounted on the glass slides. Images were acquired in Olympus Fluoview Microscope.

Confocal microscopic images of L at different [Pd^{2+}] in Hct116 cells



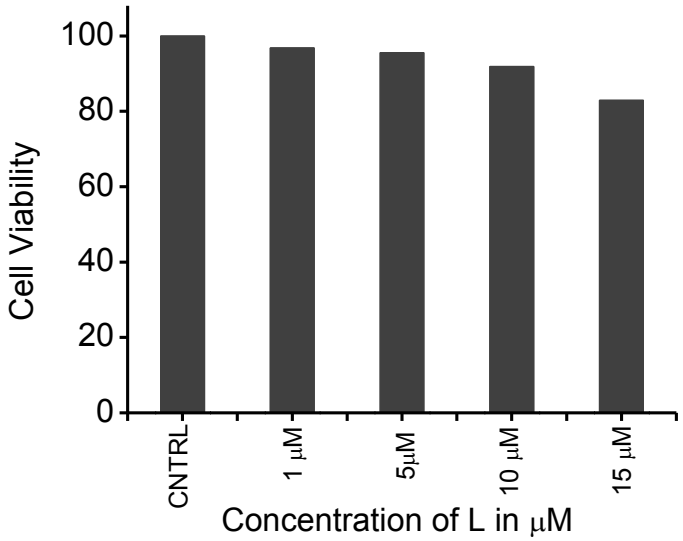
SI Figure 19: Confocal micrographs of live Hct116 cells in the presence of **L** (10 μM in aq. HEPES buffer–CH_3CN (1: 1, v/v; pH 7.2) medium). The images were acquired after 30 min of treatment of $PdCl_2$ on Hct116 cells. Bottom panels show an overlay of images with a confocal phase.

Confocal microscopic images of L at different Pd species in Hct116 cells



SI Figure 20: Confocal micrographs of live Hct116 cells in the presence of L (10 μM in aq. HEPES buffer–CH_3CN (1: 1, v/v; pH 7.2) medium). The images were acquired after 30 min of treatment of Pd(0)($Pd(Pph_3)_4$ in THF solution); Pd(ii) ($PdCl_2$ in Aq. medium) and Pd(iv) (K_2PdCl_6 in Aq. Medium) on Hct116 cells. Bottom panels show an overlay of images with a confocal phase. Note Pd $(Pph_3)_4$ in THF solution.

MTT assay for evolution of cytotoxicity of the reagent L towards Hct116 cells [1]



SI Figure 21. MTT assay to determine the cell viability percentage in Hct116 colon cancer cells. The concentration of the **L** ranges from 1- 15μM and treated for 24 hours.

References: 1. U. Reddy G, H. Agarwalla, N. Taye, S. Ghorai, S. Chattopadhyay and A. Das, *Chem. Commun*., 2014, **50**, 9899.

Evaluation of $[Pd^{2+}]$ in human urine sample

Methodology:

Urine was diluted (fresh urine sample was diluted 100 times with water: acetonitrile (1: 1, v/v) mixture) before measurement and some solutions were spiked with known concentration of Pd^{2+} as an internal standard without further treatment. These solutions along with solutions spiked with known $[Pd^{2+}]$ (1 μM, 2 μM and 3 μM) as an internal standard were used for emission measurements without further treatment. Thus fluorescence intensity of such urine samples spiked with 1 μM, 2 μM and 3μM of Pd^{2+} were $I_{urine+1}$, $I_{urine+2}$ and $I_{urine+3}$, respectively. Fluorescence intensities for aqueous HEPES buffer solution having pH of 7.2 was evaluated for $[Pd^{2+}]$ of 1, 2 and 3μM and these values were I_1, I_2 and I_3, respectively. Average of three differences ($I_{urine+1}$-I_1, $I_{urine+2}$-I_2 and $I_{urine+3}$-I_3 and the calibration plot for Pd^{2+} led us to evaluate the actual $[Pd^{2+}]$ of (0.2 μg/litre) in the urine sample.