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**Supporting Information**

# From metallodrug design to halochromic nanocarrier delivery: Revitalizing the anticancer efficacy and biocompatibility of cyclometalated Ru(II) complex

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## Synthesis of Ligands

**Synthesis of 2-phenylquinoline (L1).** To a solution of 2-nitrobenzaldehyde (5 g, 33.08 mmol) in methanol (70 mL), iron powder (7.3 g, 132.34 mmol), and 0.1 N HCl were added. The reaction mixture was stirred at 80 °C for 2 h. To this acetophenone (4.4g, 37.4 mmol) and powdered KOH (2.23 g, 39.6 mmol) is added drop wise. The mixture was reflux at 90° C for 4 h, excess iron was removed by filtration through a celite pad, and the solvent was evaporated. Then, the residue was extracted with $CH_2Cl_2$ (3 x 30 mL) and wash with water and the organic layer was dried over anhydrous $Na_2SO_4$, and concentrated. The resulting crude product was chromatographed through silica gel using ethyl acetate: hexane (15:5 v/v) as eluent to obtained pure product as white solid; yield: 45 %. $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.24-8.16 (m, 4H), 7.90-7.83 (m, 2H), 7.75-7.72 (t, 1H, $J$ = 6 Hz), 7.55-7.45 (m, 4H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 157.41, 148.31, 139.72, 136.82, 129.76, 129.70, 129.36, 127.61, 127.51, 127.21, 126.32, 119.06. IR (KBr, cm$^{-1}$): ν(C=C) 1596, ν(C=N) 1553.

**Synthesis of 2-(thiophen-2-yl) quinoline (L2).** The compound was prepared by following the same procedure for **L1** but using 2-acetylthiophene instead of acetophenone. white solid, yield 53 % $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.15-8.08 (m, 2H), 7.81-7.68 (m, 4H), 7.51-7.46 (m, 2H), 7.17-7.15 (m, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 152.36, 148.14, 145.43, 136.64, 129.85, 129.29, 128.63, 128.13, 127.53, 127.21, 126.13, 125.90, 117.66. IR (KBr, cm$^{-1}$): ν(C=C) 1593, ν(C=N) 1552.

**Synthesis of 4-methyl-2-phenylquinoline (L3).** A mixture of 2-chlorolepedine (1.786 g, 10 mmol), phenylboronic acid (1.52 g, 12.5 mmol), [$Pd(PPh_3)_4$] (0.1 g, 22 mmol, 10 mol %), potassium carbonate (17 mL), and dry tetrahydrofuran (50 mL) was taken in a round-bottom flask and heated under nitrogen atmosphere at 80 °C for 24 h. After cooling the reaction mixture was concentrated under vacuum and the residue was extracted with $CH_2Cl_2$ (3 × 30 mL), the organic layer was dried over anhydrous $Na_2SO_4$ and concentrated. The mixture was purified by column chromatography using ethyl acetate: hexane (3:2 v/v) as eluent to obtained yellow oil, yield 81 %. $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.96-7.89 (m,3H), 7.62-7.60 (d, 1H, *J* = 8 Hz), 7.44-7.40 (t, 1H, *J* = 8 Hz,), 7.34 (s, 1H), 7.24-7.16 (m, 4H), 2.35 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 152.01, 147.95, 145.52, 144.73, 129.81, 129.49, 128.37, 128.06, 127.35, 125.87, 125.69, 123.66, 118.29, 18.94. IR (KBr, $cm^{-1}$): ν(C=C) 1599, ν(C=N) 1550.

**Synthesis of 2,4-diphenyl quinoline (L4).** 2-amino benzophenone (1.97 g, 10 mmol), acetophenone (1.321 g, 11 mmol), Conc. $H_2SO_4$ (0.5 mL) and acetic acid (20 mL) were added to a 100 mL round-bottom flask. The reaction mixture was stirred at 110 °C for 24 h under inert atmosphere. After Cooling the reaction mixture was quenched with a saturated ammonia/ice mixture and extracted with $CH_2Cl_2$ (3 × 30 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under a vacuum. The mixture was purified by column chromatography through silica gel using ethyl acetate: hexane (15:5 v/v) as eluent to obtained pure product as pale-yellow solid; yield: 50 %. ($^1$H NMR (400 MHz, $CDCl_3$) δ: 8.26-8.24 (d, 1H, *J* = 8 Hz), 8.20-8.19 (d, 2H, *J* = 4 Hz), 7.93-7.90 (d, 1H, *J* = 12 Hz), 7.83 (s, 1H), 7.76-7.72 (t, 1H, *J* = 8Hz), 7.57-7.45 (m, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 156.94, 149.19, 148.86, 139.70, 138.44, 130.18, 129.61, 129.57, 129.39, 128.89, 128.64, 128.45, 127.63, 126.38, 125.80, 125.69, 119.41. IR (KBr, $cm^{-1}$): ν(C=C) 1587, ν(C=N) 1543.

**Lipophilicity**

The lipophilicity of the complexes was determined using the "shake-flask" method in octanol-water phase partitioning.[1,2] The complexes were dissolved in a mixture of water and n-octanol and shaken for 24 hours. After settling for 30 minutes, the two phases were collected separately to prevent cross-contamination. The concentration of the complexes in each phase was measured by UV-Vis absorption spectroscopy at room temperature. The results represent the mean values from three independent experiments. The concentration of the sample solution

was used to calculate the log P values. The partition coefficients for the four complexes were calculated with the formula: log P = log[octanol]/[water].

**In vitro cytotoxicity**

MCF-7 and HEK-293 were procured from National Centre for Cell Science (NCCS), Pune. Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) culture media supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) (Invitrogen Corporation, CA, USA) in a humidified atmosphere of 5% $CO_2$ at 37˚C until confluent then the cells were trypsinized and plated at a density of ~20, 000 cells/well in a 96-well plate and incubated at 37 °C in the $CO_2$ incubator. MCF-7 and HEK-293 were purchased from the National Centre for Cell Science (NCCS), Pune. A humidified atmosphere of 5% $CO_2$ was used to cultivate the cells in Dulbecco's modified Eagle's medium (DMEM) culture media supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) (Invitrogen Corporation, CA, USA) until confluent. The cells were then trypsinized and plated at a density of approximately 20,000 cells/well in a 96-well plate, and they were then incubated at 37 °C in the $CO_2$ incubator. Following a 24-hour incubation period with varying doses of compounds in both cell lines, the cell viability % was determined using the MTT assay. The stock solutions of the compounds were prepared in a 1% DMSO/10 mM PBS (pH = 7.2) mixture immediately before dilution. The final DMSO concentration in the wells did not exceed 0.2% and the same amount of DMSO was maintained in all the cellular experiments. Prior to performing these experiments, the stability of the compounds in 1% DMSO/10 mM PBS was assured by UV-Vis absorption spectroscopy over 24 hours (**Fig. S24**). However stock solution of PDL-Ru(1) was prepared in 10 mM PBS. Different concentrations of compound solution in μM were prepared by the dilution of the stock solution using culture media in triplicate. The MTT insoluble formazan was dissolved in DMSO and the MTT reduction was quantified by measuring the absorbance at 570 nm (Multiskan Spectrophotometer, USA). The obtained data were plotted and fitted using origin and GraphPad Prism software. The data were obtained for three biological replicates each and used to calculate the mean. The $IC_{50}$ values provided are mean ± standard deviation. The statistical significance (p-value) of the data, which was determined using GraphPad prism software with t-test, is $< 0.05$ or better.

**Preparation of PDL-Ru(1) nanoformulation**

PCDA and DMPC were dissolved in $CHCl_3$ at a 4:1 molar ratio in a round bottom flask and mixed with a methanol solution of complex (stock solution concentration = 1mg/1ml). A milky white layer was obtained by solvent evaporation using a rotary evaporator at 45 °C. The white layer obtained was hydrated with 10 mM PBS (pH = 7.2) and sonicated for 30 minutes at 70°C and filtered using a 0.4 μm syringe filter. Then the non-encapsulated complex was removed by centrifugation and the assemblies were re-dispersed in 10 mM PBS (pH = 7.2) solution. The samples were irradiated using a UV lamp (254 nm, 400 μW/cm2, Luzchem photo reactor) for 30 minutes to get the polymerized PCDA/DMPC vesicle solution, which is green in color. The stability of the vesicles was monitored for 24 h time intervals up to 7 days by recording the nature of the UV-Vis absorption spectrum of the assembly **(Fig.S25).**

**AO/EB staining assay**

MCF-7 breast cancer cells ($1 \times 10^5$ cells/well) were seeded in 12-well plates containing 10% FBS medium and cultured for 24 hours. Following treatment, cells were incubated for an additional 24 hours at 37°C to induce apoptosis. Subsequently, cells were stained with acridine orange (AO, 200 μM) and ethidium bromide (EB, 100 μM) for 1 hour. Live cells were stained green by AO, while dead cells were stained red by EB. Cellular and nuclear morphology were then examined under a fluorescence microscope (Biorevo, BZ-9000, Keyence)

**Zebrafish Embryo Toxicity (ZET)**

The embryos of wild-type Danio rerio (zebrafish) have been staged and nurtured at 27 ± 1 °C as previously described and by following OECD 2013 guidelines.[3,4] The viable embryos were seeded in 24-well plates and exposed to five different concentrations (50, 100, 150, 200, 250, and 500 μM) of **Ru(1)** and **PDL-Ru(1)** together with untreated control and vehicle control. The stock solution of the compounds was prepared in DMSO and then diluted using E3-medium but the final DMSO concentration was kept constant at 0.1% (v/v). The zebrafish embryo's mortality, malformations, and hatching rates were scrutinized under a stereo zoom microscope (Leica SAP0) up to 96 h with a time interval of 24 h. Experiments were performed in triplicate to get the mean values ± standard deviation. The percentage of the hatching rate was calculated according to OECD guidelines as below.[5] The Lethal Concentration 50 ($LC_{50}$) was determined from the plot of the percentage of mortality vs. concentration using Origin software.

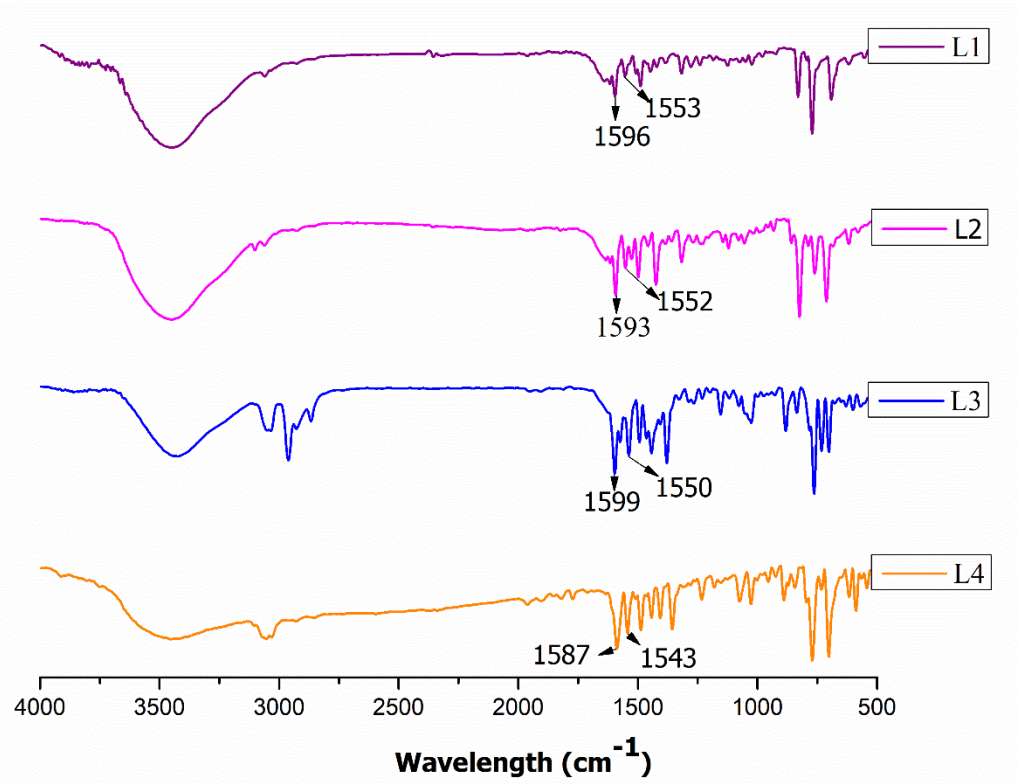


**Figure S1**. IR spectra of Ligands (**L1-L4**).

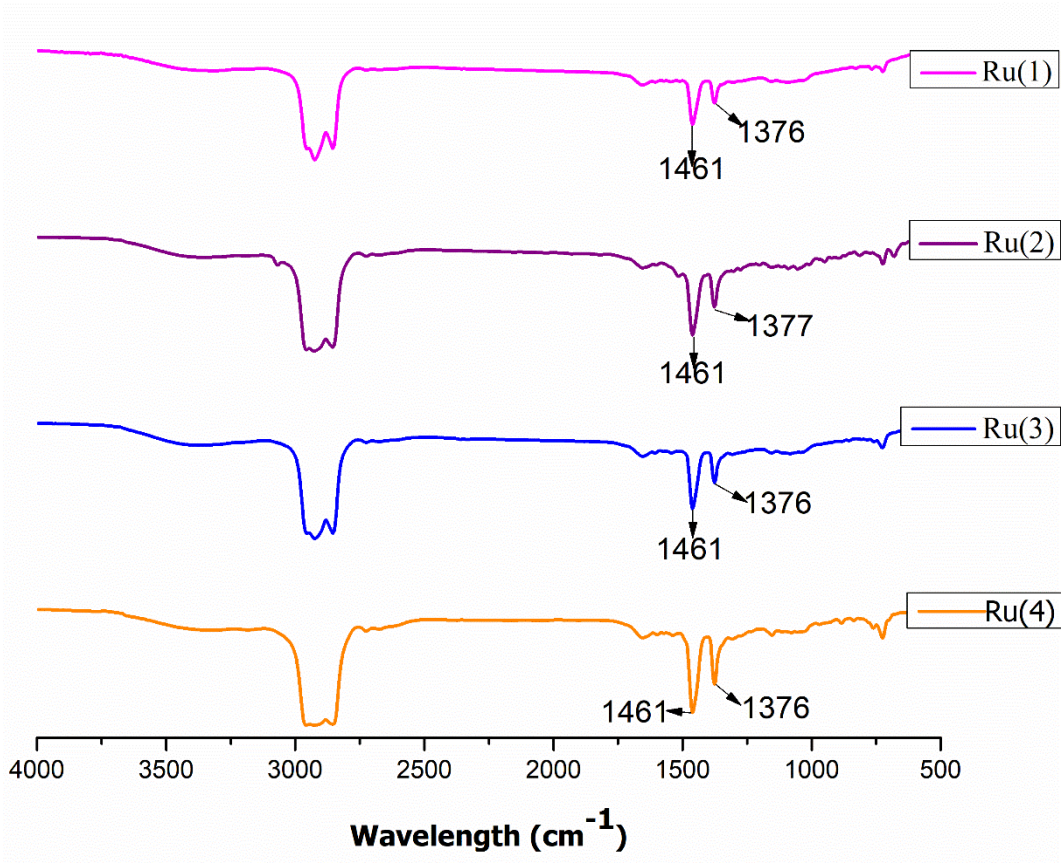


**Figure S2**. IR spectra of Complexes [(**Ru(1)-Ru(4)**].

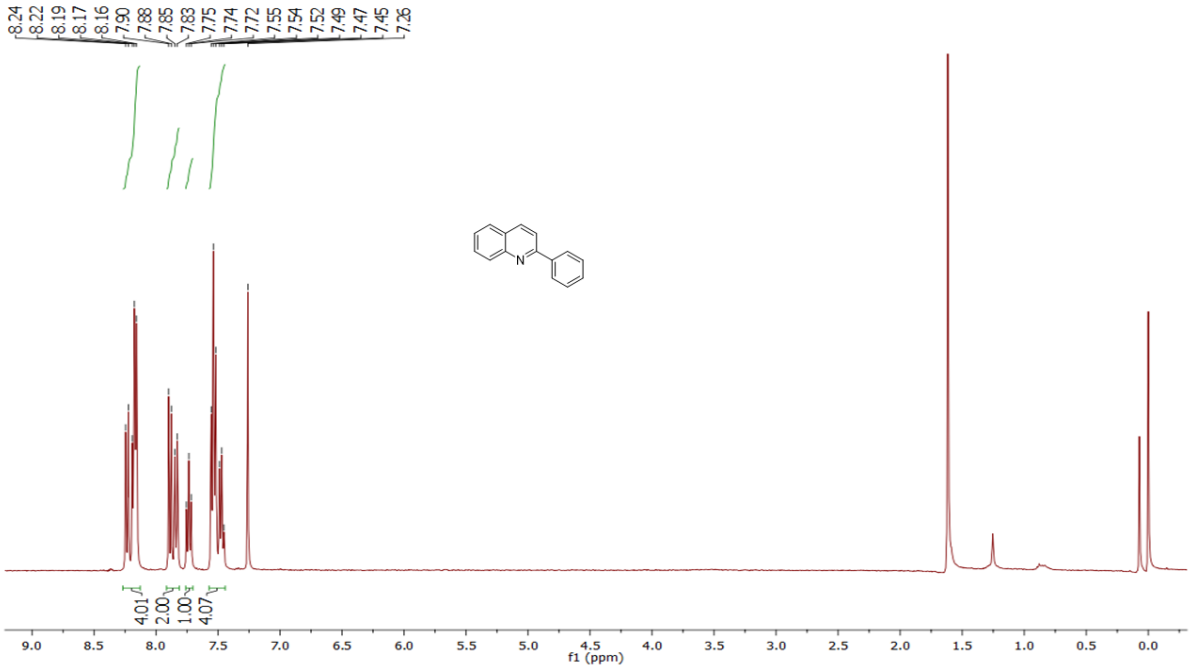


**Figure S3**: $^{1}$H NMR of 2-phenylquinoline (**L1**) in $CDCl_3$.

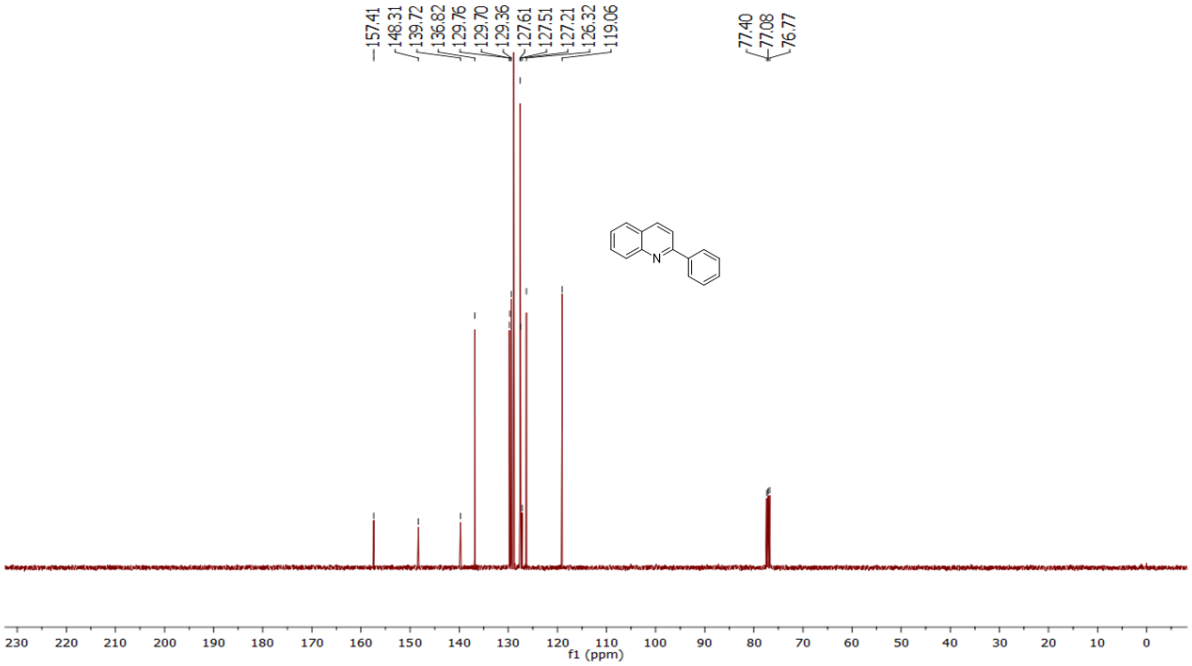


**Figure S4**: $^{13}$C NMR of 2-phenylquinoline (**L1**) in $CDCl_3$.

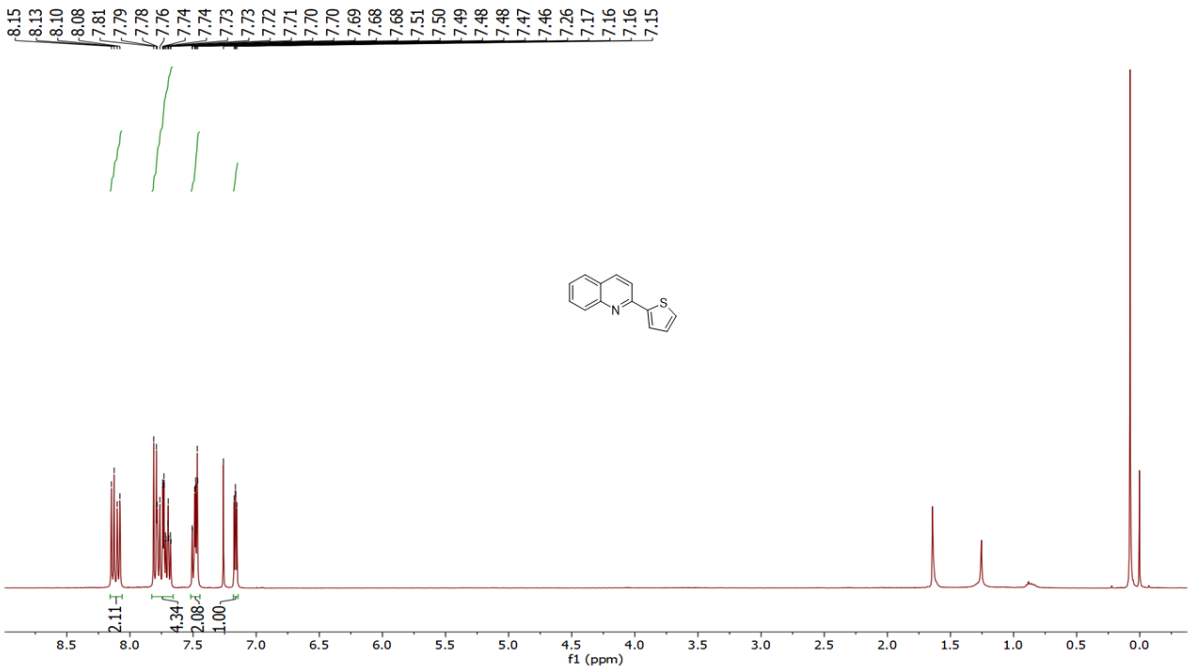


**Figure S5.** $^{1}$H NMR of 2-(thiophen-2-yl)quinoline (**L2**) in $CDCl_3$.

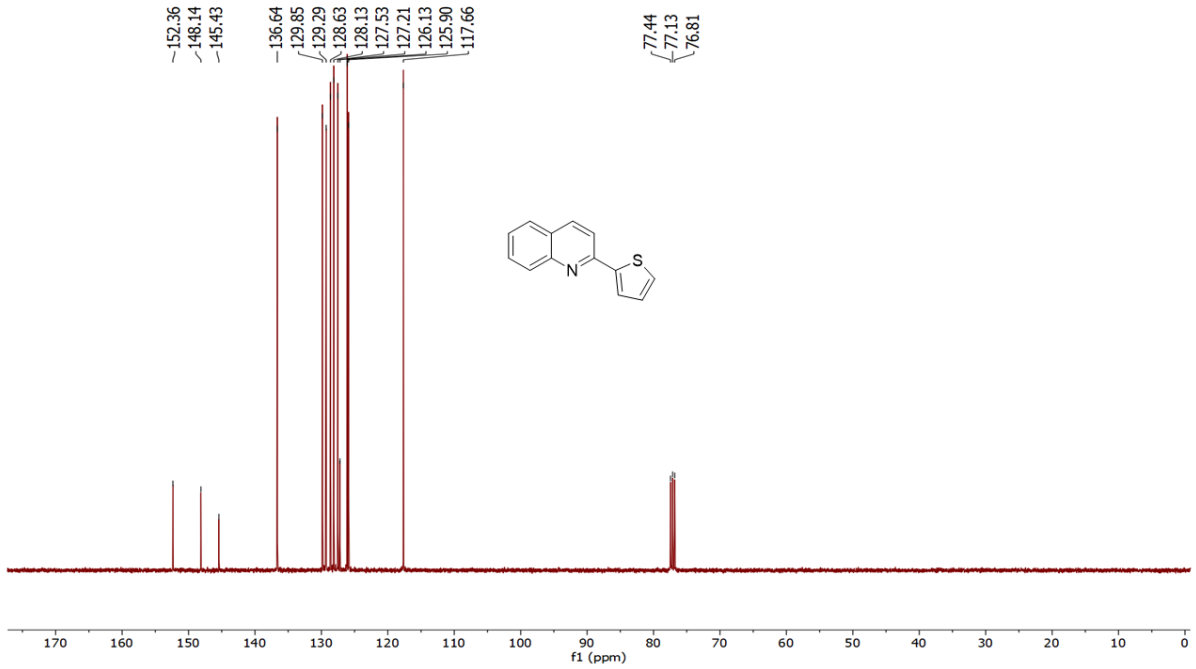


**Figure S6.** $^{13}$C NMR of 2-(thiophen-2-yl)quinoline (**L2**) in $CDCl_3$.

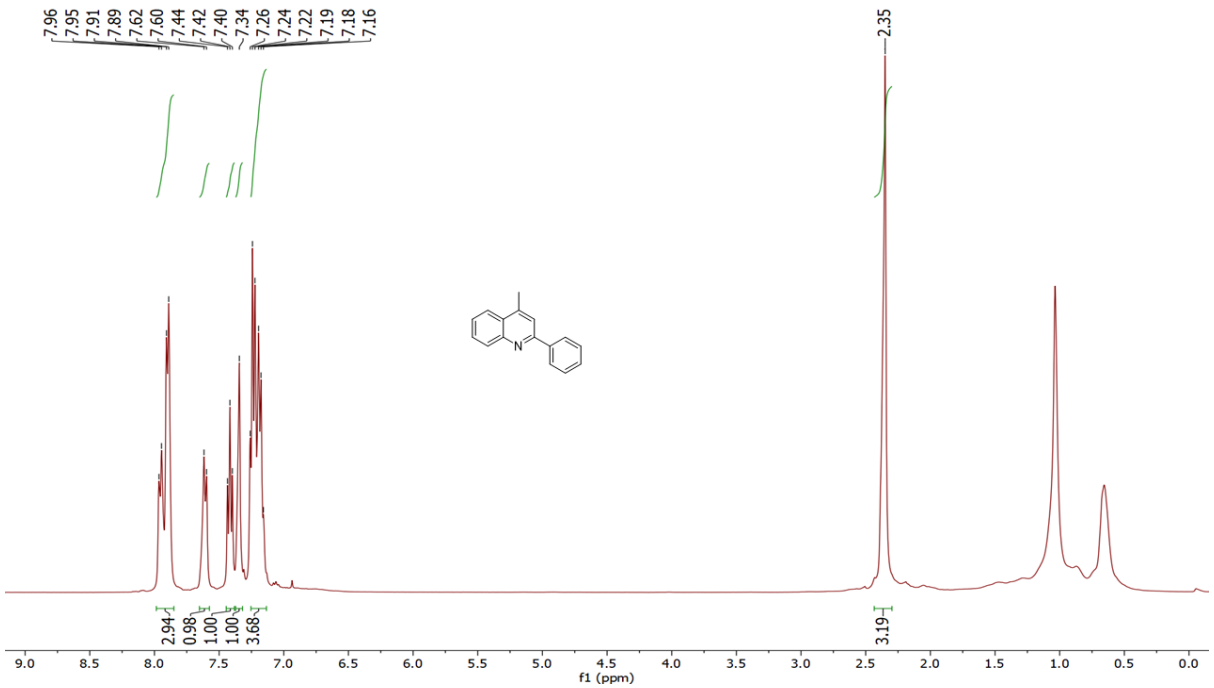


**Figure S7.** $^{1}$H NMR of 1-methyl-3-phenylnaphthalene (**L3**) in $CDCl_3$.

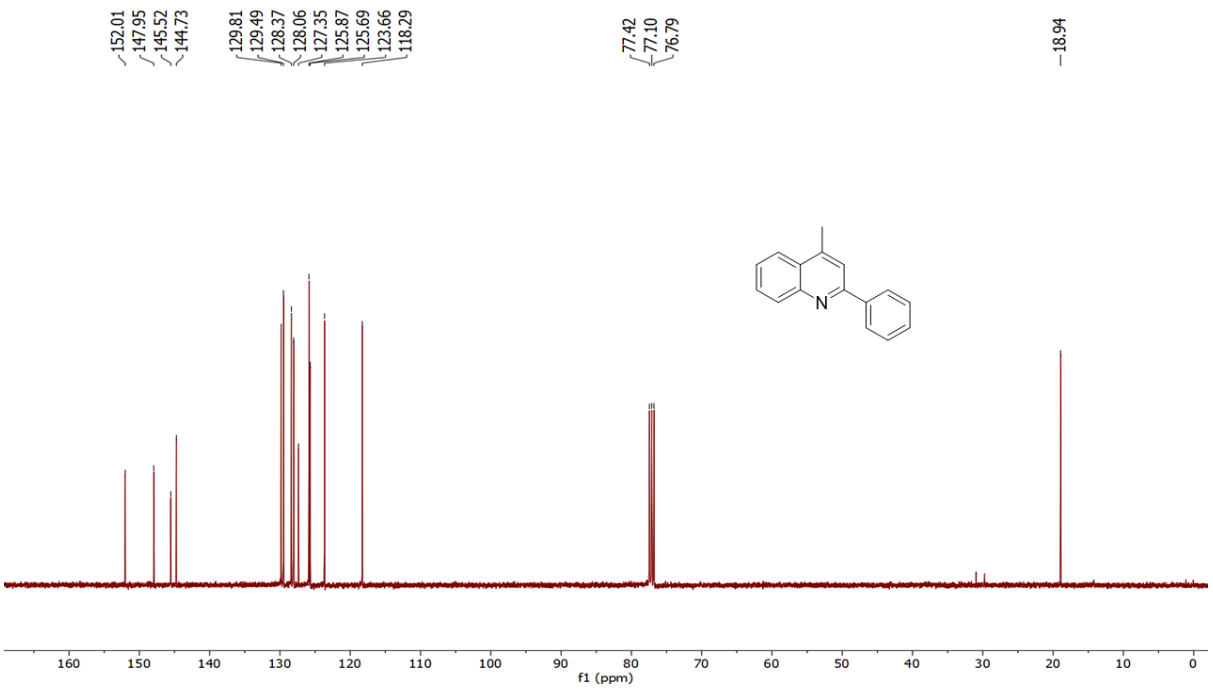


**Figure S8.** $^{13}$C NMR of 1-methyl-3-phenylnaphthalene (**L3**) in $CDCl_3$.

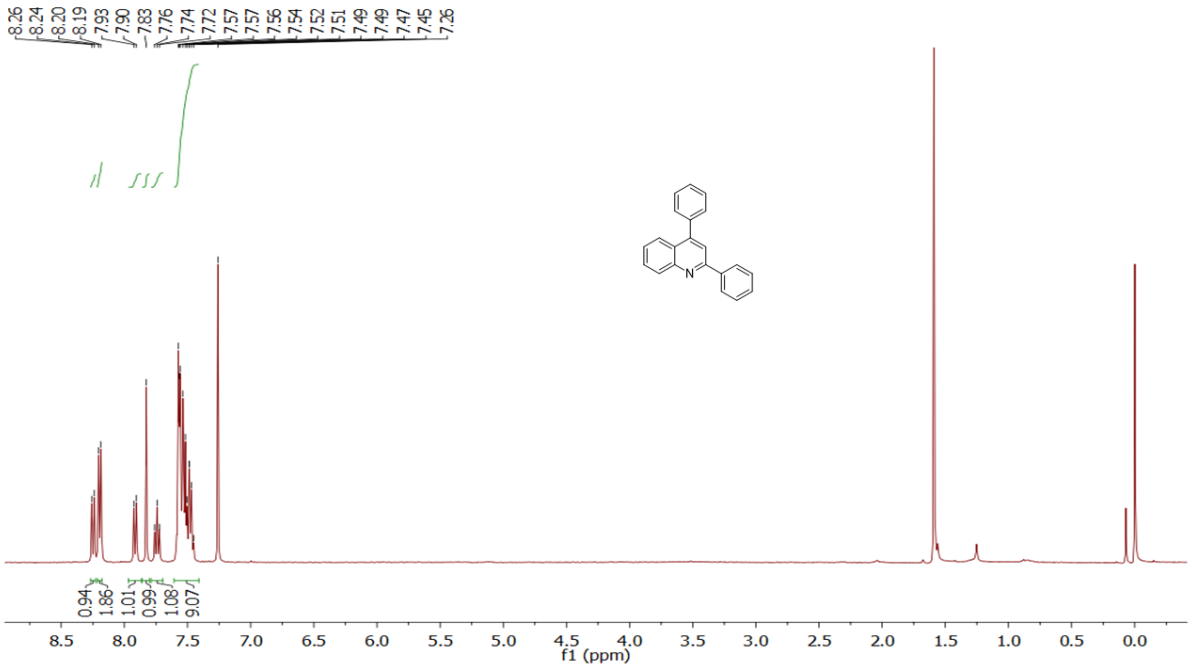


**Figure S9.** $^{1}$H NMR of 2,4-diphenylquinoline (**L4**) in $CDCl_3$.

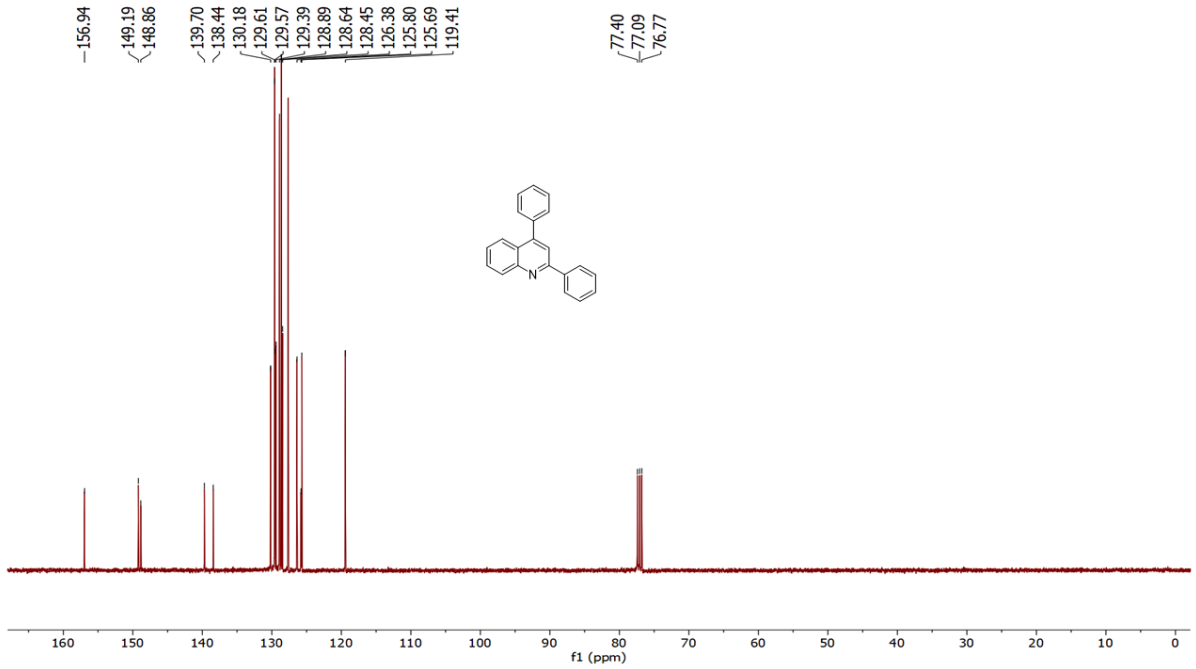


**Figure S10.** $^{13}C$ NMR of 2,4-diphenylquinoline (**L4**) in $CDCl_3$.

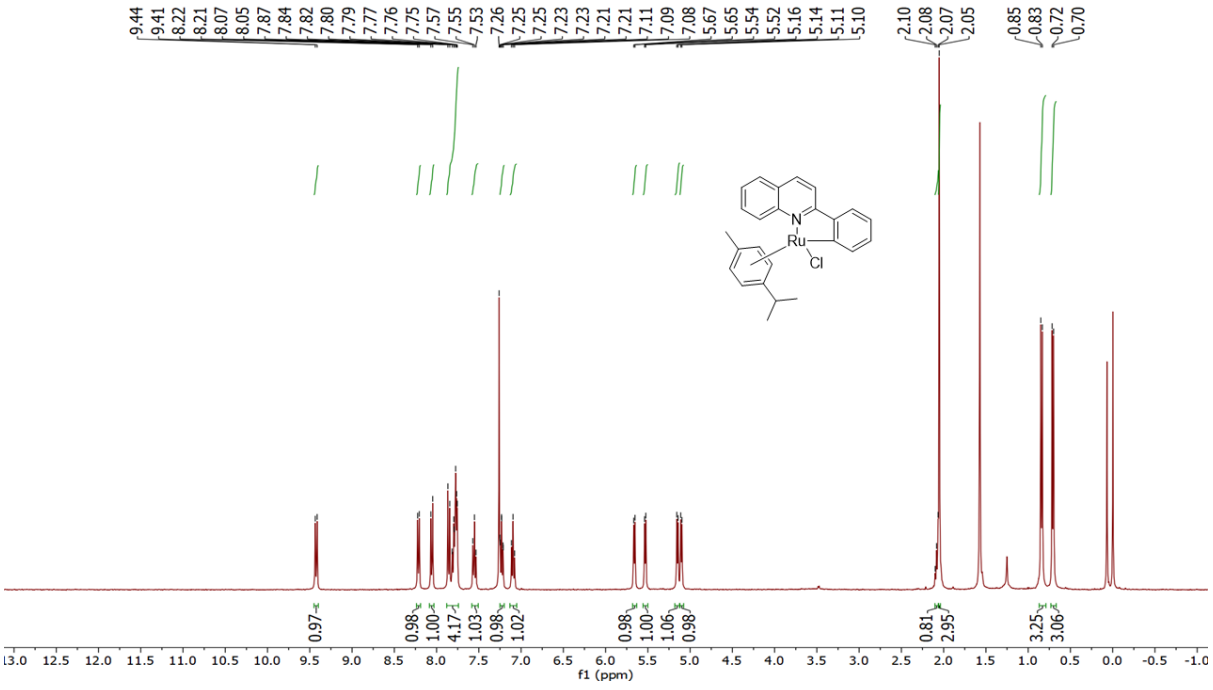


**Figure S11.** $^{1}H$ NMR of **Ru(1)** complex in $CDCl_3$.

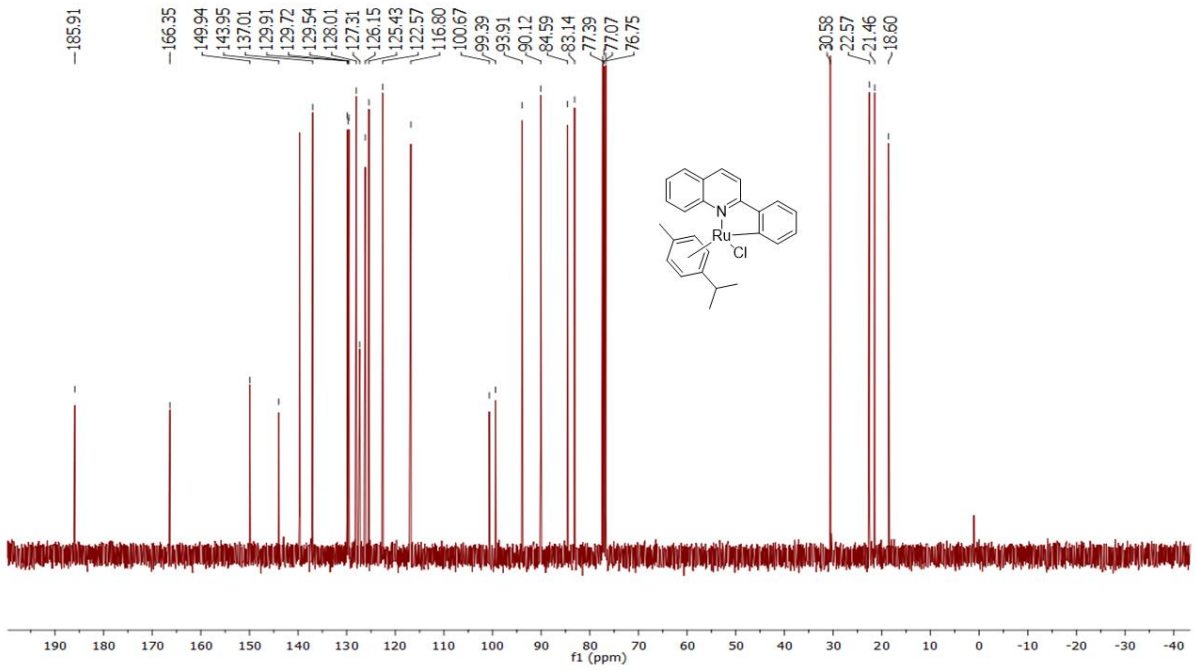


**Figure S12.** $^{13}C$ NMR of **Ru(1)** complex in $CDCl_3$.

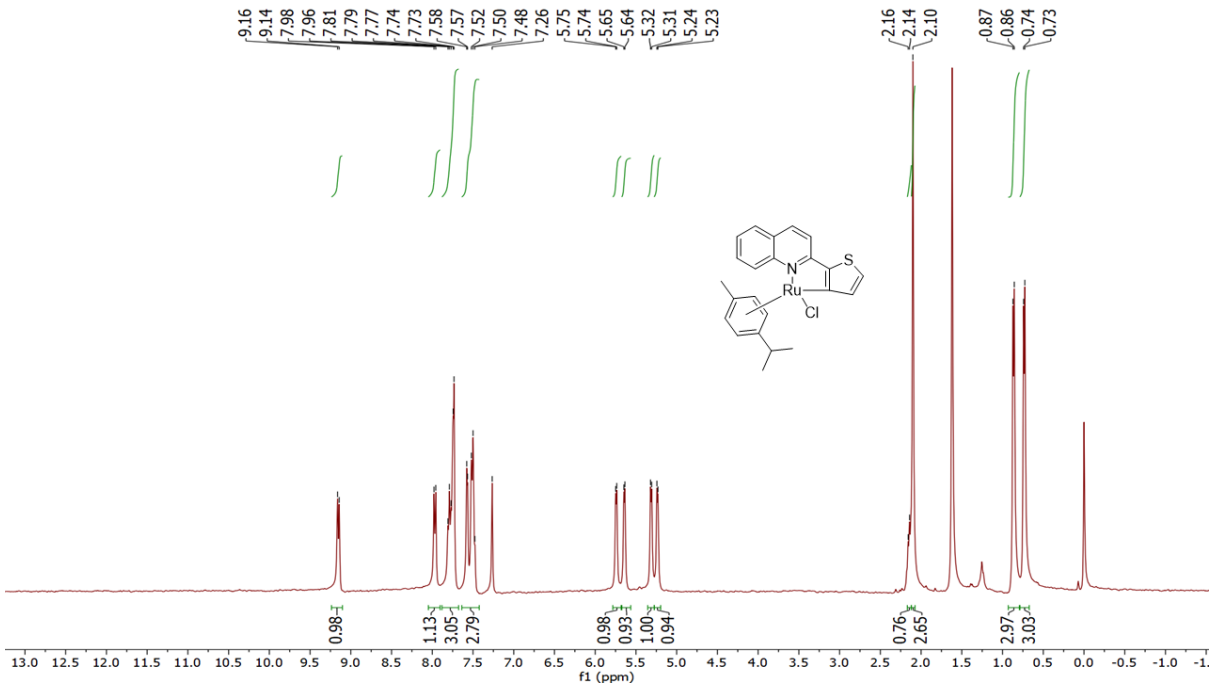


**Figure S13.** $^{1}H$ NMR of **Ru(2)** complex in $CDCl_3$.

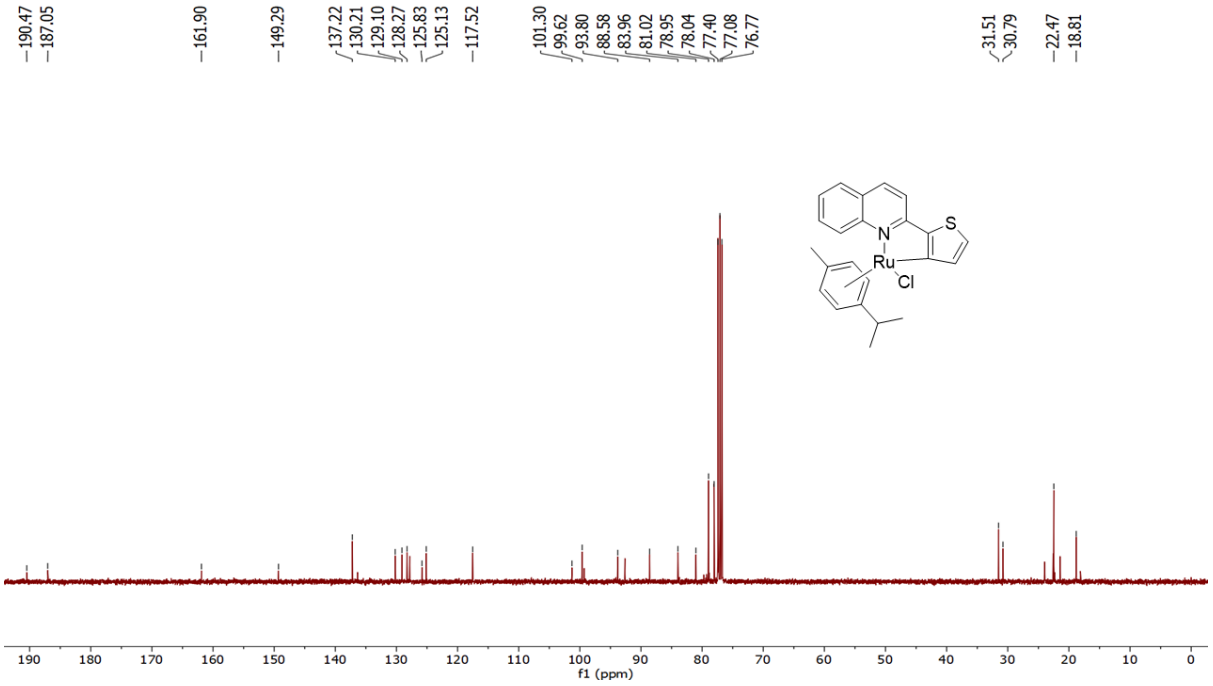


**Figure S14.** $^{13}C$ NMR of **Ru(2)** complex in $CDCl_3$.

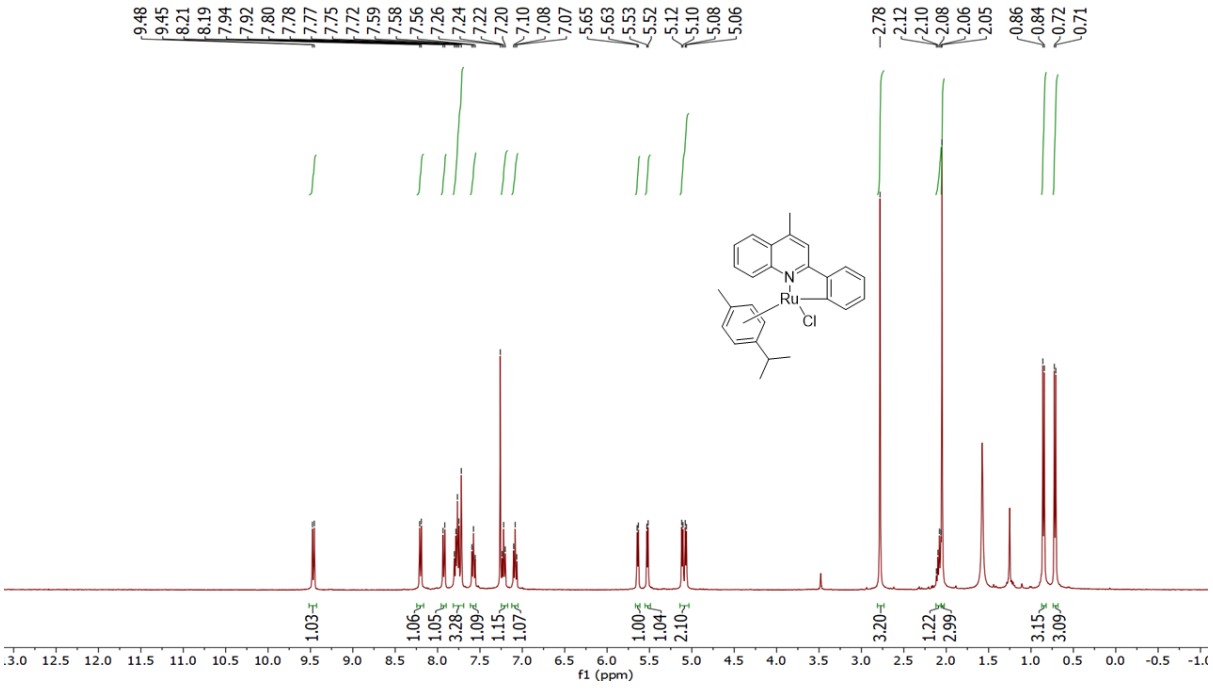


**Figure S15.** $^{1}H$ NMR of **Ru(3)** complex in $CDCl_3$.

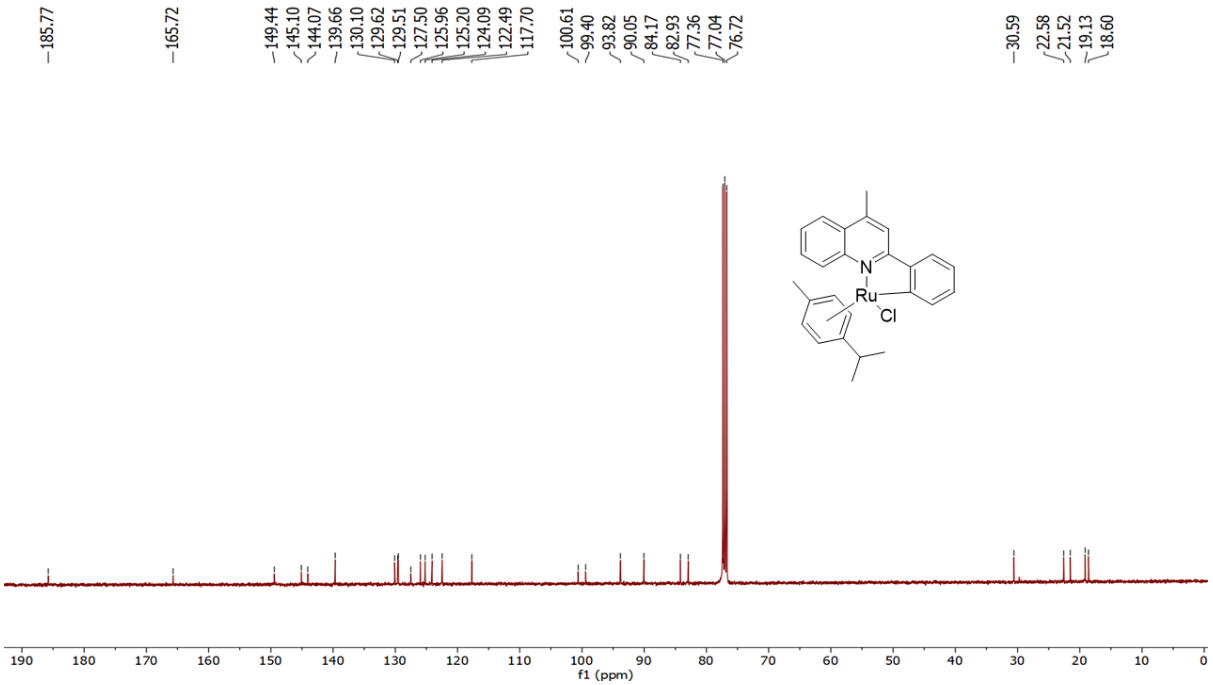


**Figure S16.** $^{13}C$ NMR of **Ru(3)** complex in $CDCl_3$.

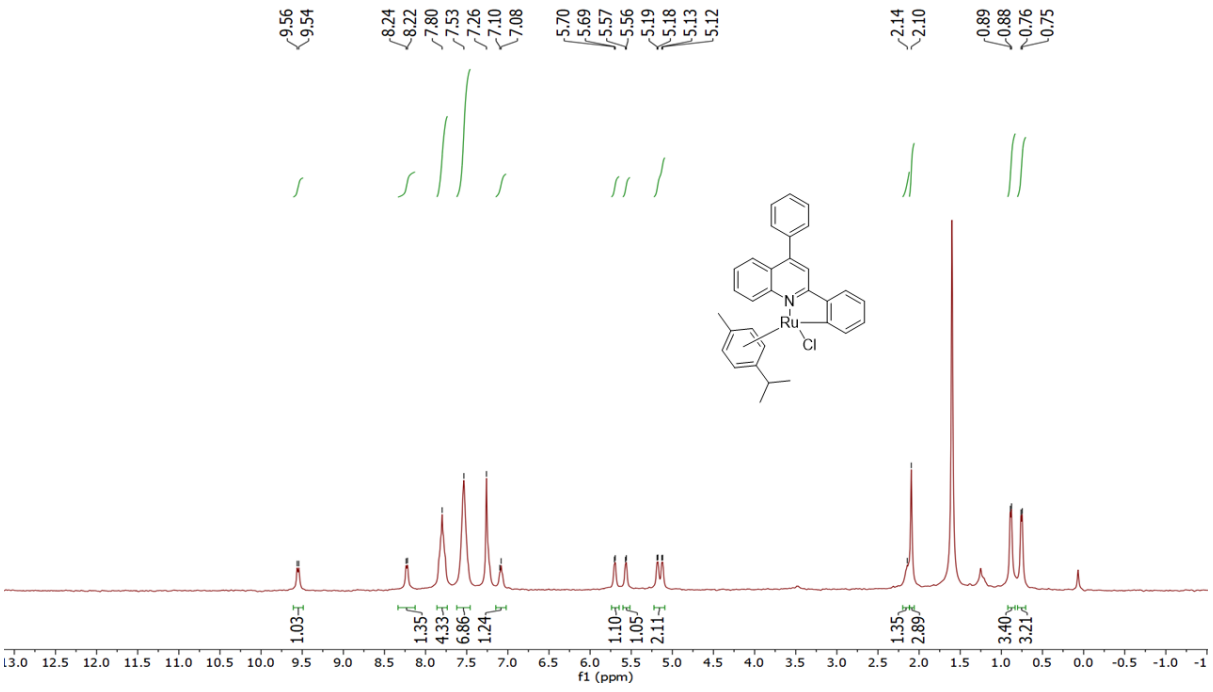


**Figure S17.** $^{1}H$ NMR of **Ru(4)** complex in $CDCl_3$.

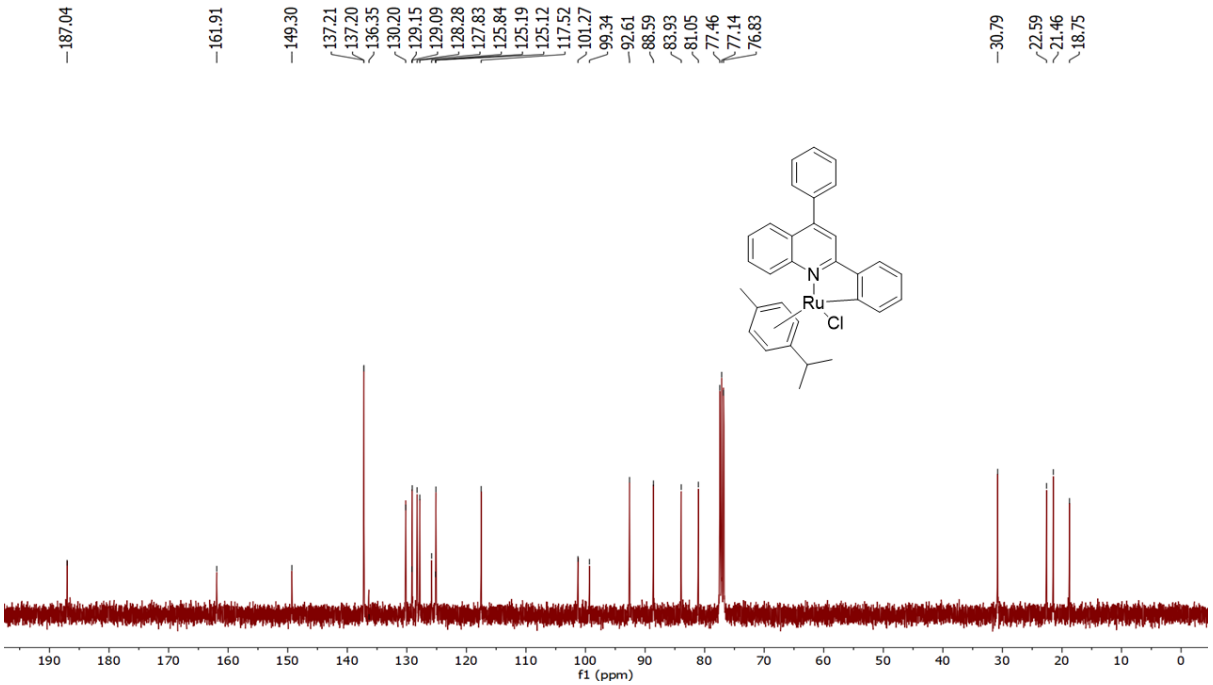


**Figure S18.** $^{13}C$ NMR of **Ru(4)** complex in $CDCl_3$.

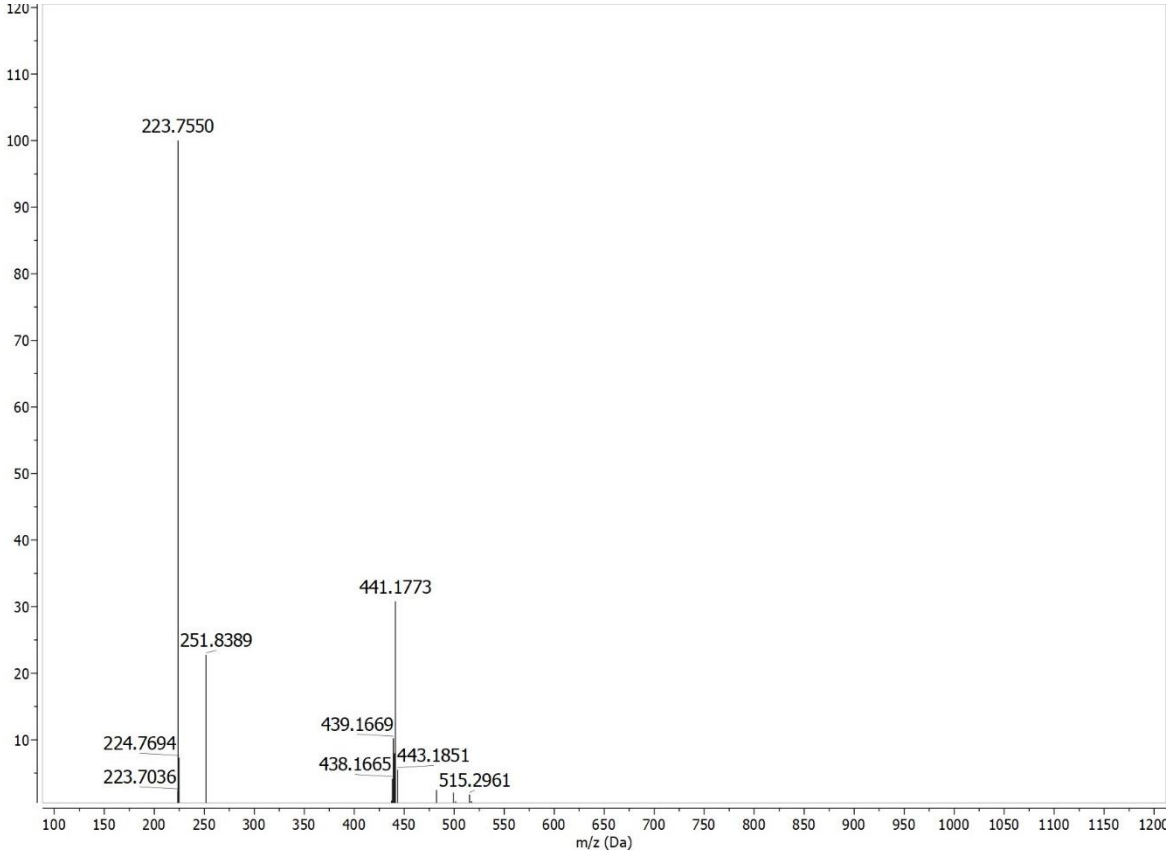


**Figure S19**: HRMS of [Ru($\eta^6$-*p*-cymene)(L1)Cl] [**Ru(1)**].

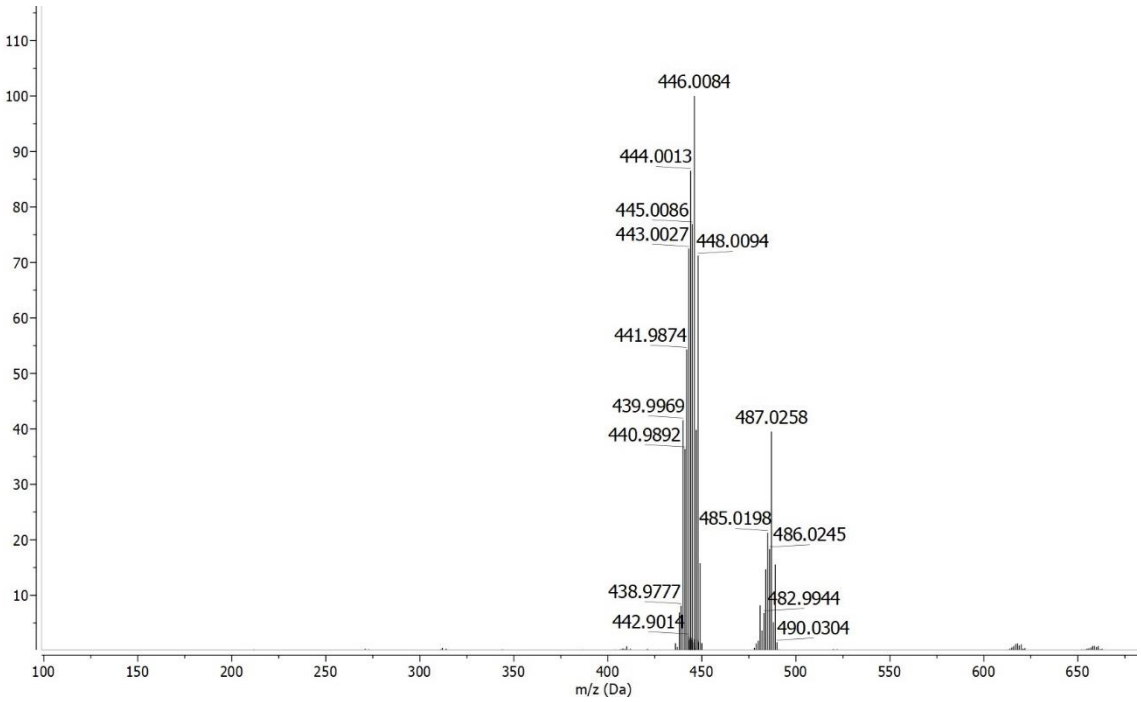


**Figure S20**: HRMS of [Ru($\eta^6$-*p*-cymene)(L2)Cl] [**Ru(2)**].

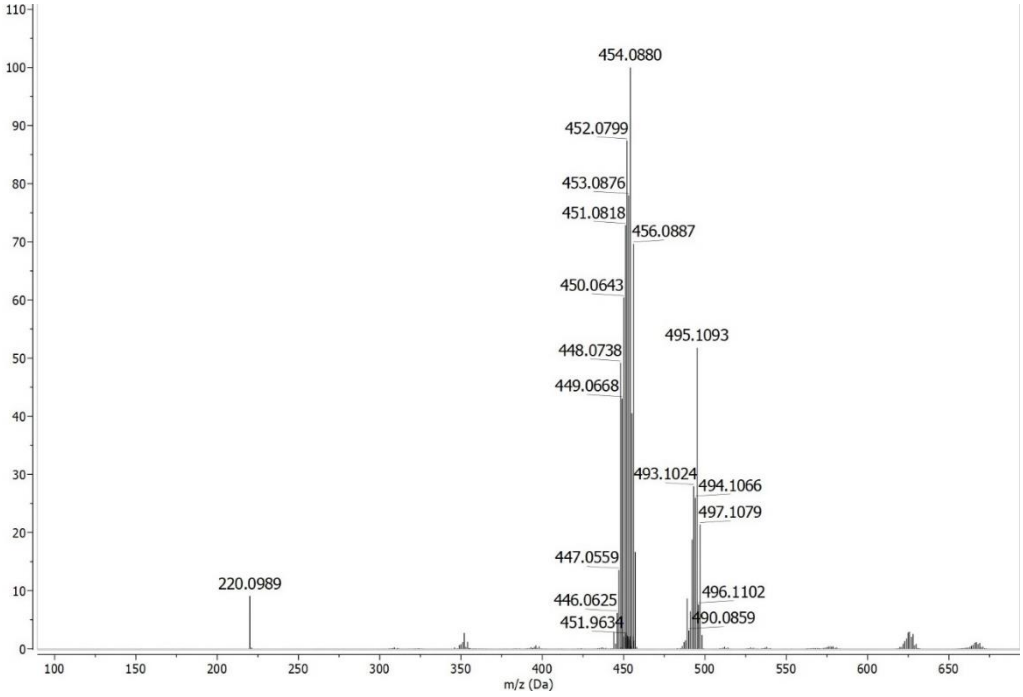


**Figure S21**: HRMS of [Ru($\eta^6$-*p*-cymene)(L3)Cl] [**Ru(3)**].

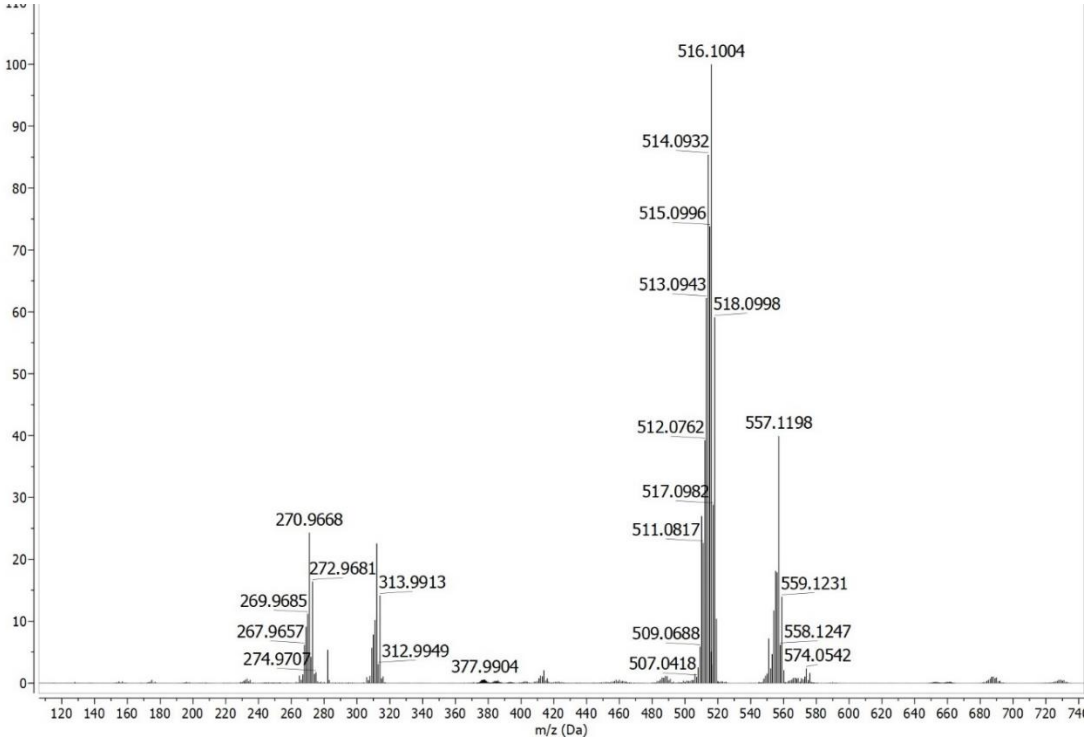


**Figure S22**: HRMS of [Ru($\eta^6$-*p*-cymene)(L4)Cl] [**Ru(4)**].

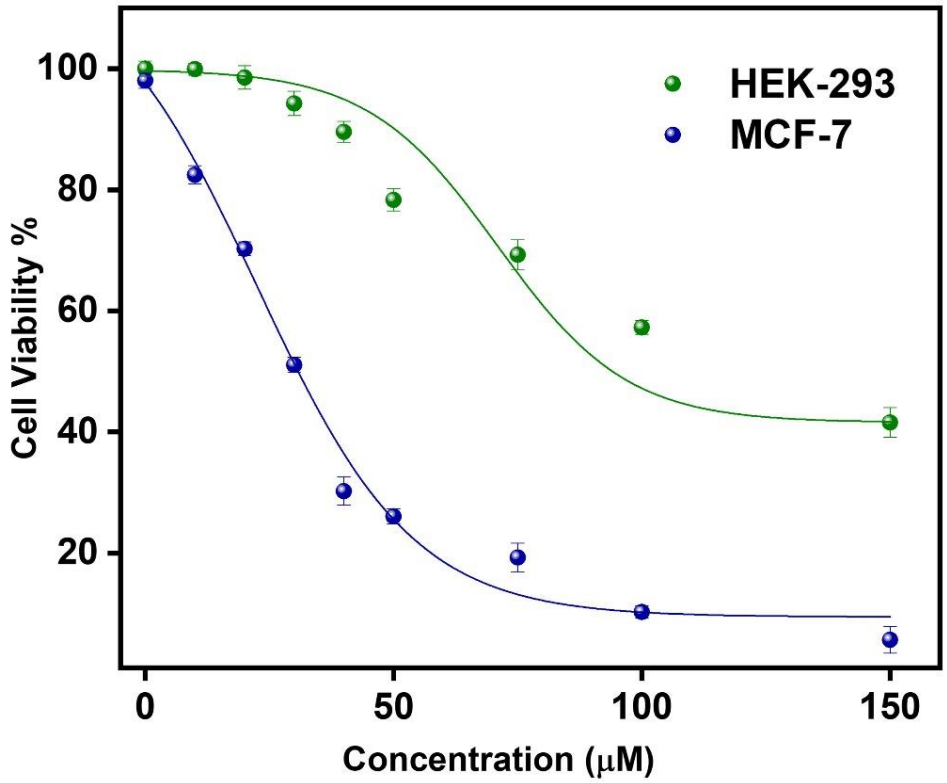


**Figure S23.** Dose responsive curves for cytotoxicity of PDL-Ru(1) against HEK-293 and MCF-7 cancer cell lines after 24 h incubation

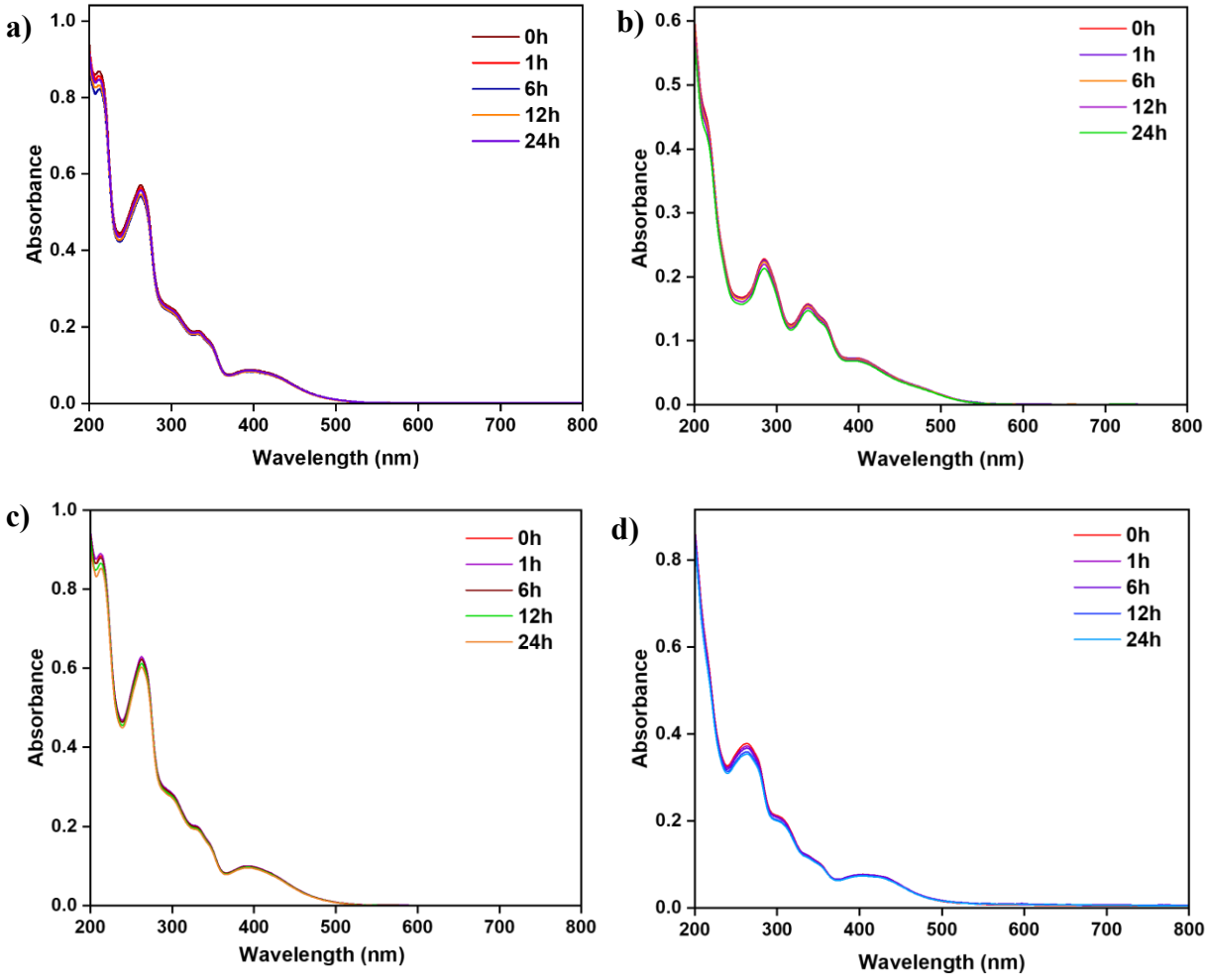


**Figure S24.** Stability studies of the a) Ru(1) b) Ru(2) c) Ru(3) and d) Ru(4) in 1% DMSO/10 mM PBS (pH = 7.2) solution at various time intervals up to 24 h.

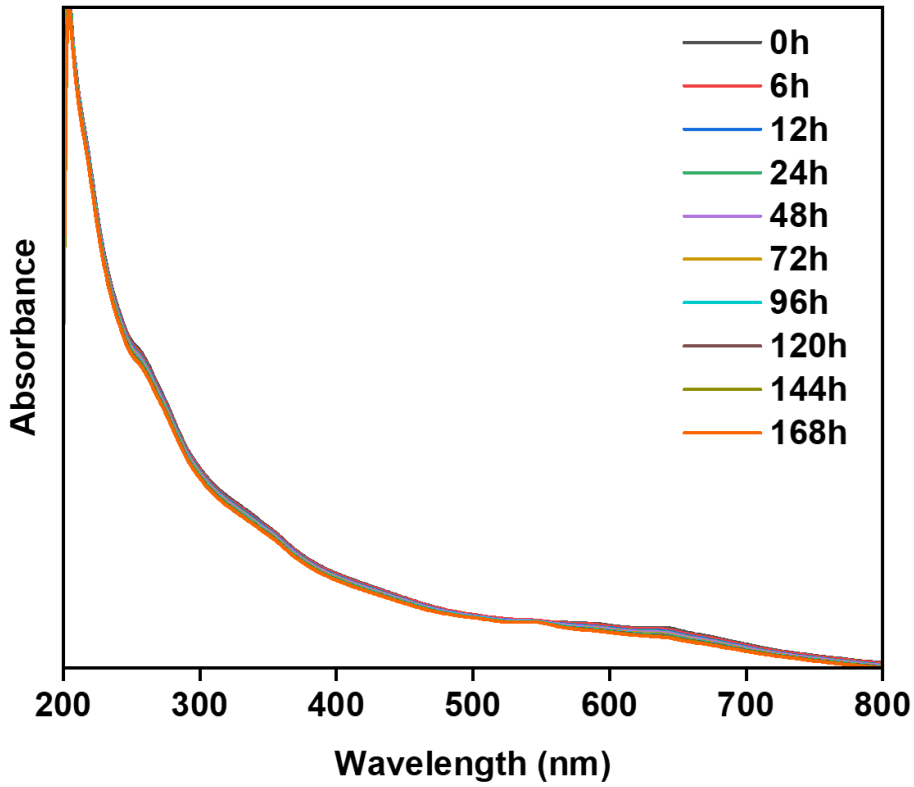


**Figure S25.** Stability profile of PDL-Ru(1) in PBS at various time intervals up to 7 days.

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