Electronic Supplementary Information

N,O-chelating quinoline-based half-sandwich organorhodium and -iridium complexes: synthesis, antiplasmodial activity and preliminary evaluation as transfer hydrogenation catalysts for the reduction of NAD^+

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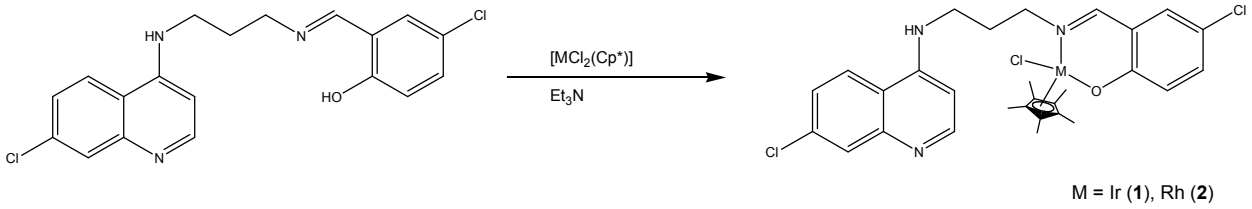


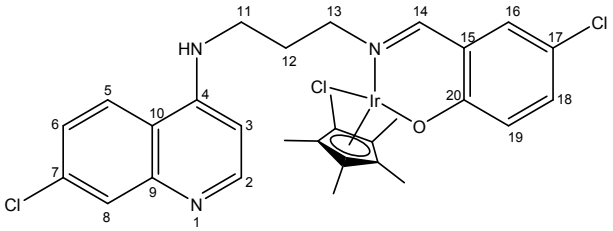
Scheme S1. Synthesis of Iridium (**1**) and Rhodium (**2**) quinoline complexes

General methods:

Synthetic procedures were performed at ambient temperatures unless otherwise stated. All reagents were purchased from Sigma Aldrich and used as received. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Inova 300 or 500 MHz spectrometer using the solvent resonance as internal standard. Infrared (IR) absorptions were measured on a Nicolet Avatar 360 FT-IR spectrometer using attenuated total reflectance (ATR). High resolution (HR) ESI-mass spectrometry was used to further characterize all new compounds and determinations were carried out using a Waters API Quattro instrument in the positive mode. The complexes were prepared using a similar method described in literature.[1]

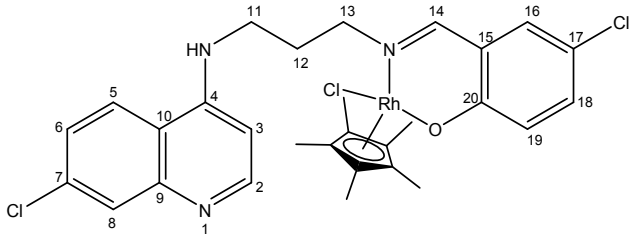
A solution of the ligand (2 eq.) and Et_3N (1.5 eq.) in DCM (20 mL) was left to stir for 30 minutes at room temperature. The mixture was cooled in a liquid nitrogen/acetone slurry. The metal dimer $[MCl_2Cp^*]_2$ (1 eq.) in DCM (1 mL) was added dropwise to the mixture. The reaction mixture was stirred overnight at ambient temperature. After this time, the solvent was removed and the crude product purified using silica gel column chromatography (DCM:MeOH (9:1)). The solvent was then removed, the product dissolved in minimal DCM and diethyl ether added to precipitate the product. The solid was collected by filtration. The resulting filtrate was left to stand to allow more of the product to precipitate, which was then collected.

Complex 1



^{1}H NMR (300.077 MHz, $CDCl_3$): (δ, ppm) 1.55 (15H, s, Cp*); 2.29-2.63 (2H, m, H_{12}); 3.43-3.54 (2H, m, H_{11}); 4.03-4.38 (2H, m, H_{13}); 6.31 (1H, d J = 5 Hz, H_3); 6.57-6.70 (2H, m, $H_{16,19}$); 6.80 (1H, d, J = 9 Hz, H_{18}); 7.13 (1H, dd, J = 2 Hz, 9 Hz, H_6); 7.63 (1H, m, H_8); 7.86 (1H, s, H_{14}); 8.05 (1H, d, 9 Hz, H_5); 8.47 (1H, d, J = 5 Hz, H_2).^{13}C{^{1}H} NMR (125.68 MHz, $CDCl_3$): (δ, ppm) 8.74, 27.23, 41.27, 65.66, 85.11, 97.79, 116.89, 119.06, 121.56, 123.16, 123.95, 125.22, 126.44, 131.24, 134.21, 135.18, 147.27, 150.02, 150.36, 160.21, 163.22. IR (ATR): (v_{max}/cm^{-1}) 1616, 1580 cm^{-1}. ESI-MS (HR): m/z 736.1222 ((M+H)$^+$, requires 736.1240)

Complex 2



^{1}H NMR (499.76 MHz, $CDCl_3$): (δ, ppm) 1.52 (15H, s, Cp*), 2.30-2.46 (2H, m, H_{12}); 3.39-3.44 (2H, m, H_{11}); 3.99-4.10 (1H, m, H_{13}); 4.21-4.30 (1H, m, H_{13}); 6.28 (1H, d, J = 5 Hz, H_3); 6.68 (1H, s, H_{16}); 6.83 (1H, d, J = 9 Hz, H_{19}); 6.88 (1H, br s, NH); 7.04 (1H, dd, J = 2 Hz, 9 Hz, H_6); 7.20 (1H, d, J = 8 Hz, H_{18}); 7.70 (1H, m, H_8); 7.84 (1H, s, H_{14}); 8.14 (1H, d, J = 9 Hz, H_5); 8.41 (1H, d, J = 5 Hz, H_2).^{13}C{^{1}H} NMR (125.68 MHz, $CDCl_3$): (δ, ppm) 8.56, 27.66, 40.54, 61.78, 93.05, 97.74, 117.01, 118.02, 121.45, 123.29, 125.01 (2C); 126.78, 131.85, 134.16, 134.90, 147.82, 150.20, 150.46, 163.19, 165.06. IR (ATR): (v_{max}/cm^{-1}) 1615, 1579 cm^{-1}. ESI-MS (HR): m/z 646.0670 ((M+H)$^+$, requires 646.0666)

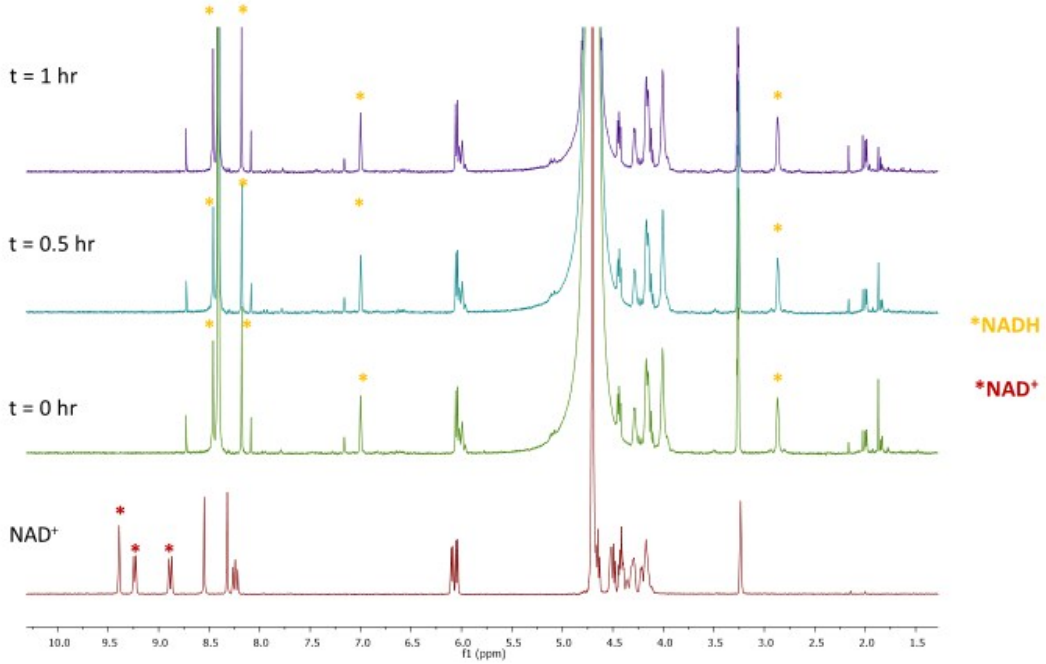


Figure S1. ^{1}H NMR spectra of a mixture of iridium-quinoline complex **1**, NAD^+ and sodium formate in $MeOD/D_2O$ at 310K. * = signals for NAD^+ and * = signals for 1,4-NADH.

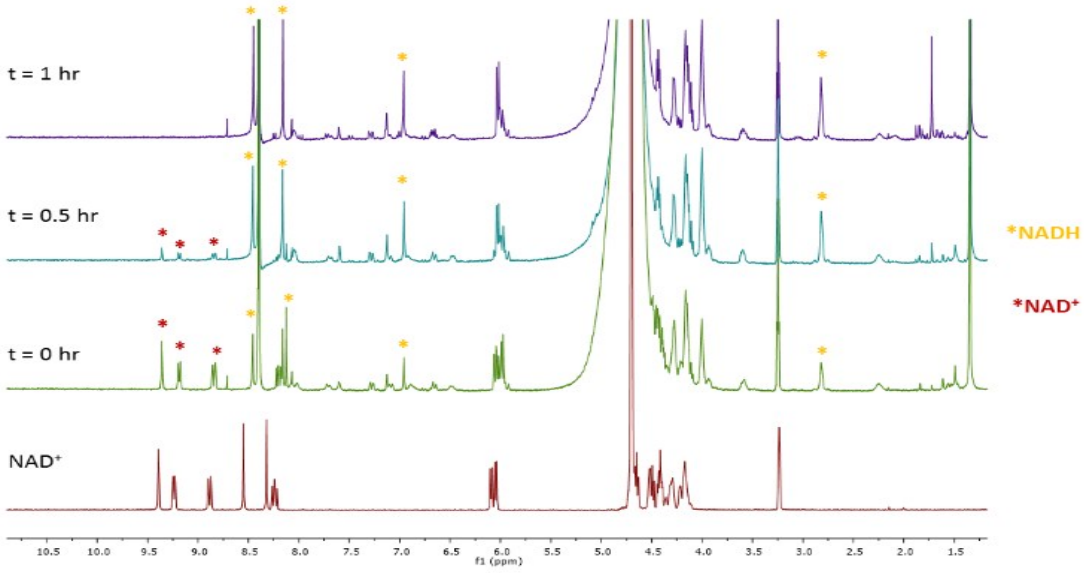


Figure S2. ^{1}H NMR spectra of a mixture of rhodium-quinoline complex **2**, NAD^+ and sodium formate in MeOD/D_2O at 310K. * = signals for NAD^+ and * = signals for 1,4-NADH

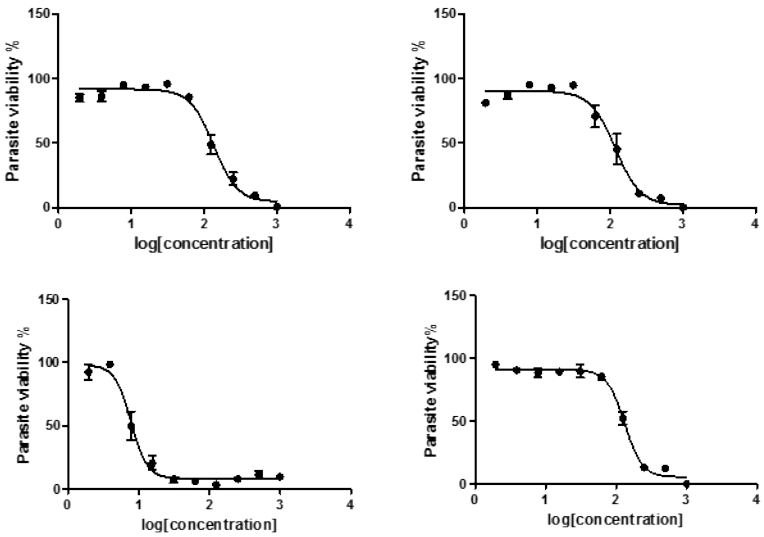


Figure S3. Dose-response curves obtained for complex **1** (top left), complex **2** (top right) and CQ (bottom left) in the NF54 strain, along with CQ (bottom right) in the K1 strain.

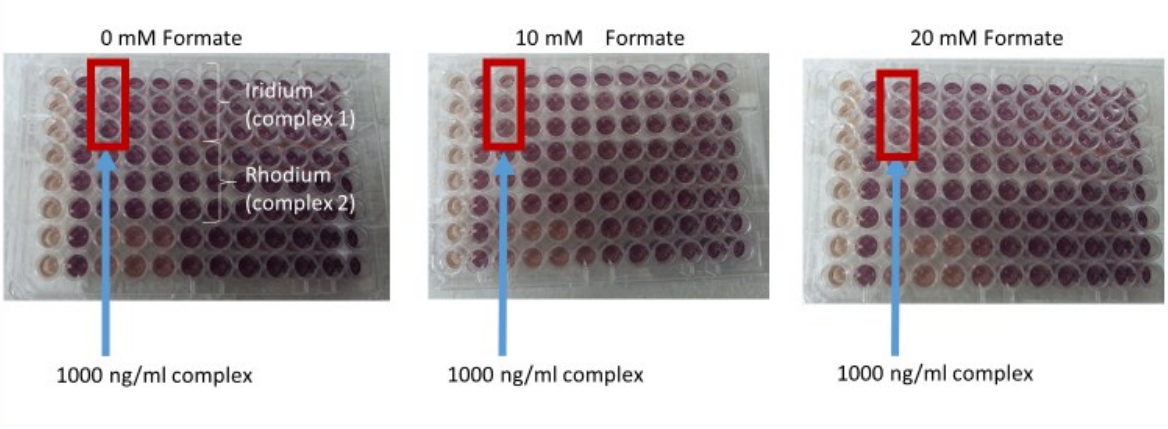


Figure S4. Images taken from parasite viability studies using the pLDH assay, indicating the decrease in parasite viability as the amount of formate increases for 1000 ng/ml of complex **1** in the K1 strain.

Method for qualitative ^{1}H NMR studies for the conversion of NAD$^+$ to NADH by complexes 1 and 2

Approximately 6 mg of the complex, 20 mg of NAD$^+$ and 40 mg of sodium formate were weighed. The NAD$^+$ and sodium formate were each dissolved separately in 600 ul of D_2O. The complex was then suspended in a mixture of 100 ul of the sodium formate solution and 300 ul of the NAD$^+$ solution. To this, 300 ul of MeOD was added. The suspension was then mixed well, any solid matter that remained was filtered off and the pH of the resulting filtrate adjusted to 7.4 using a NaOH solution. The solution was then added to an NMR tube. The ^{1}H NMR spectrum was recorded prior to incubation at 37°C. The tube was then incubated at 37°C and the spectra recorded at the 30-minute mark and at the 60-minute mark.

Cell-free assay to detect transfer hydrogenation of NAD$^+$ to NADH facilitated by PGM complexes

- A 50 ml solution containing NAD$^+$ at pH 7.4 was prepared as follows: Sodium formate (0.31 g, 4.56 mmol); trizma base (0.33g, 2.72 mmol) and NAD$^+$ (5.50 mg, 0.00829 mmol) were dissolved in 25 ml of distilled water. The pH was then adjusted to 7.4 using HCl. The volume was made up to 50 ml with distilled water.
- The tetrazolium solution (NBT) was made up using nitroblue tetrazolium (NBT) (80 mg, 0.0978 mmol) and (4 mg, 0.0120 mmol) phenazine ethosulfate in 50 ml distilled water.

Plate set-up:

6 mM Stocks of each complex were prepared in DMSO (to 1 ml). In a 96-well plate (labelled plate 1), 200 ul of the drug solution was added to column 3 in triplicate. 100 ul DMSO was added to each well from column 4 to column 12. The compounds were serially diluted giving 10 concentrations. 75 ul of the solution from each well was transferred into a second 96-well plate (plate 2) into the corresponding well (eg. 75 ul C1 of plate 1 into C1 of plate 2 etc). 100 ul of the NAD^+ solution was then added to each well. In column 1, 75 ul DMSO and 100 ul NAD^+ solution were added to each well only. In column 2, 175 ul DMSO was added to each well only. The plate was read at 600 nm to account for the absorbance of the compounds (pre-read). The plate was then covered with a foil plate cover to prevent evaporation and was incubated for 6-8 hours at 37°C. After this time, 25 ul of the NBT solution was added and the plate incubated for a further 20 minutes (if required) to aid plate developing. After this time, the absorbance was read at 600 nm again, the data from the pre-read accounted for and the data plotted using Prism v5 at the various concentrations (2250 uM to 4 uM).

Antiplasmodial studies against Plasmodium falciparum and formate studies

The samples were tested in triplicate on one experiment against the chloroquine-sensitive NF54 strain and chloroquine-resistant K1 of *P. falciparum*. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.[2]The antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase (pLDH) assay using a modified method described by Makler et al.[3] The samples were prepared as a 2 mg/ml stock solution using DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -20°C. Further dilutions were prepared on the day of the experiment. Chloroquine was used as the reference drug in all experiments. A full dose-response measurement was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} value). The samples were tested at a starting concentration of 1000 ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 2 ng/ml. The same dilution technique was used for all samples. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability. The IC_{50} values were obtained using a nonlinear dose-response curve fitting analysis via Graph Pad Prism v.5.0 software.

For the formate experiments in the K1 strain, medium containing 0 mM, 10 mM, 20 mM and 40 mM sodium formate was used instead of complete medium (After addition of the medium to the 96-well plates, the final formate concentrations are 0 mM, 5 mM, 10 mM and 20 mM, respectively). The same procedure was followed as the pLDH assay described above.

^{1}H NMR study into the long-term stability of complexes 1 and 2 in biological medium

Approximately 8 mg of the complex was weighed and dissolved in 400 µL DMSO-d_6. To this, 300 µL of the biological medium* was added. The suspension was then sonicated and any solid matter that remained was filtered off prior to addition to an NMR tube. The initial ^{1}H NMR spectrum at 0 hours was recorded prior to incubation at 37 °C. The tube was then incubated at 37°C and the spectra recorded at the 1 hour mark and then again at the 24 hour and 48 hour mark.

*Biological medium used for parasite culture and for the pLDH assay. The biological medium is made up as follows:

- 10.4 g/L RPMI 1640 with glutamine but without $NaHCO_3$, 4 g/L glucose, 6 g/L Hepes (buffer), 0.088 g/L Hypoxanthine, 5 g/L albumax, and 1.2 mL/L gentamycin
- Medium is pre-filtered (0.45 µM) then filter sterilised (0.22 µM)
- 5 % Bicarbonate is added before use (17 mL per 400 mL medium)
- The complete medium is stored at 4 °C

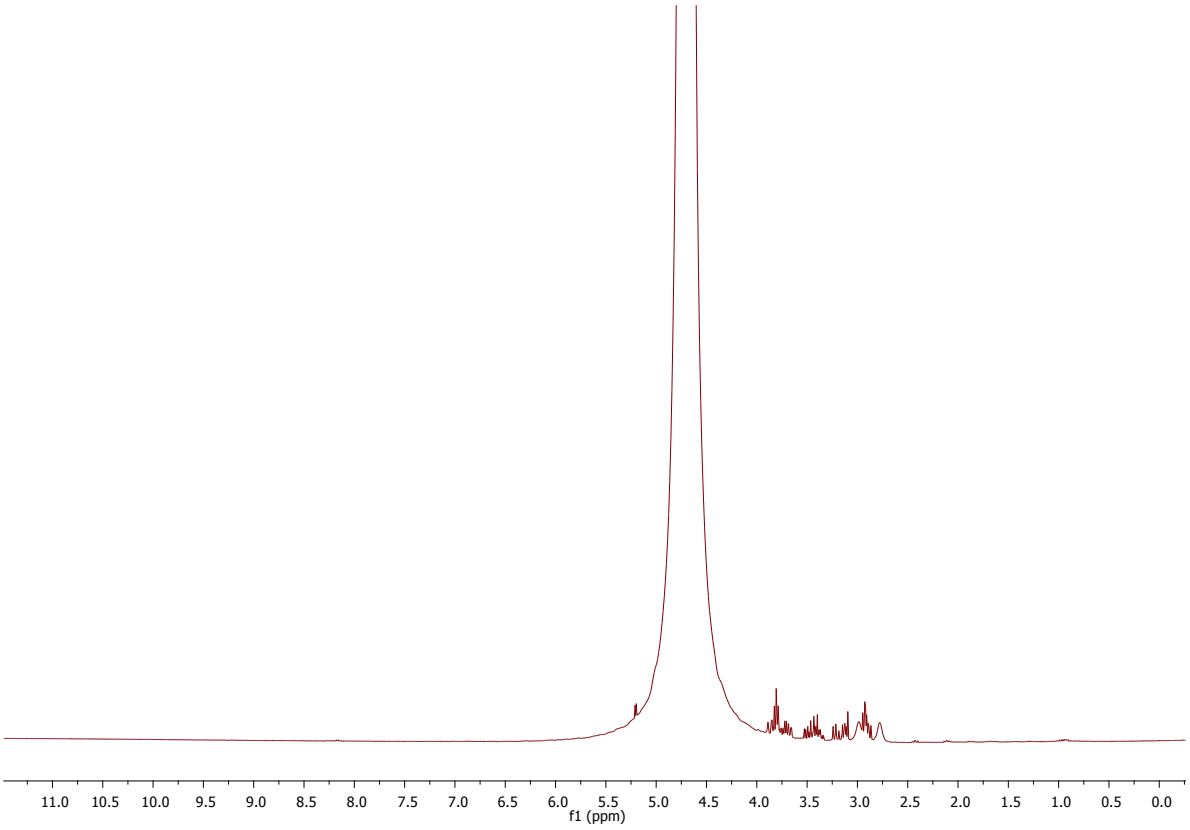


Figure S5. ^{1}H NMR spectrum of the biological medium in D_2O.

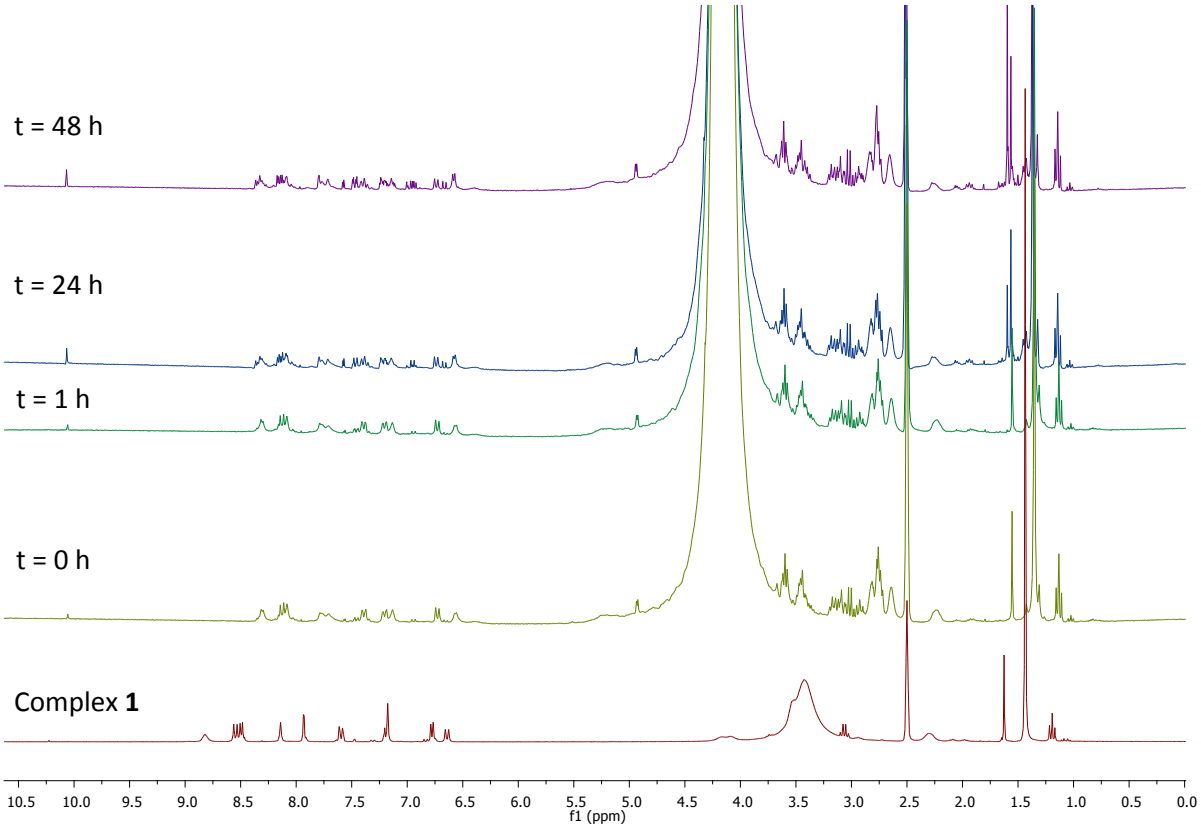


Figure S6. ^{1}H NMR spectra of a mixture of Iridium quinoline complex **1** and biological medium in DMSO-d_6 at 37 °C over 48 hours.

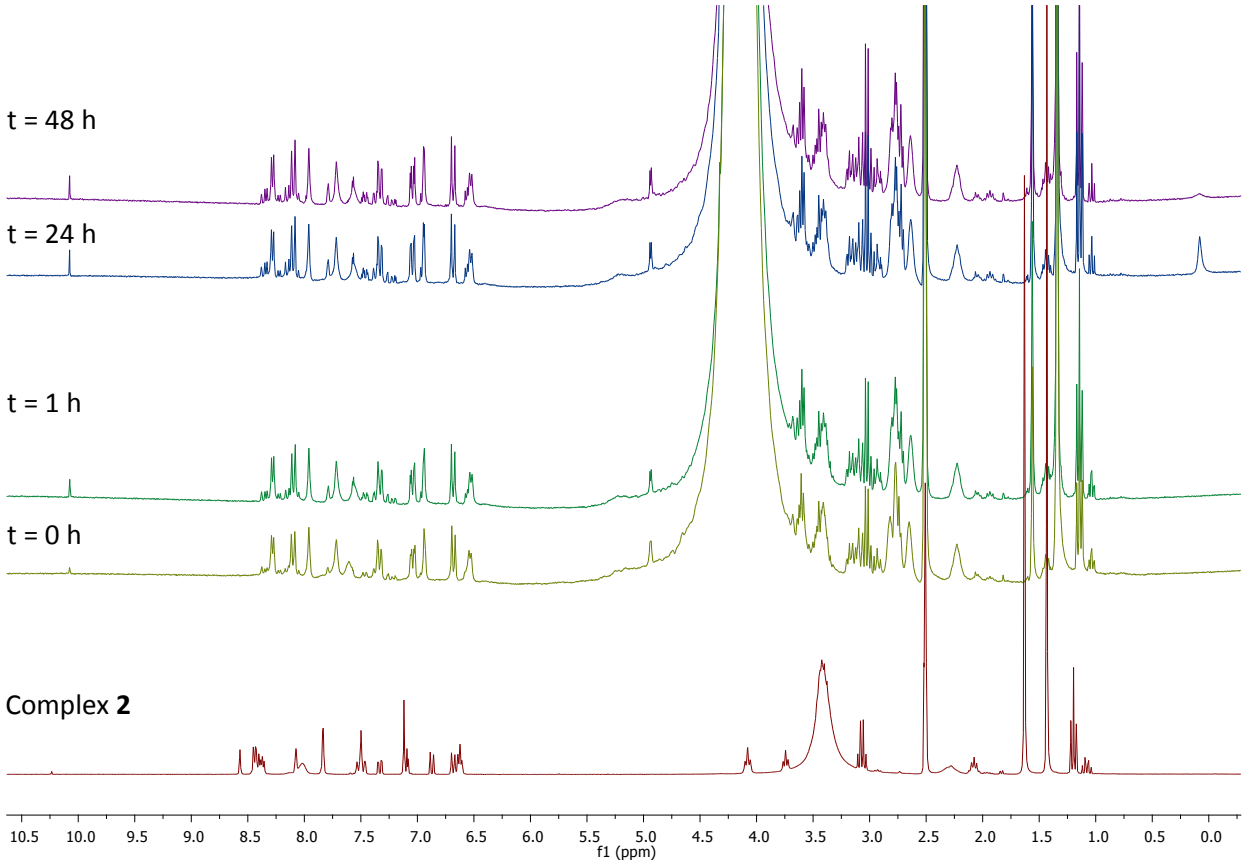


Figure S7. ^{1}H NMR spectra of a mixture of Rhodium quinoline complex **2** and biological medium in DMSO-d_6 at 37 °C over 48 hours.

References

(1) Ekengard, E.; Kumar, K.; Fogeron, T.; De Kock, C., Smith, P. J., Haukka, M.; Monari, M.; Nordlander, E., *Dalton Trans.* **2016**, 45, 3905-317.

(2) Trager, W; Jensen, J. B., *Science* **1976**, *193*, 673-675.

(3) Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. J., *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739-741.