Electronic Supplementary Material (ESI) for Dalton Transactions.

Synthesis, Structure, Properties, and Cytotoxicity of a (Quinoline)$RuCp^+$ Complex

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Supporting Information

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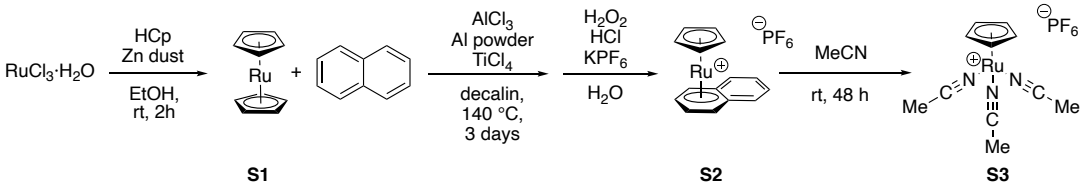
Modified Synthesis of $\{RuCp(NCMe)_3\}^+PF_6^-$



S1 **S2** **S3**

The procedure was adapted from a literature procedure.[1] Ruthenium trichloride hydrate (1g, 4.8 mmol) was dissolved in absolute ethanol (20 mL) in a 250 mL Schlenk flask with a stir bar. Freshly distilled cyclopentadiene (5 mL, 60 mmol) was added to the stirring solution. Then, zinc dust (3.1 g, 48 mmol) was added in 5 portions over 10 mins. The reaction mixture was stirred at room temperature for 2 h. The mixture was filtered on celite through a frit, and the solids were washed with 50 mL of hexanes. The filtrate was evaporated, and the resulting solid extracted with 50 mL of hexanes. The mixture in hexanes was passed through a plug of silica gel, and the plug was washed with hexanes until the filtrate was almost colorless. The solution was evaporated to afford ruthenocene (**S1**) as a grey solid (405 mg, 36%). ^{1}H NMR ($CDCl_3$, 500 MHz, 21 °C): δ 4.55 (s, 10H). All characterization data was consistent with reported values.[1]

In a glovebox, a 100 mL Schlenk tube was charged with **S1** (400 mg, 1.7 mmol), naphthalene (2.2g, 17 mmol), $AlCl_3$ (230 mg, 1.7 mmol), Al powder (23 mg, 0.9 mmol), dry decalin (20 mL), and a stir bar. To the mixture was added $TiCl_4$ (95 μL, 0.9 mmol) dropwise. The Schlenk tube was sealed, taken out from

glovebox, and heated in a 140 °C silicon oil bath for 3 d. The mixture turned dark green over the course of the reaction. Then, the Schlenk tube was cooled to room temperature and a premade solution of water (30 g), 32% hydrogen peroxide (2.5 mL), and 30% HCl (2.5 mL) was added with stirring. The mixture turned red after addition. The aqueous layer was separated, and the organic layer was washed with water (3 × 30 mL). To the combined aqueous layers was added KPF_6 (460 mg, 2.5 mmol) resulting in the formation of a precipitate. The precipitate was extracted with dichloromethane (40 mL). Evaporation of the volatiles afforded the product $\{RuCp(\eta^6\text{-naphthalene})\}^+PF_6^-$ (**S2**) as an orange solid (304 mg, 40%). ^1H NMR (acetone-d_3, 500 MHz, 21 °C): δ 7.86 (dd, J = 6.6, 3.2 Hz, 2H), 7.67 (dd, J = 6.7, 3.2 Hz, 2H), 7.18 (dd, J = 4.3, 2.5 Hz, 2H), 6.41 (dd, J = 4.4, 2.4 Hz, 2H), 5.09 (s, 5H). Characterization data was consistent with reported values.[1]

In a glovebox, a 20 mL glass vial was charged with a stir bar, **S2** (300 mg, 0.7 mmol), acetonitrile (5 mL), and hexanes (5 mL). The mixture was stirred at room temperature for 24 h. The hexanes layer was decanted. Another portion of hexanes was added, and the mixture was stirred for 24 h. After decanting the hexanes layer, the acetonitrile solvent was removed in vacuo to afford $\{RuCp(NCMe)_3\}^+PF_6^-$ (**S3**) as a yellow solid (280 mg, 95%). ^1H NMR ($CDCl_3$, 500 MHz, 21 °C): δ 4.21 (s, 5H), 2.41 (s, 9H). Characterization data was consistent with reported values.[1]

Stability of 1 in DMSO-d_6/D_2O

In a 20 mL glass vial, **1** (1.2 mg) was dissolved in DMSO-d_6 (60 μL), which was then diluted with D_2O (540 μL). The solution was mixed via pipet and transferred to an NMR tube. Proton NMR spectra were taken after 0, 24, 48, and 72 h. No appreciable change was observed in the spectra over this time period.

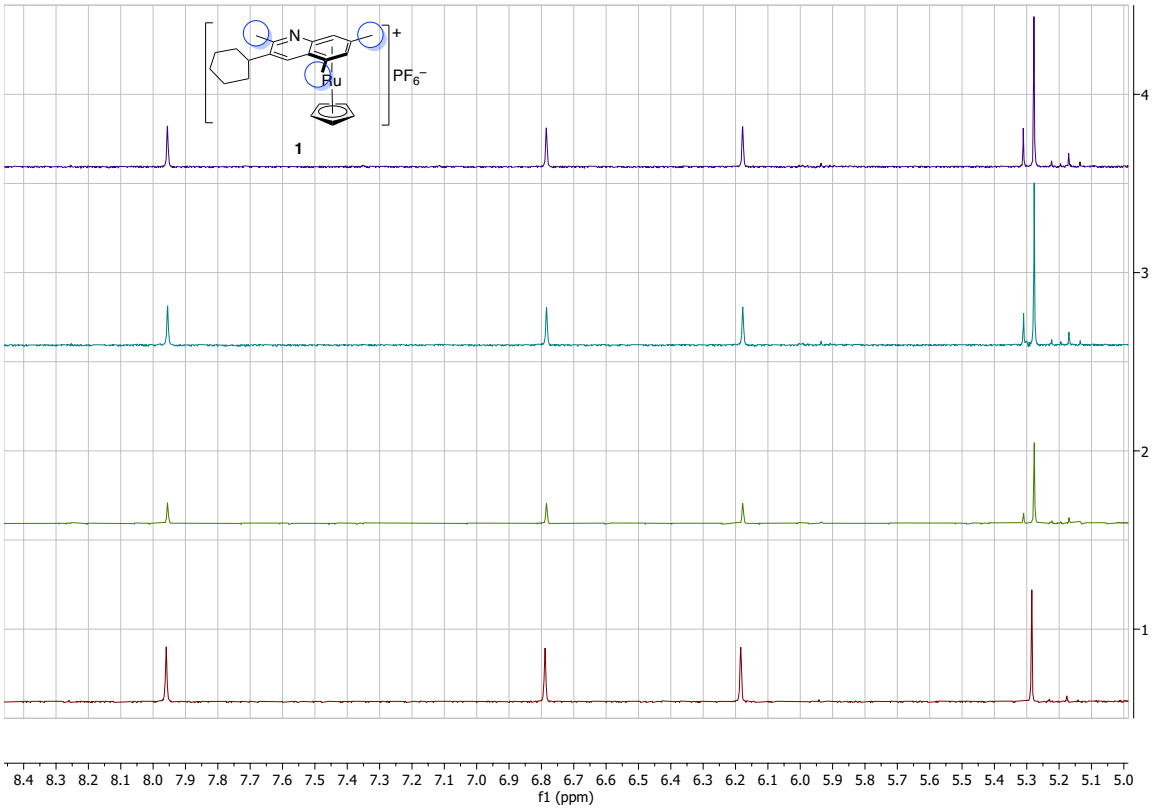


Fig. 1. From bottom to top, ^{1}H NMR spectra of **1** (aromatic region) in 10% DMSO-d_6/D_2O after 0, 24, 48, and 72 h.

Stability of 1 in DMSO-d_6

In a 20 mL glass vial, **1** (1.3 mg) was dissolved in DMSO-d_6 (600 µL). The solution was mixed via pipet and transferred to an NMR tube. Proton NMR spectra were taken d_6 after 0, 48, and 72 h.

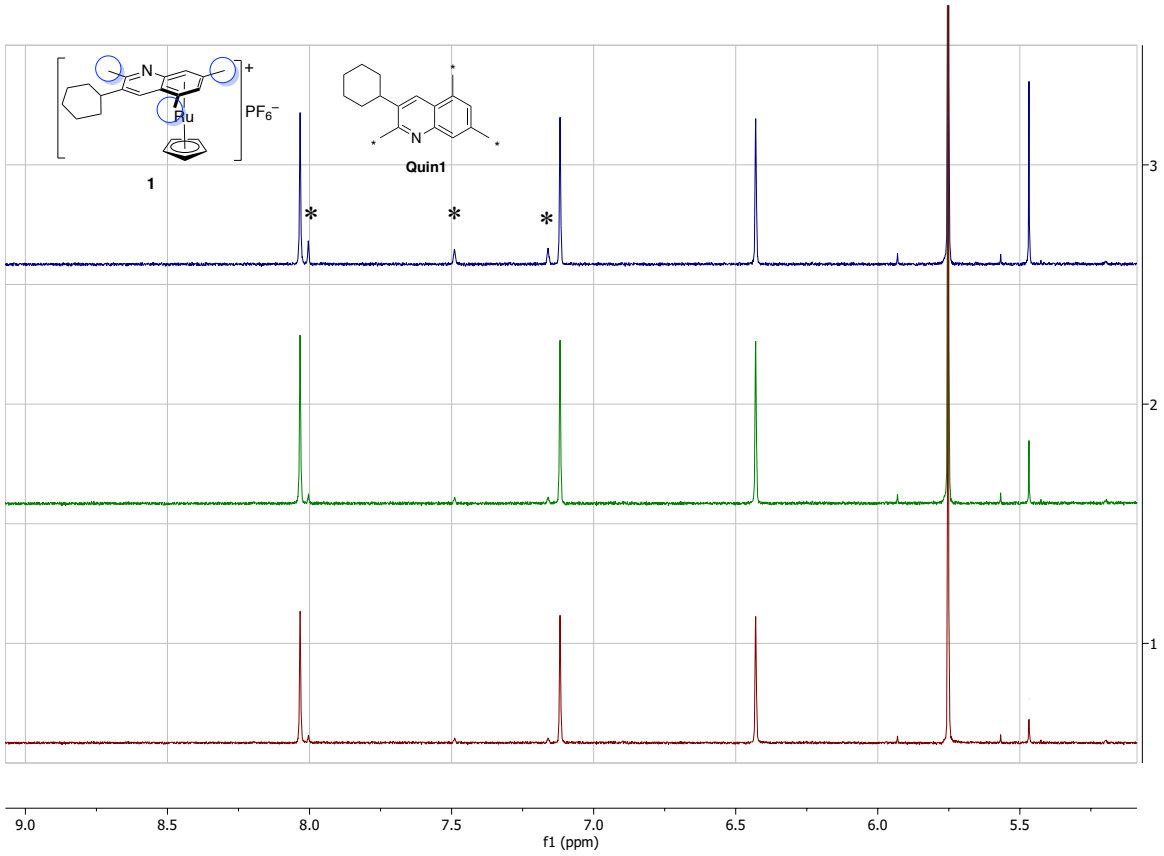


Fig. 2. From bottom to top, ^{1}H NMR spectra of **1** (aromatic region) in DMSO-d_6 after 0, 48, and 72 h. Asterisk represents dissociation product Quin 1.

Stability of 2 in DMSO-d_6/D_2O

In a 20 mL glass vial, **2** (3.8 mg) was dissolved in DMSO-d_6 (60 µL), which was then diluted with D_2O (540 µL). The solution was mixed via pipet and transferred to an NMR tube. Proton NMR spectra were taken after 0, 24, 48, and 72 h. No appreciable change was observed in the spectra over this time period.

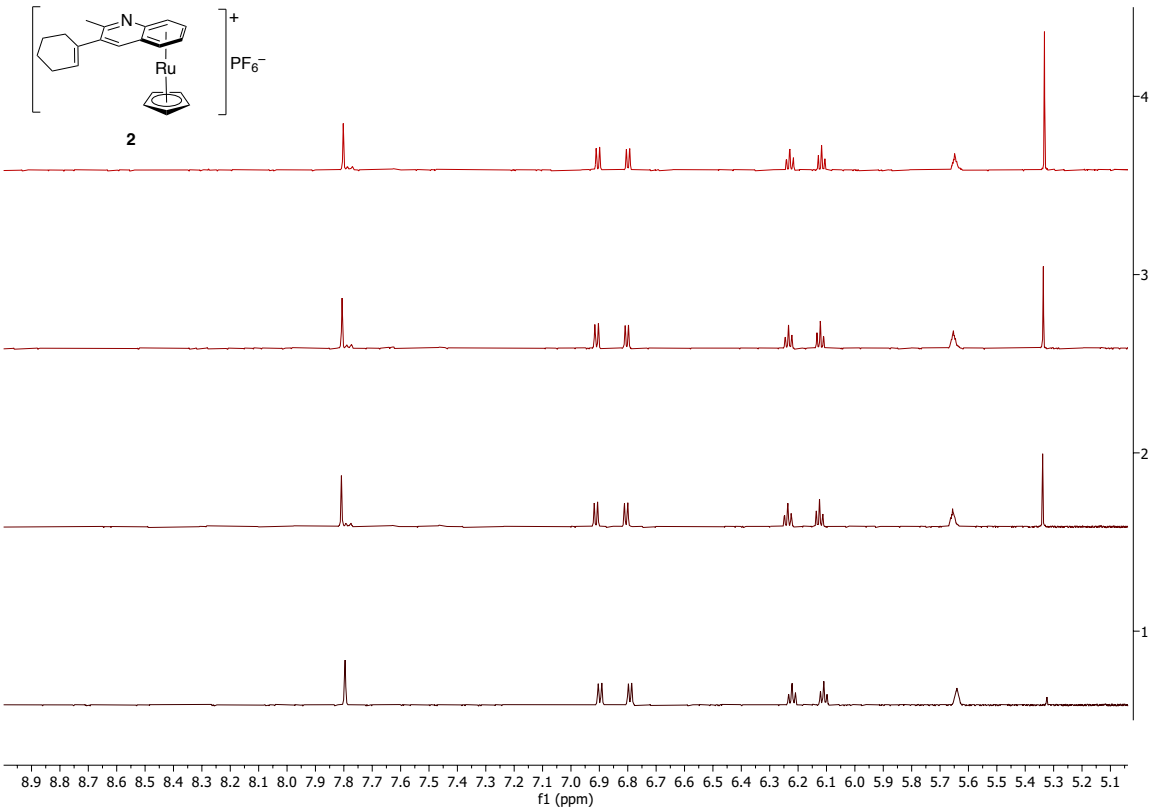
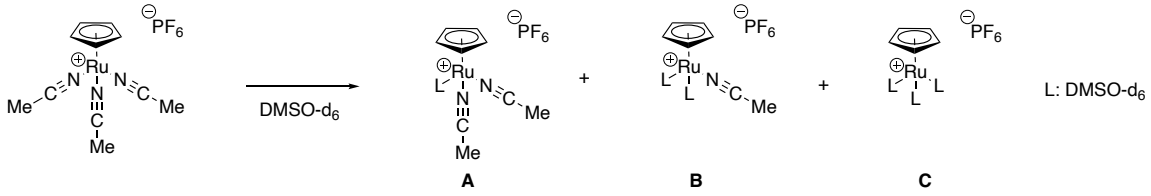


Fig. 3. From bottom to top, ^{1}H NMR spectra (zoom in 5-9ppm) of **2** in 10% DMSO-d_6/D_2O after 0, 24, 48, and 72 h.

Stability of {RuCp(NCMe)$_3$}$^+$ PF$_6^-$ in DMSO-d$_6$



In a 20 mL glass vial, {RuCp(NCMe)$_3$}$^+$ PF$_6^-$ (1.0 mg) was dissolved in DMSO-d_6 (600 µL). The solution was mixed via pipet and transferred to an NMR tube. Proton NMR spectra were taken periodically. According to NMR integration, chemical shifts of products were assigned as following: **A**: ^{1}H NMR (DMSO-d_6, 500 MHz, 21 °C): δ 4.76 (s, 5H), 2.54 (s, 6H). **B**: ^{1}H NMR (DMSO-d_6, 500 MHz, 21 °C): δ 5.13 (s, 5H), 2.58 (s, 3H). **C**: ^{1}H NMR (DMSO-d_6, 500 MHz, 21 °C): δ 5.47 (s, 5H).

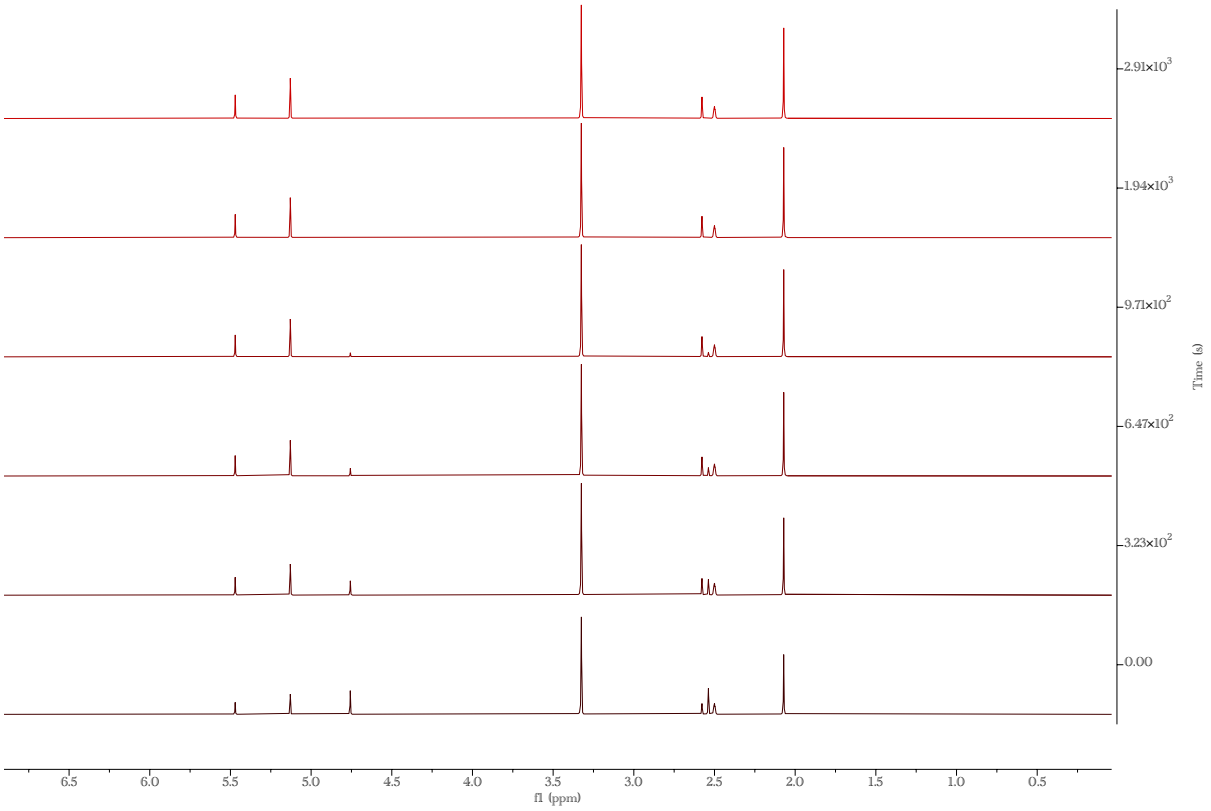


Fig. 4. ^{1}H NMR spectra of $\{RuCp(NCMe)_3\}^+$ PF_6^- after dissolved in DMSO-d_6 at different times (Y axis).

Stability of $\{RuCp(NCMe)_3\}^+$ PF_6^- in DMSO-d_6/D_2O

In a 20 mL glass vial, $\{RuCp(NCMe)_3\}^+$ PF_6^- (1.1 mg) was dissolved in pre-mixed 10% DMSO-d_6/D_2O (600 µL). The solution was mixed via pipet and transferred to an NMR tube. A, B, and C were observed at 0 h. B and C were observed at time 72 h with A having been converted.

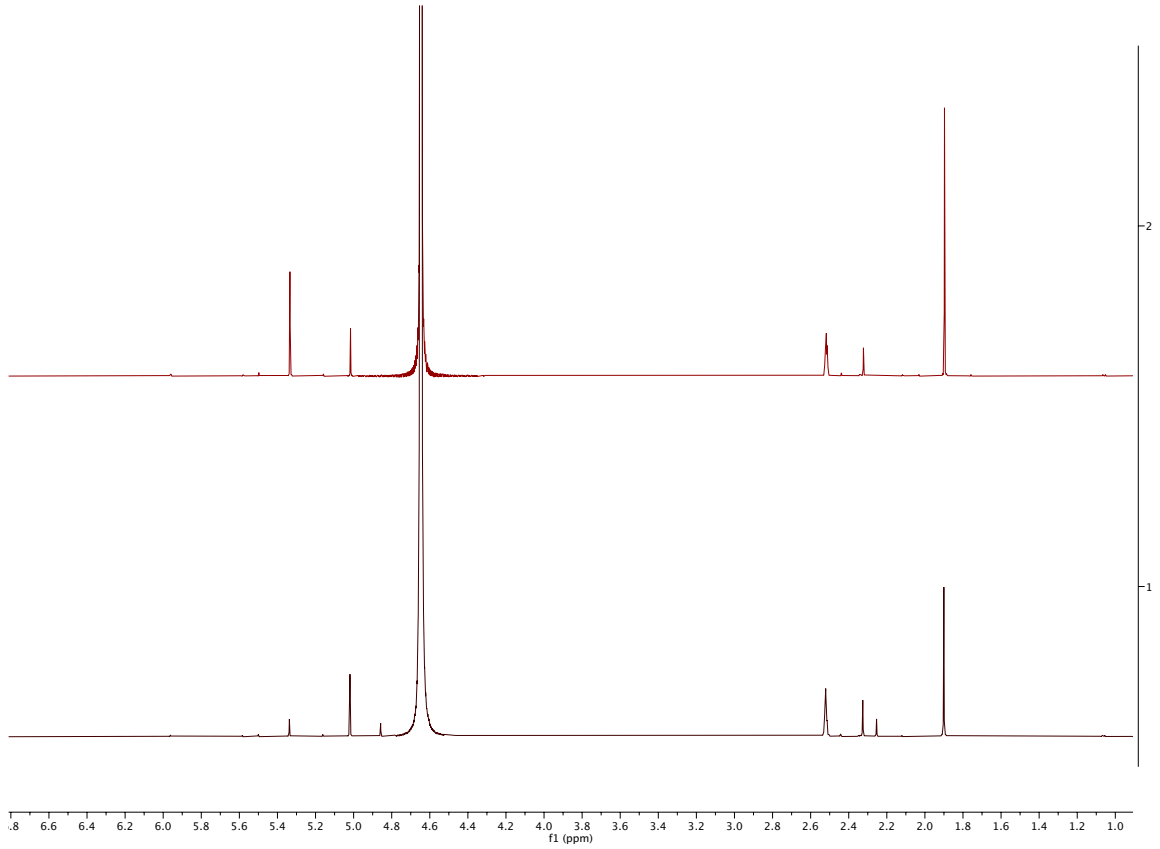


Fig. 5. From bottom to top, ^{1}H NMR spectra of $\{RuCp(NCMe)_3\}^+$ PF_6^- in 10% DMSO-d_6/D_2O after 0 and 72 h.

***Measurement of the kinetics of quinoline replacement by DMSO-d_6*:**

In an inert atmosphere glove box, a 20 mL glass vial was loaded **2** (3.1 mg, 5.8 μmol), ferrocene (3.7 mg, 19.9 μmol), and DMSO-d_6 (600 μL). The solution was mixed by drawing in and out of a pipet until homogeneous. The reaction was transferred into an NMR tube, which was carefully capped and sealed with electrical tape. Then, the NMR tube was removed from the glovebox and placed in room temperature a silicon oil bath. Periodically, an NMR tube was removed from the oil bath, and ^{1}H NMR spectra were measured. The relative concentration of **2** vs ferrocene was monitored as a function of time. The fits of the exponential decay of **2** were done using the scientific program KaleidaGraph v5.0.1. The expression used to fit the data was $Y_t = Y_\infty + (Y_0 - Y_\infty)e^{-k_{obs}t}$, where Y = concentration at time t (Y_t), infinity (Y_∞), or at the start of the reaction (Y_0).[2] In the figure, m1=Y_∞, m2=$Y_0 - Y_\infty$, m3=k_{obs}.

An example of a plot of concentration vs time and its fit is shown in Fig. 8. The disappearance of **2** has a rate constant of 4×10^{-4} s^{-1}, while the appearance of $CpRu(DMSO\text{-}d_6)_3^+$ has a rate constant of 3.8×10^{-4} s^{-1}. The difference in the starting concentration of **2** versus the ending concentration of $CpRu(DMSO\text{-}d_6)_3^+$ likely reflects the creation of some byproduct but none was observed by ^1H NMR spectroscopy.

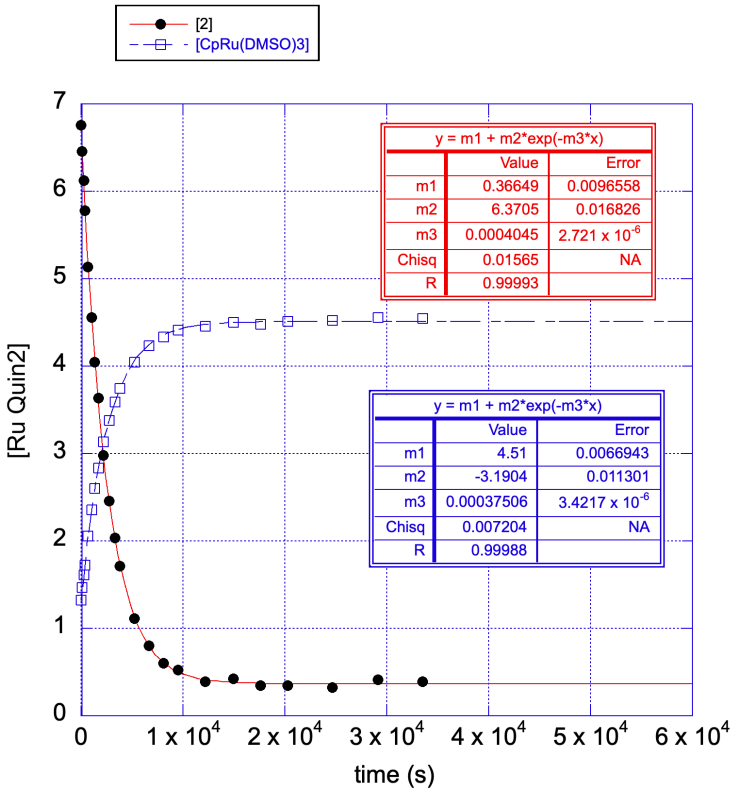
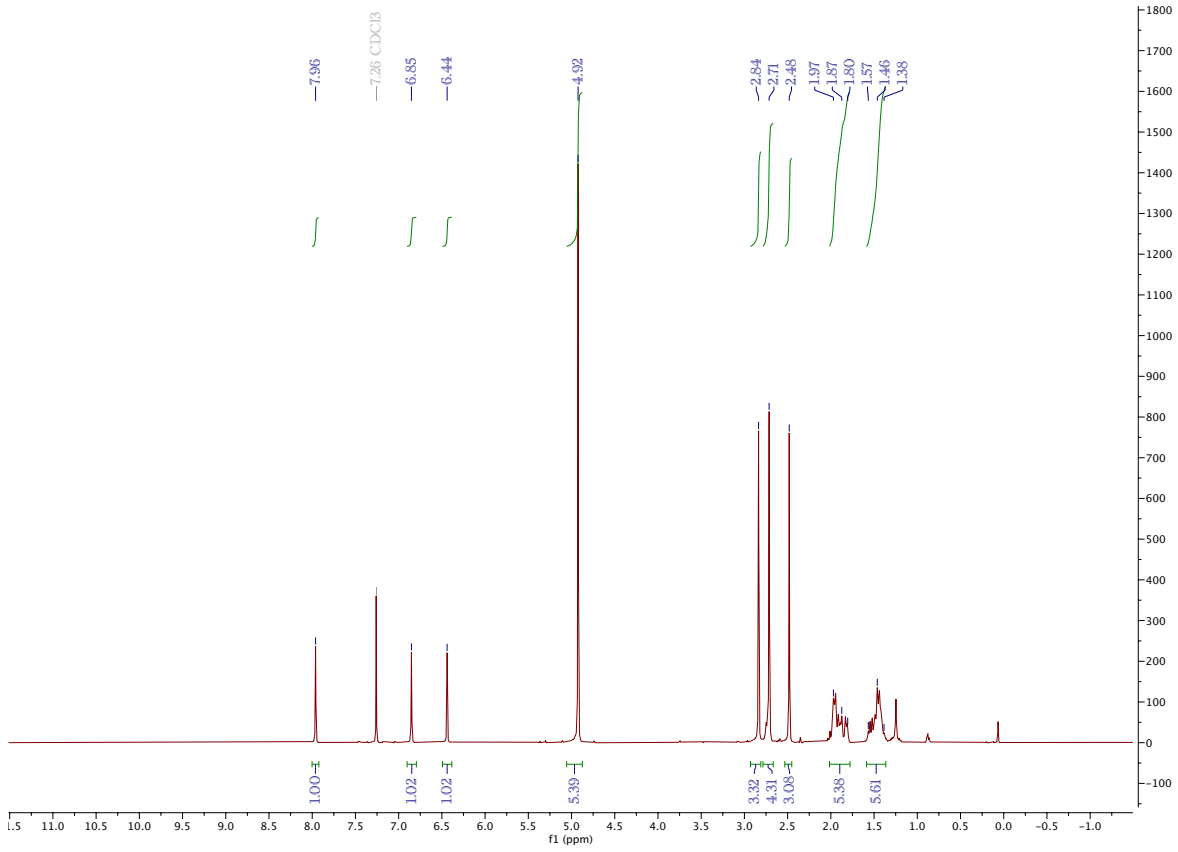


Fig. 6. Fit of [**2**] (block dots) as a function of time (red line) and fit of [$CpRu(DMSO\text{-}d_6)_3^+$] (blue squares) as a function of time (blue line). Concentrations are relative to ferrocene internal standard. See text above for details on the fit.

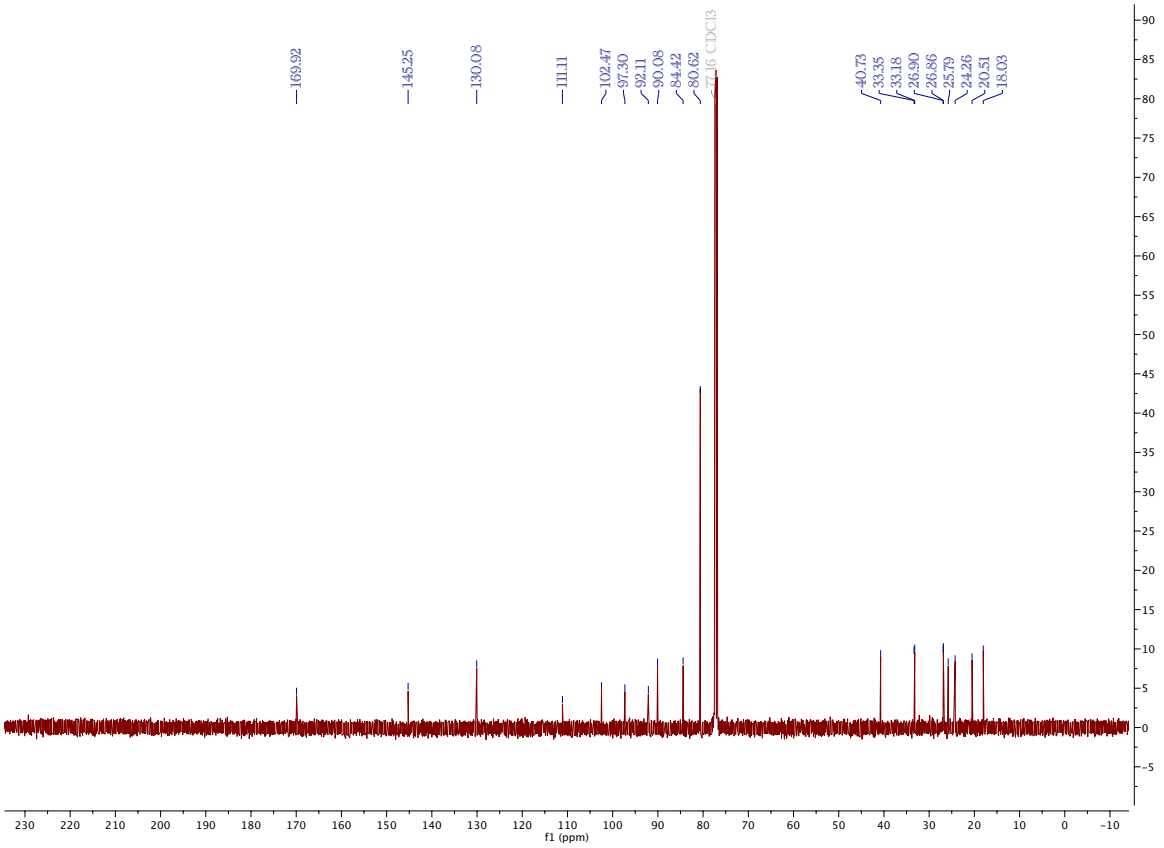
NMR Spectra

(η^6(C6)-3-cyclohexyl-2,5,7-trimethylquinoline)(η^5-cyclopentadienyl)ruthenium(II) hexafluorophosphate ***(1)***

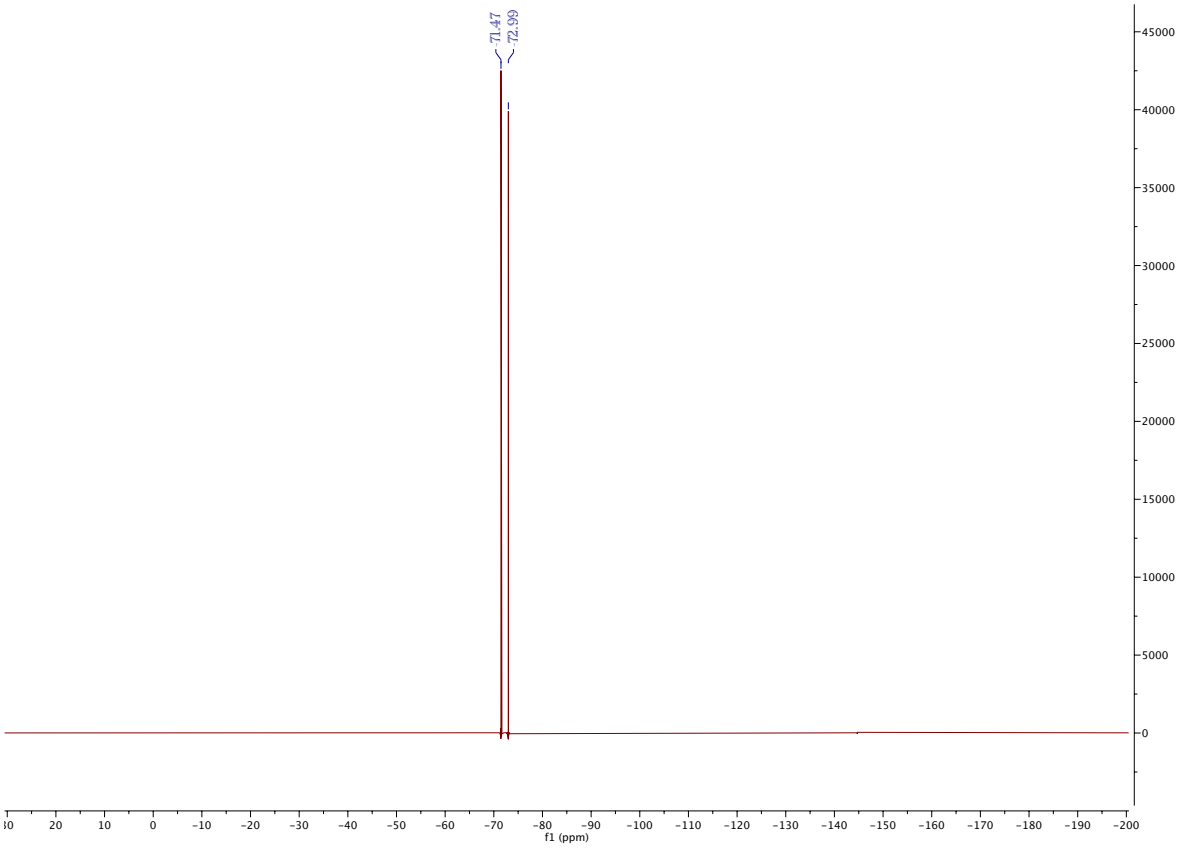
^{1}H NMR



^{13}C NMR


169.92
145.25
130.08
111.11
102.47
97.30
92.11
90.08
84.42
80.62
77.16 CDCl3
40.73
33.35
33.18
26.90
26.86
25.79
24.26
20.51
18.03
f1 (ppm)

^{19}F NMR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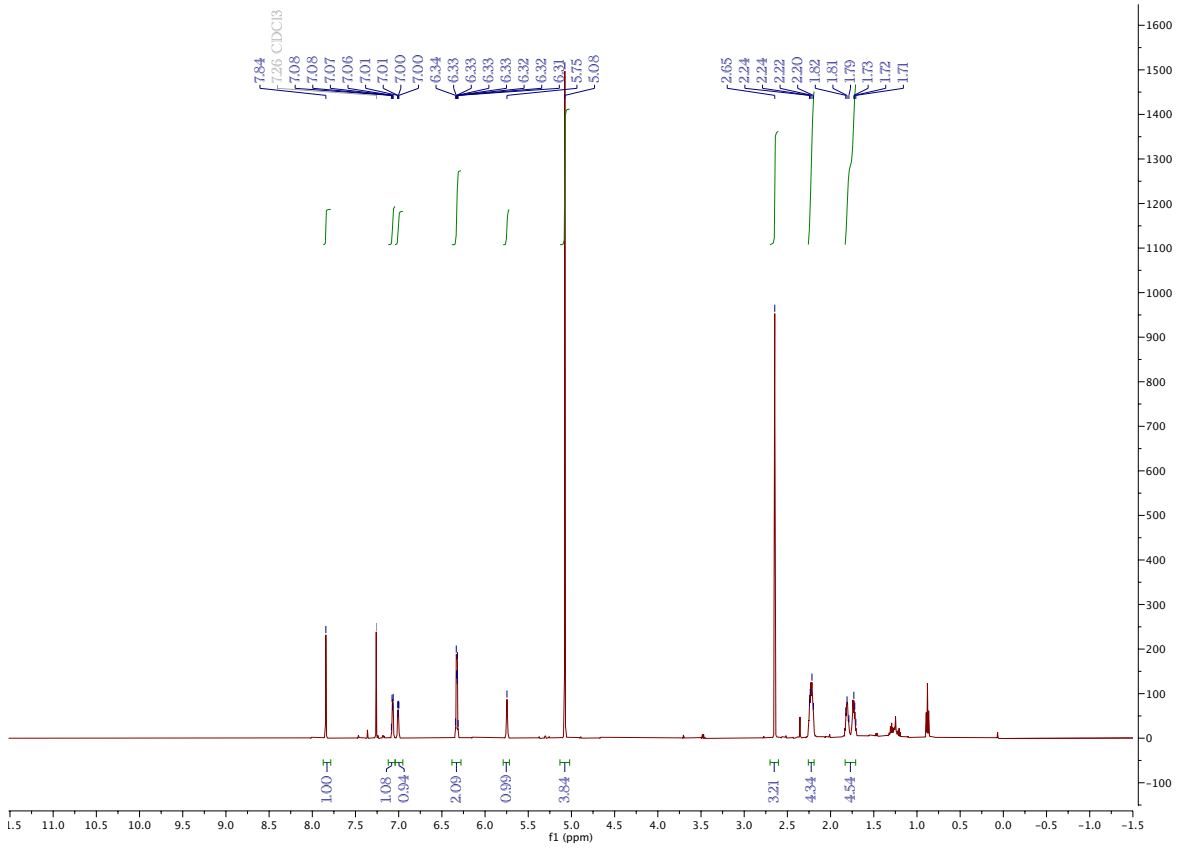
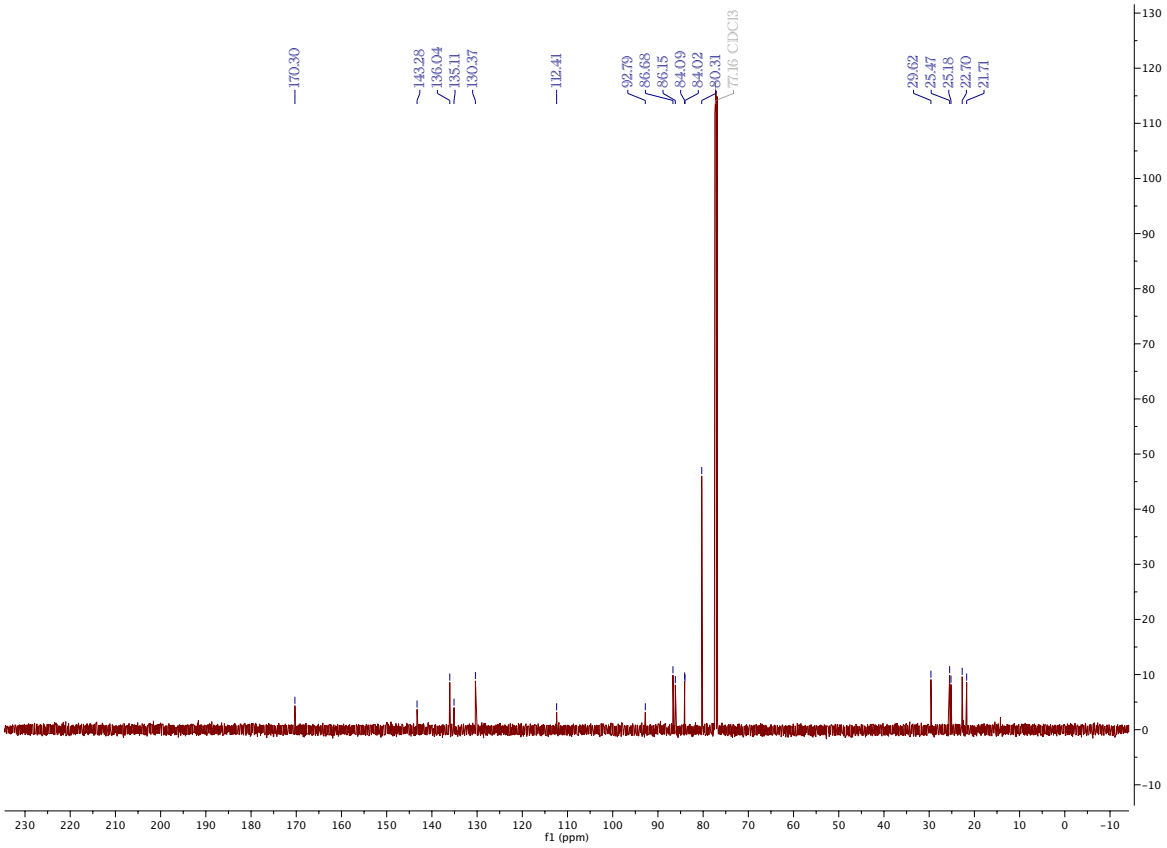
-72.99
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

*(η^6(C6)-3-(cyclohex-1-en-1-yl)-2-methylquinoline)(η^5-cyclopentadienyl)ruthenium(II) hexafluorophosphate (**2**)*

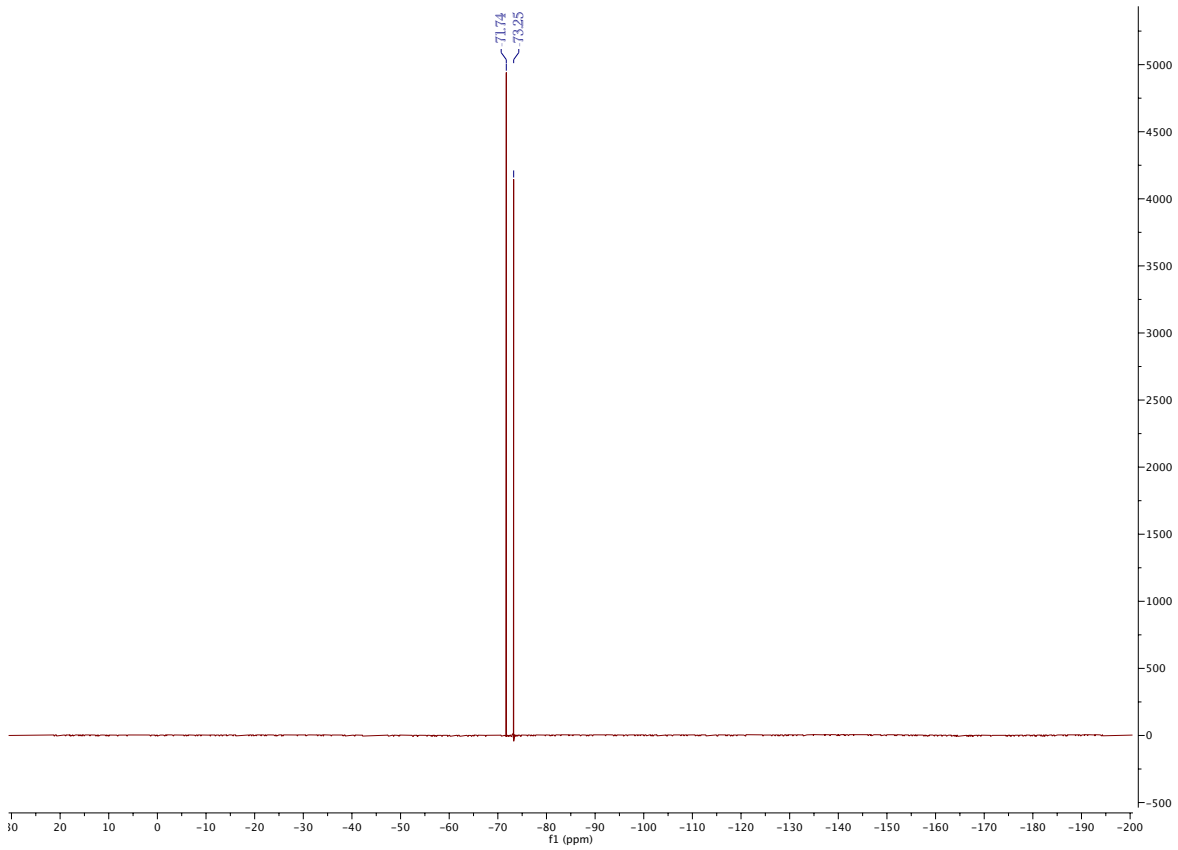
^{1}H NMR



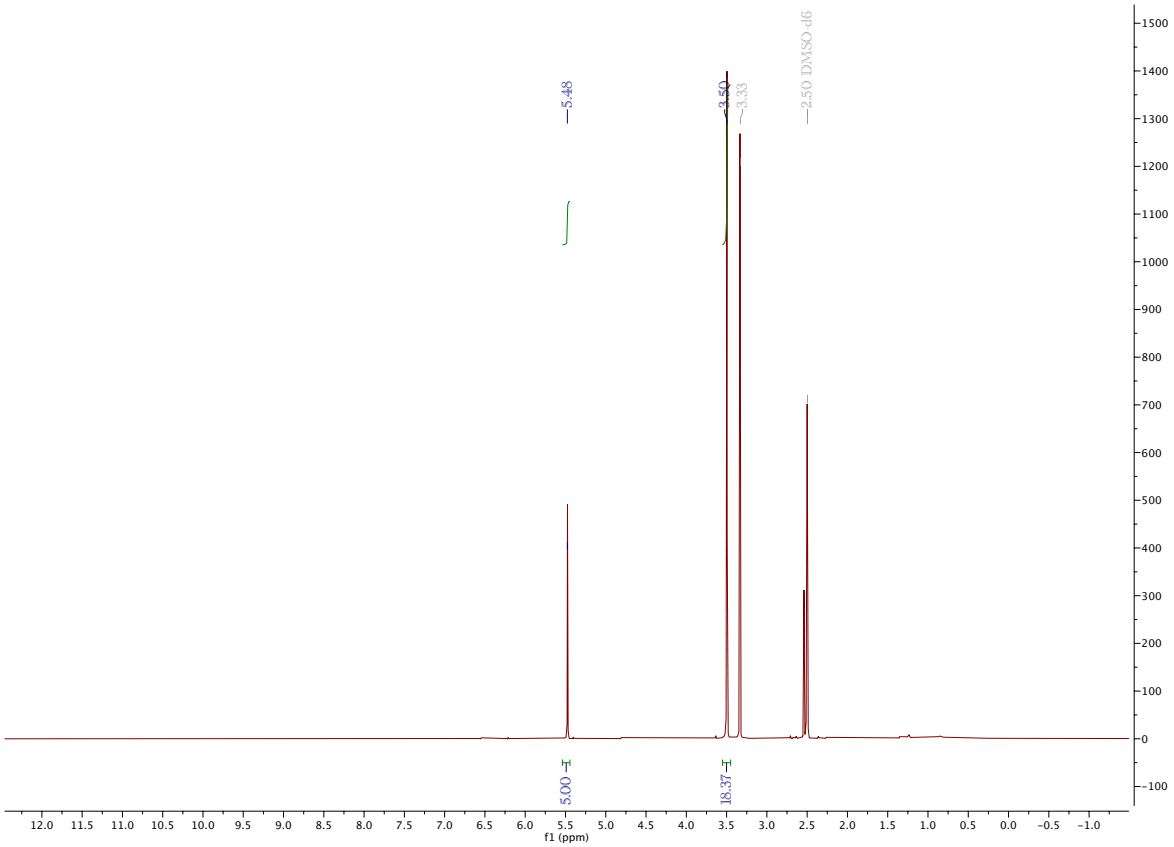
^{13}C NMR


170.30
143.28
136.04
135.11
130.37
112.41
92.79
86.68
86.15
84.09
84.02
80.31
77.16 CDCl3
29.62
25.47
25.18
22.70
21.71
f1 (ppm)

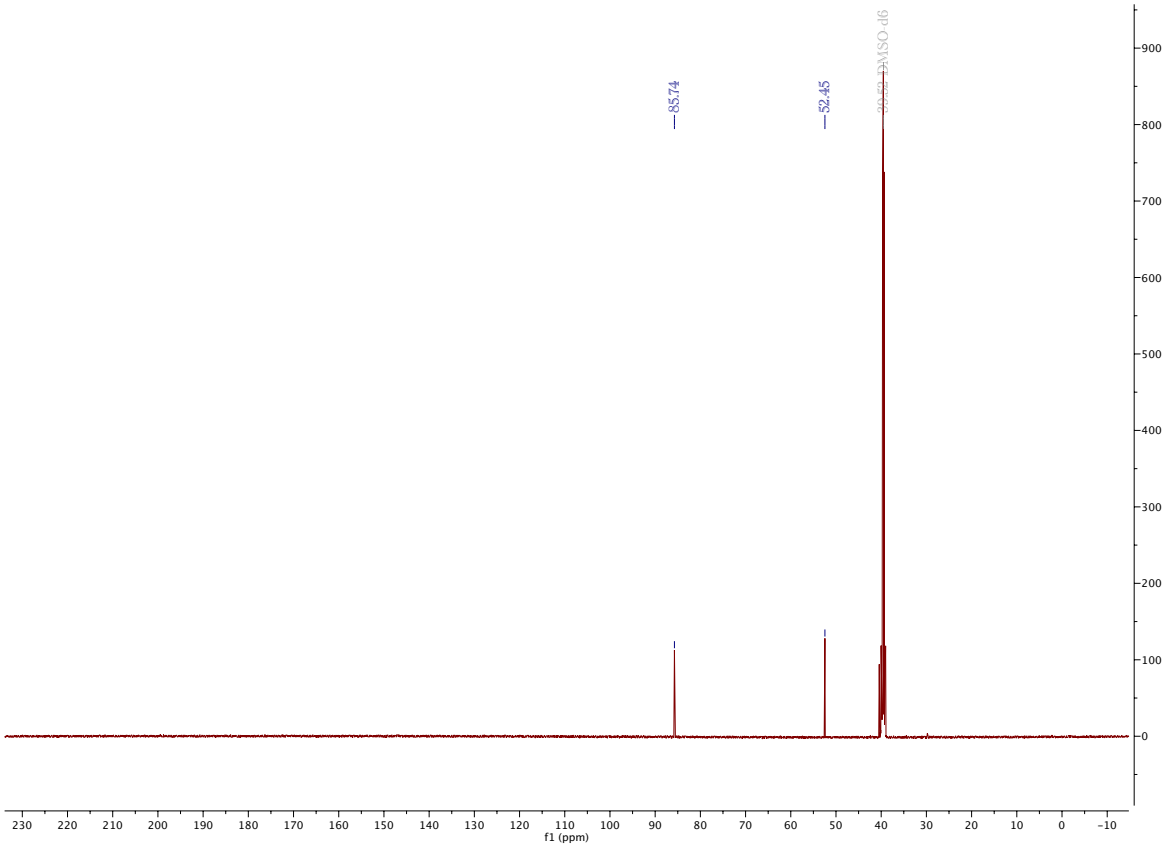
^{19}F NMR


71.74
73.25
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
f1 (ppm)

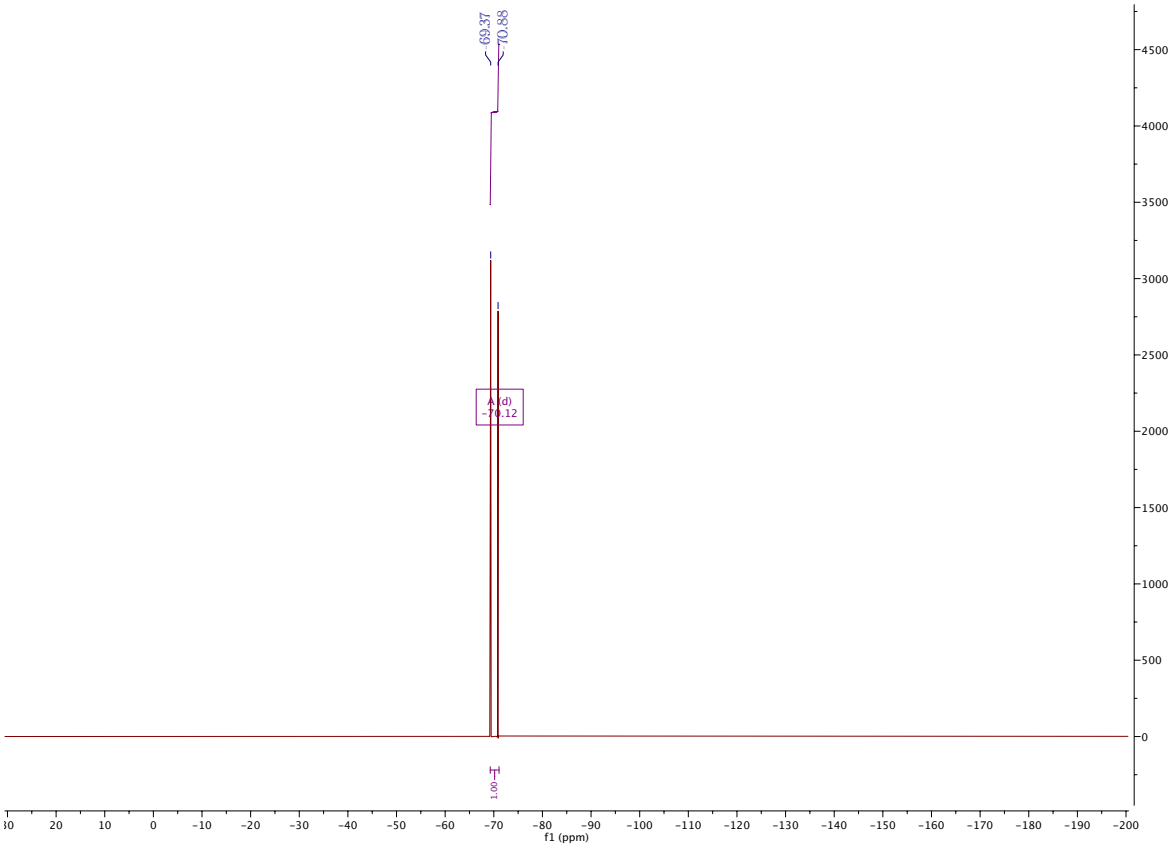
tris(dimethyl sulfoxide)(η^5-cyclopentadienyl)ruthenium(II) hexafluorophosphate
^{1}H NMR



^{13}C NMR


85.74
52.45
39.52 DMSO-d6
900
800
700
600
500
400
300
200
100
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{19}F NMR


69.37
70.88
A (d)
-70.12
1.00
4500
4000
3500
3000
2500
2000
1500
1000
500
0
30 20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200
f1 (ppm)

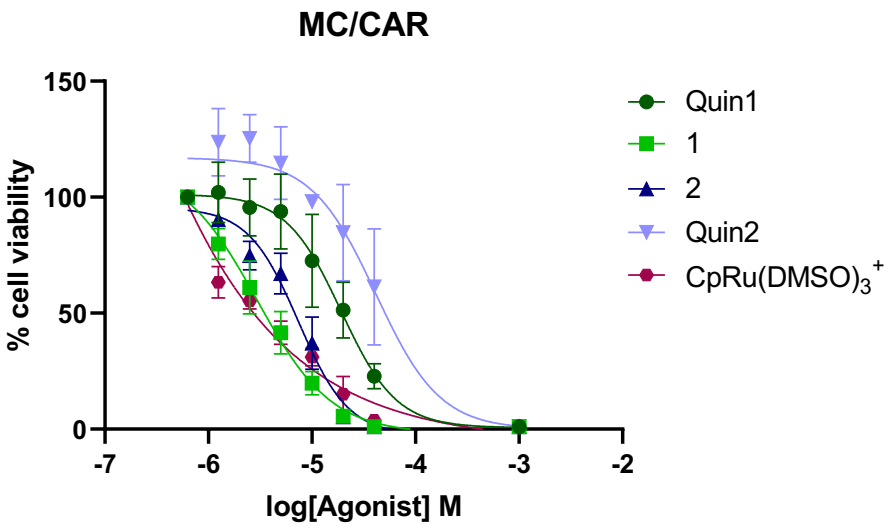
Cytotoxicity – MC/CAR cells

MC/CAR cells (5,000 cells per well) were seeded in a 96-well plate (100 µL volume). The compounds were added for 72 h (0.5% DMSO). Cell Titer Glo assay was used to determine the percent cell viability.

Percent Viable Cells

	Vehicle	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	CC_{50} (µM)
Quin1	100	102	95	94	72	51	23	**19.5**
1	100	80	61	41	20	6	1	**3.4**
2	100	90	75	67	37	5	1	**6.8**
Quin2	100	124	125	114	98	85	61	**>40.0**
$CpRu(DMSO)_3^+$	100	63	55	41	31	15	4	**2.8**

(n = 4)



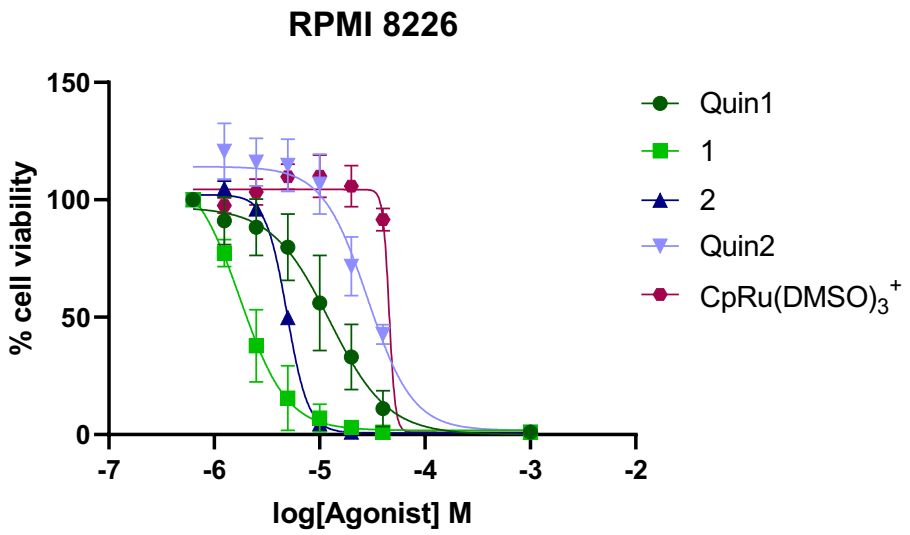
Cytotoxicity – RPMI 8226 cells

RPMI 8226 cells (25,000 cells per well) were seeded in a 96-well plate (100 µL volume). Cell Titer Glo assay was used to determine the percent cell viability.

Percent Viable Cells

	Vehicle	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	CC_{50} (µM)
Quin1	100	91	88	80	56	33	11	**11.8**
1	100	77	38	16	7	3	1	**2.0**
2	100	105	96	50	5	1	1	**5.0**
Quin2	100	121	116	115	107	72	43	**32.4**
$CpRu(DMSO)_3^+$	100	97	103	109	110	106	92	**>40.0**

(n = 4)

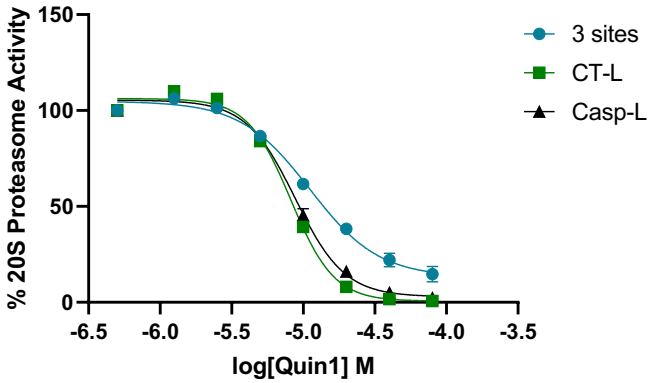


20S Proteasome Inhibitor Data for Quin1

% 20S proteasome activity

	Vehicle	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	IC_{50} (µM)
3 Sites	100	106	101	87	62	38	22	15	13.9
CT-L	100	110	106	84	39	8	2	1	8.5
Casp-L	100	108	104	85	45	16	5	2	9.4
T-L	100	88	75	78	95	105	129	135	>80.0

(n = 3)

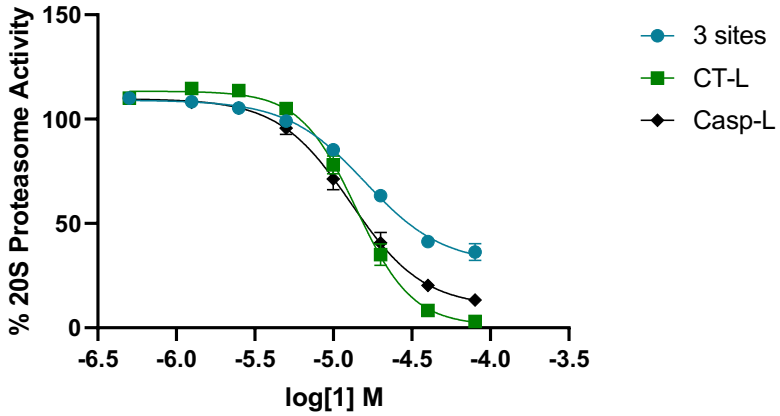


20S Proteasome Inhibitor Data for 1

% 20S proteasome activity

	Vehicle	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	IC_{50} (µM)
3 Sites	100	108	105	99	85	63	41	36	29.7
CT-L	100	115	114	105	78	35	8	3	15.4
Casp-L	100	108	105	96	71	41	20	13	16.0
T-L	100	100	93	91	88	85	91	87	>80.0

(n = 3)

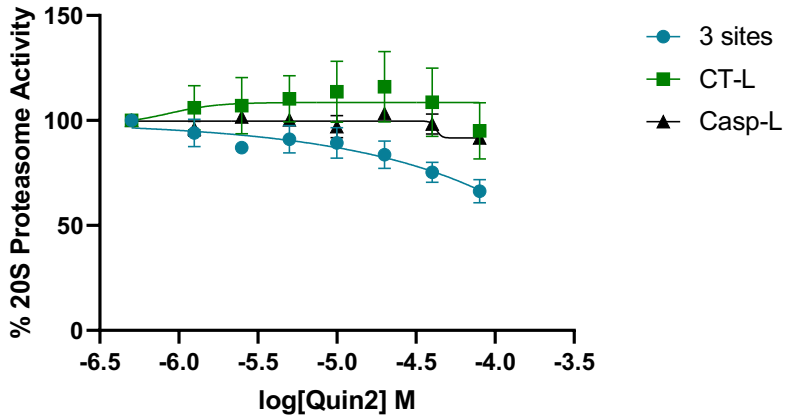


20S Proteasome Inhibitor Data for Quin2

% 20S proteasome activity

	Vehicle	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	IC_{50} (µM)
3 Sites	100	94	87	91	89	84	75	67	>80.0
CT-L	100	106	107	110	113	116	109	95	>80.0
Casp-L	100	96	102	100	97	103	98	91	>80.0
T-L	100	89	95	87	90	104	109	121	>80.0

(n = 3)

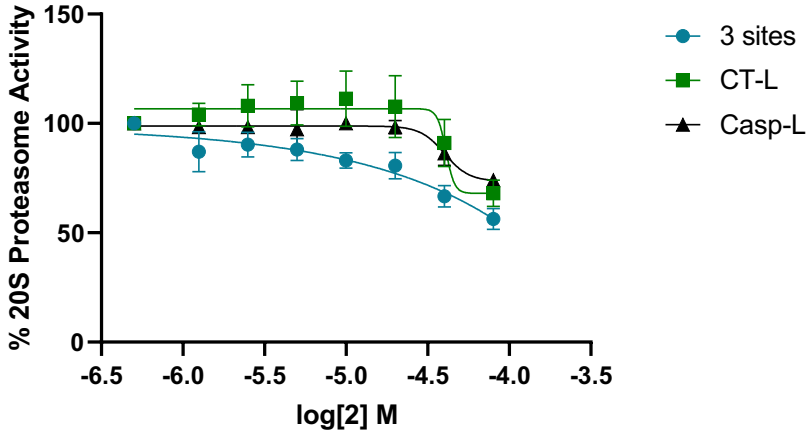


20S Proteasome Inhibitor Data for 2

% 20S proteasome activity

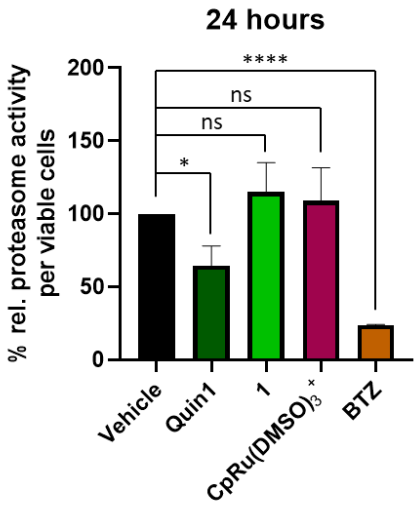
	Vehicle	1.25 µM	2.5 µM	5 µM	10 µM	20 µM	40 µM	80 µM	IC_{50} (µM)
3 Sites	100	87	90	88	83	81	67	56	>80.0
CT-L	100	104	108	109	111	108	91	68	>80.0
Casp-L	100	98	99	97	100	98	86	74	>80.0
T-L	100	91	80	83	87	88	100	116	>80.0

(n = 3)



Cellular Proteasome Activity Assay

RPMI 8226 cells (10,000 cells per well) were seeded in a 96-well plate (100 µL volume). Cells were treated with DMSO, 3 or 5 µM test compound, or 3 nM BTZ for 24 hours. Proteasome-Glo cell-based (CT-L site) activity assay was used to measure proteasome activity. Cell Titer Glo assay was run in parallel to determine the percent cell viability at this treatment time.

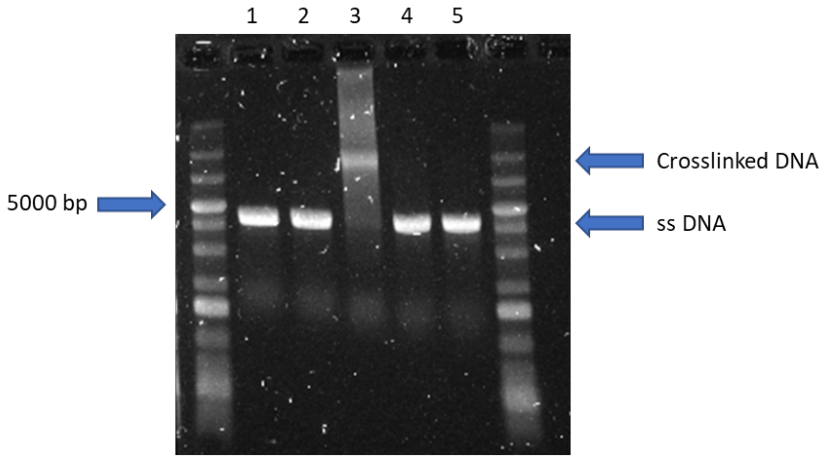


	Percent Proteasome Activity	Percent Cell Viability	Percent Relative Proteasome Activity per Viable Cells
Vehicle	100	100	100
5 µM Quin1	56	87	64
3 µM 1	69	60	115
3 µM $CpRu(DMSO)_3^+$	96	89	109
3 nM BTZ	22	95	24

(n=3)

DNA Crosslinking Assay

Plasmid pBR322 was linearized with *Eco*R1 for 1 h at 37 °C. Linearized DNA (250 µg) was incubated with DMSO or 50 µM drug for 16 h at 37 °C. An alkaline agarose gel was prepared using 20 mL of buffer (50 mM NaCl, 2 mM EDTA pH 8.0) and 0.2 g of agarose. The suspension was heated in a microwave for 45 seconds to dissolve, then 2 µL of GelGreen stain was added. The gel was poured and allowed to cool at room temperature and then soaked in alkaline running buffer (25 mL of 2 N NaOH, 2 mL of 0.5 M EDTA in 1 L of H_2O) for 1 h. The samples were then loaded into the wells with loading dye (5 µL) and the gel was run at 4 °C for 1.5 h. Gel was visualized on an Azure Biosystems Imager (green LED/orange filter).



Lanes:

1 – Vehicle
2 – 50 µM **1**
3 – 50 µM Cisplatin
4 – 50 µM $CpRu(NCMe)_3^+$
5 – 50 µM **2**

Cited References

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