

Supporting Information for

A Cancer Cell-Selective and Low-Toxic Bifunctional Heterodinuclear

Pt(IV)-Ru(II) Anticancer Prodrug

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Table S1 Wound closure ratios after 8 h or 24 h treatment of different concentrations of complex **5**.

Table S2. Toxicity assessments of cisplatin and complex **5** using zebrafish embryos.

Supplementary Figures and Tables

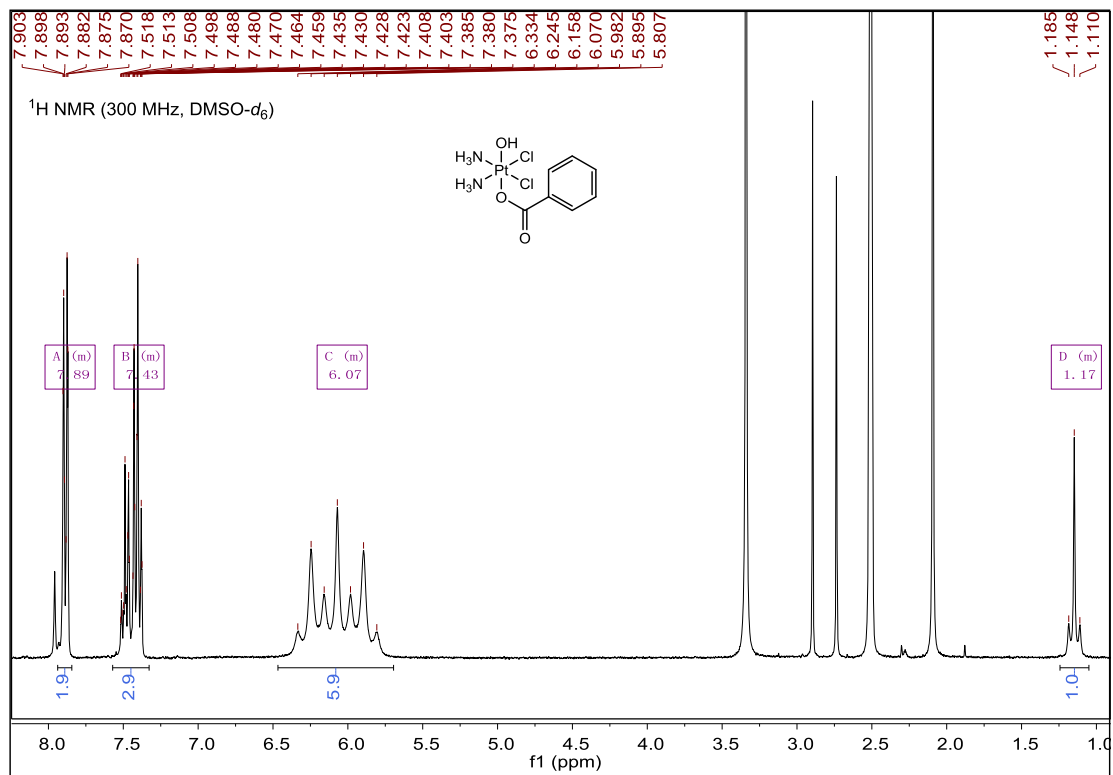


Figure S1. ¹H NMR of compound **1**.

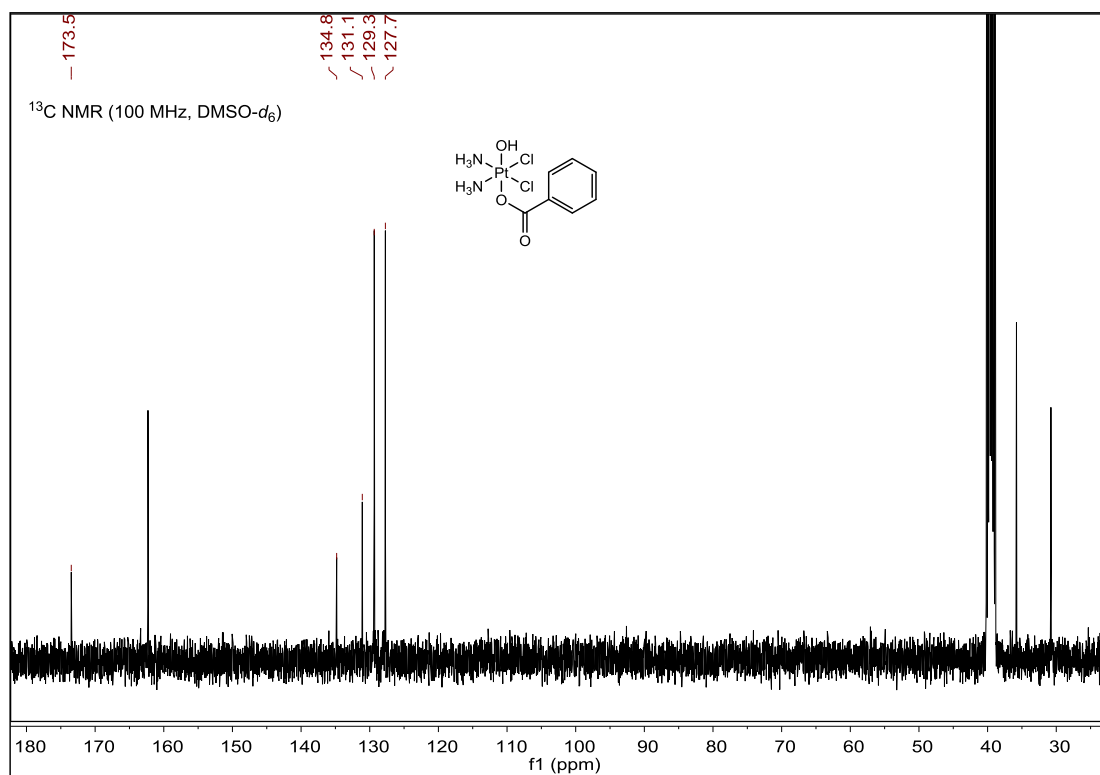


Figure S2. ¹³C NMR of compound **1**.

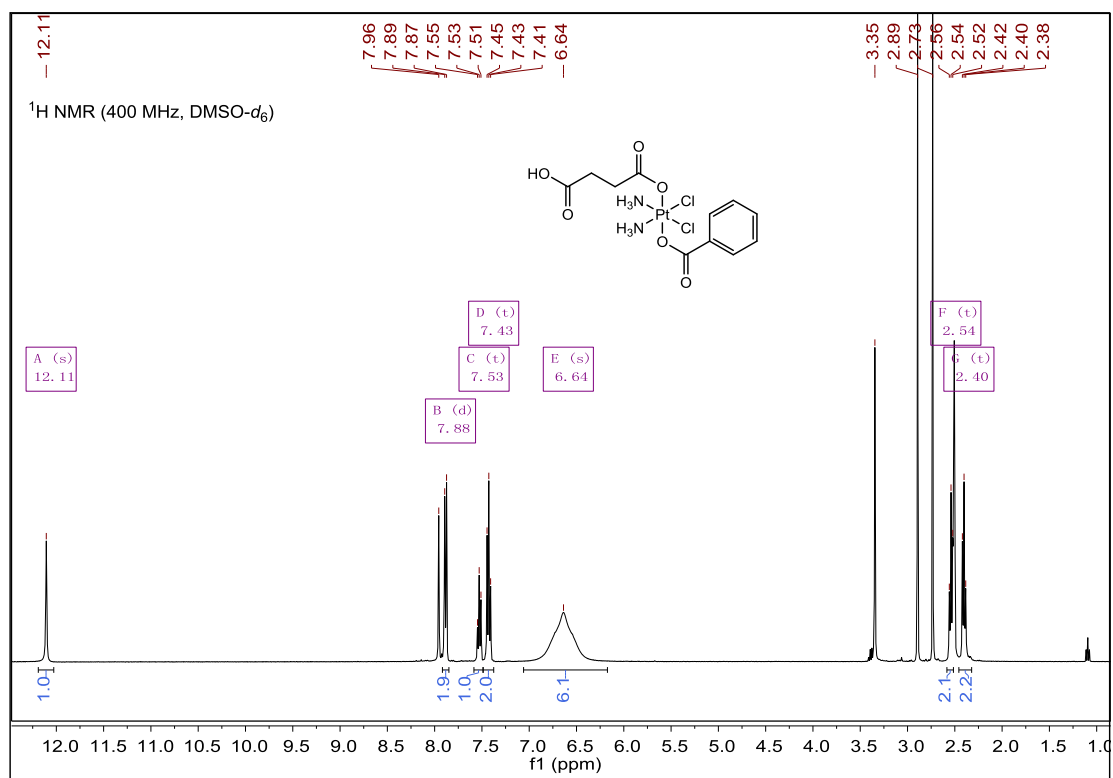


Figure S3. ¹H NMR of compound **2**.

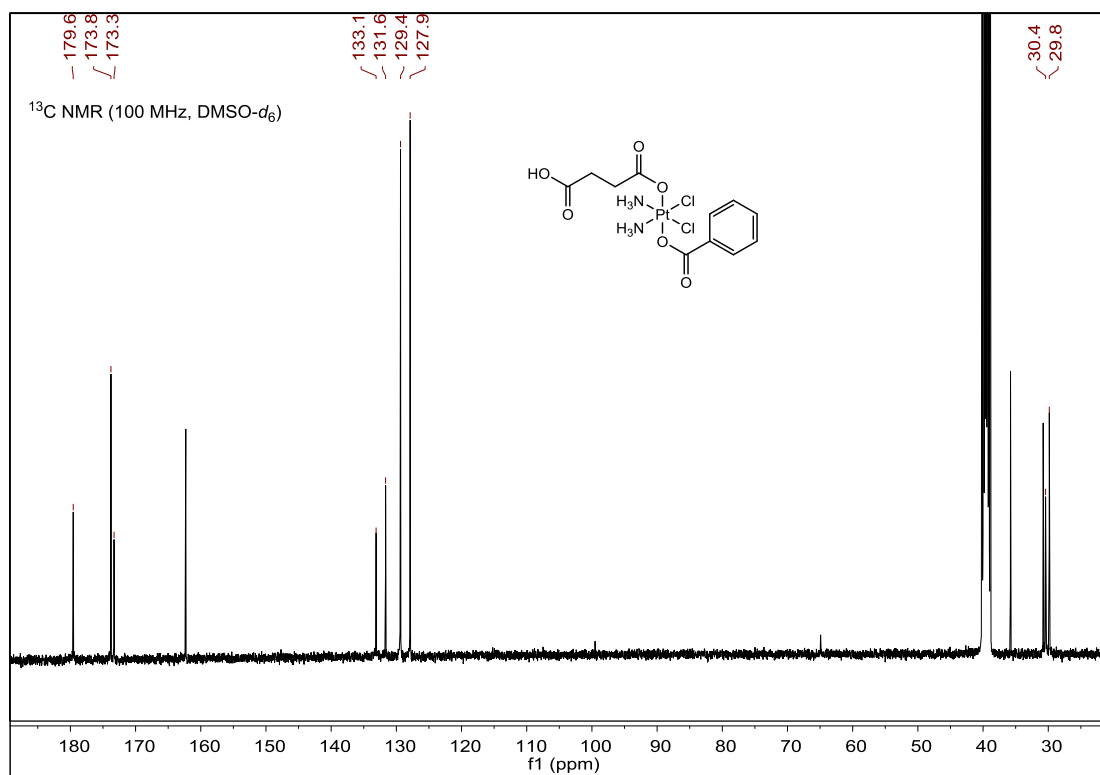


Figure S4. ¹³C NMR of compound **2**.

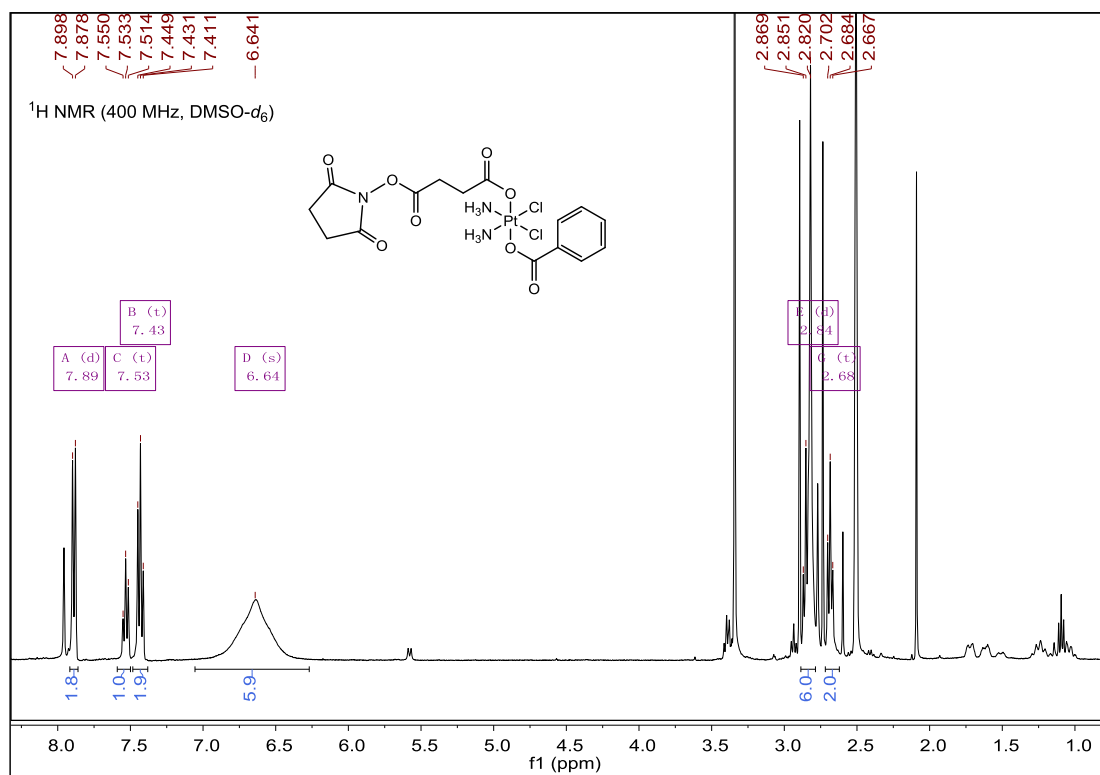


Figure S5. ¹H NMR of compound **3**.

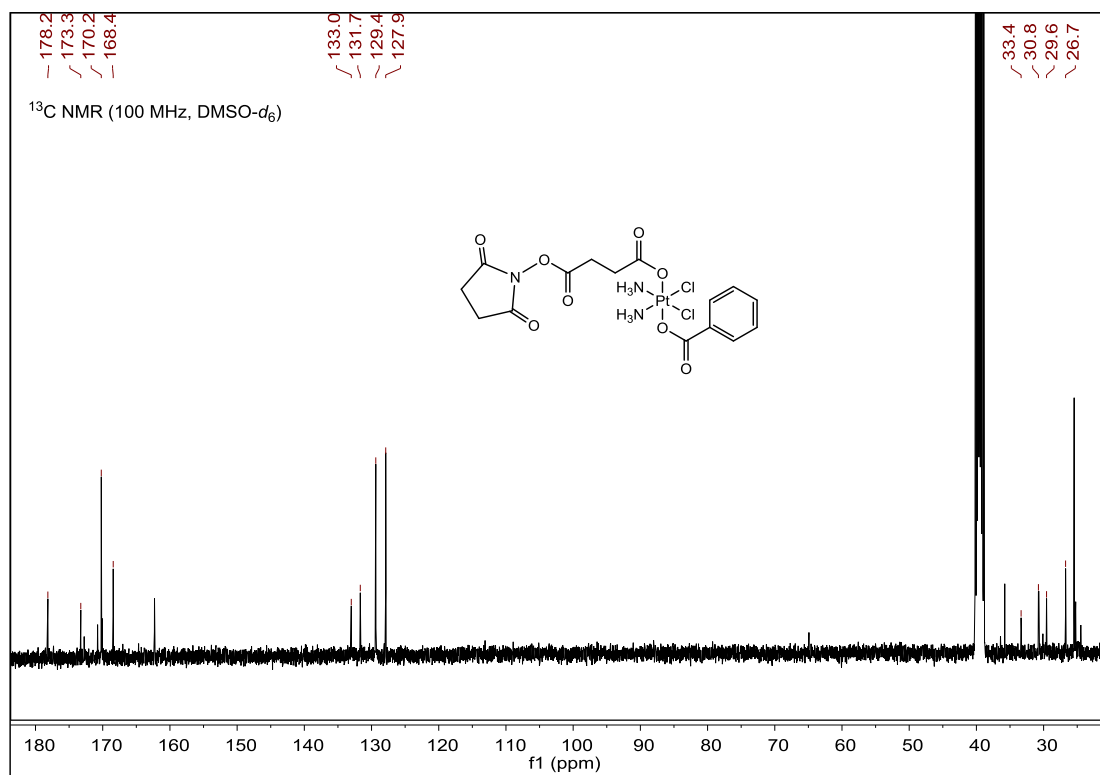


Figure S6. ¹³C NMR of compound **3**.

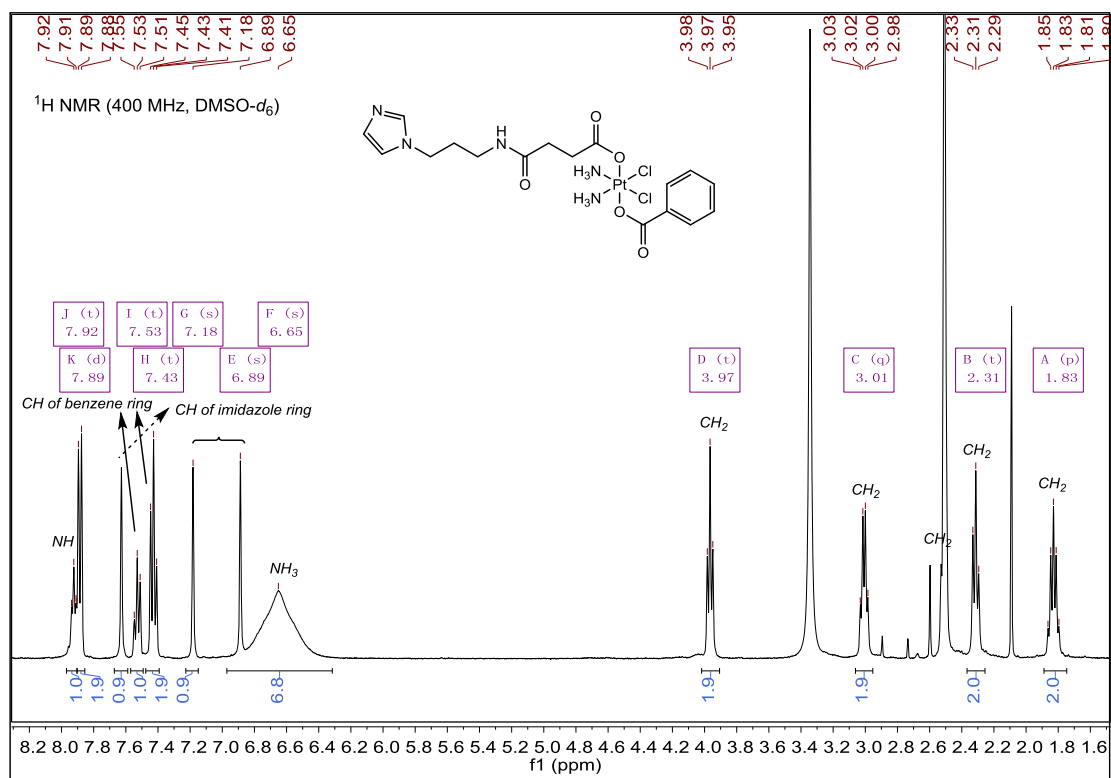


Figure S7. ¹H NMR of compound 4.

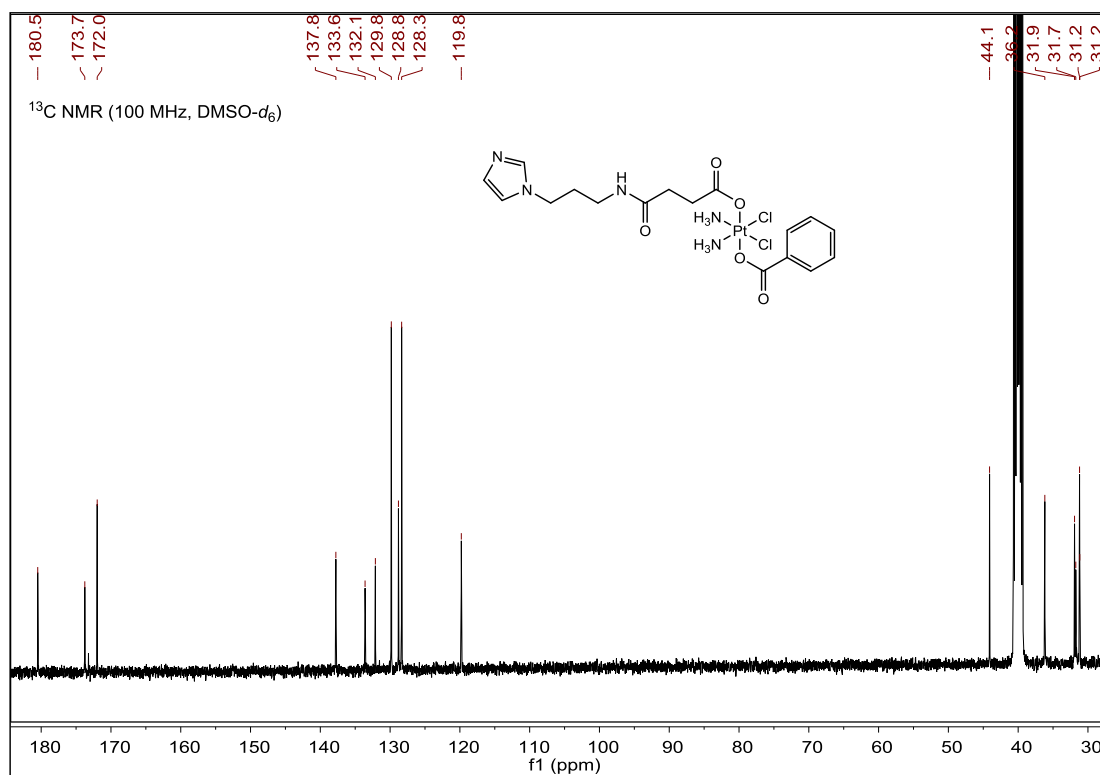


Figure S8. ¹³C NMR of compound **4**.

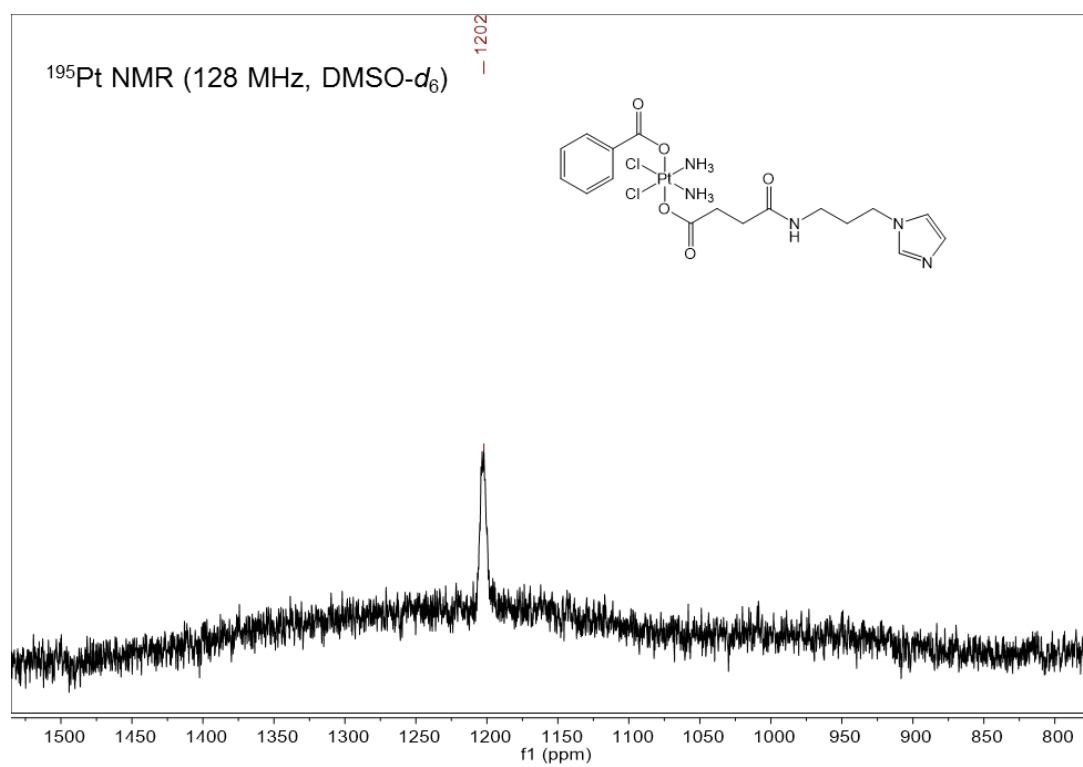


Figure S9. ^{195}Pt NMR of compound 4.

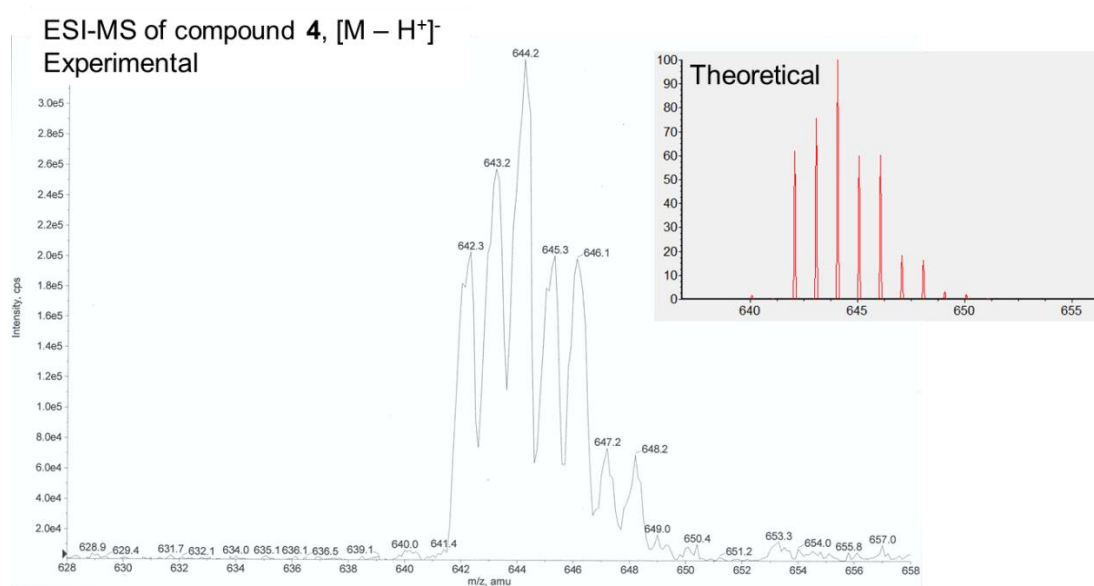


Figure S10. ESI-MS of compound **4**, negative mode in methanol. m/z $[M - H]^-$, calcd. 644.1, found 644.2.

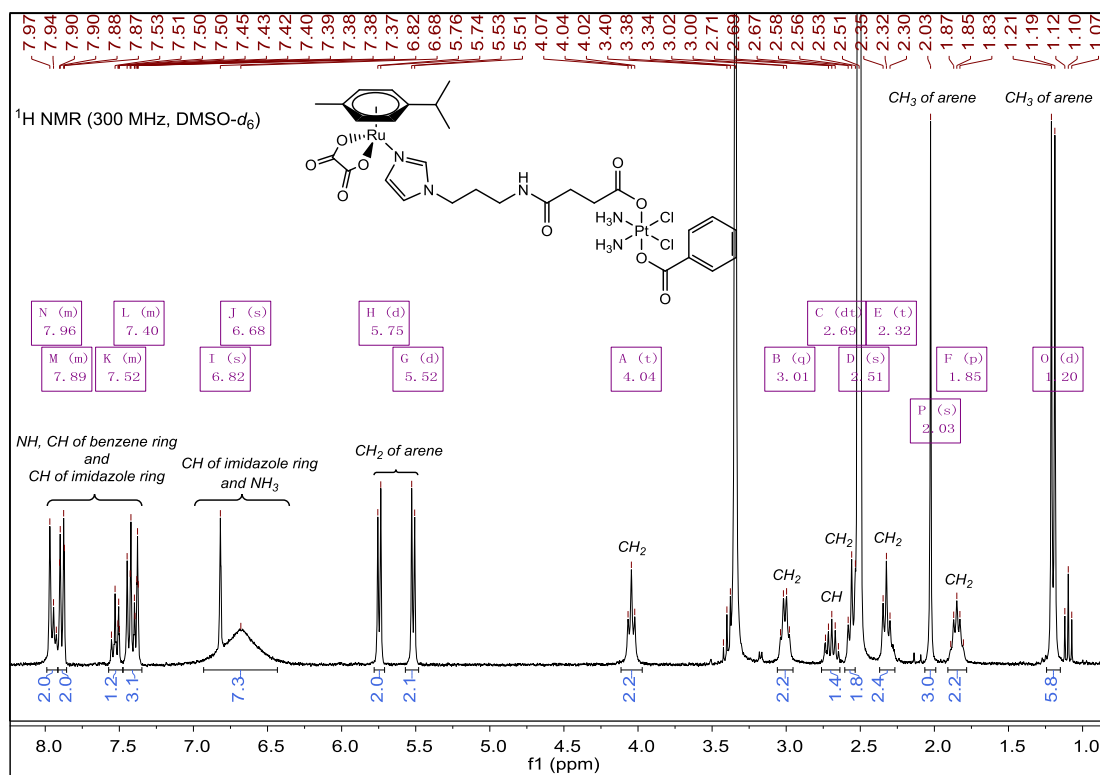


Figure S11. ¹H NMR of complex 5.

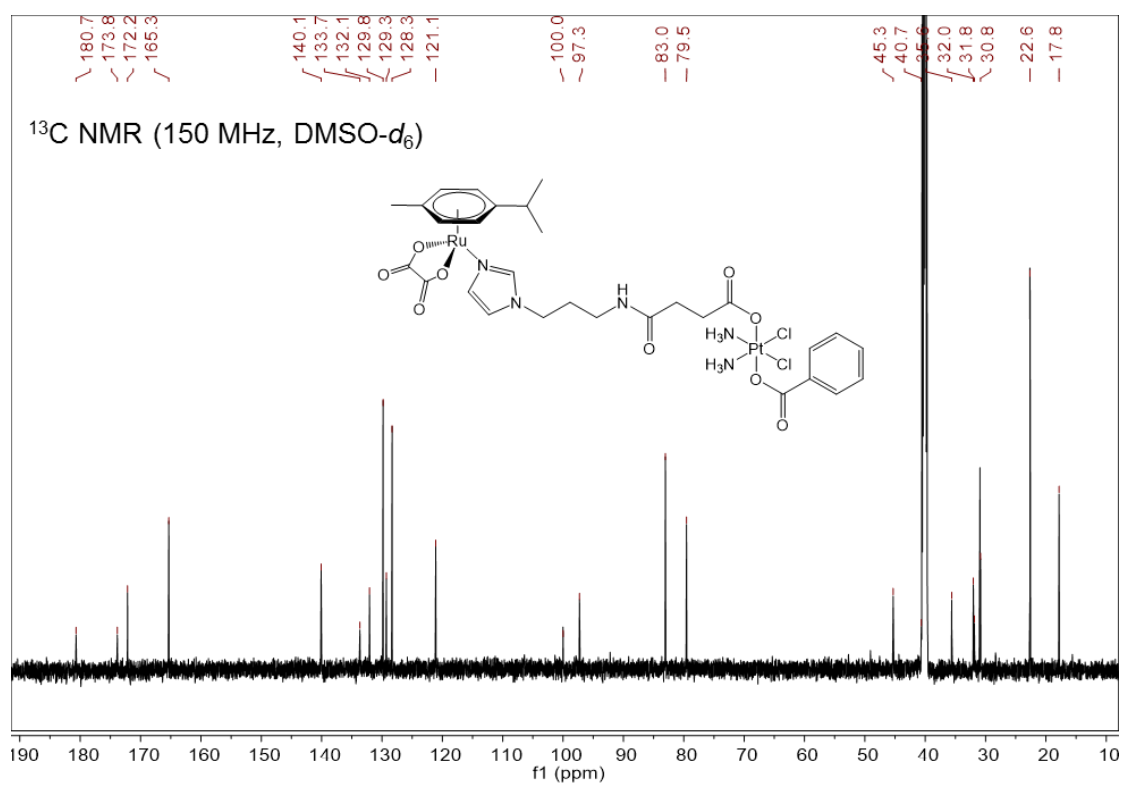


Figure S12. ¹³C NMR of complex 5.

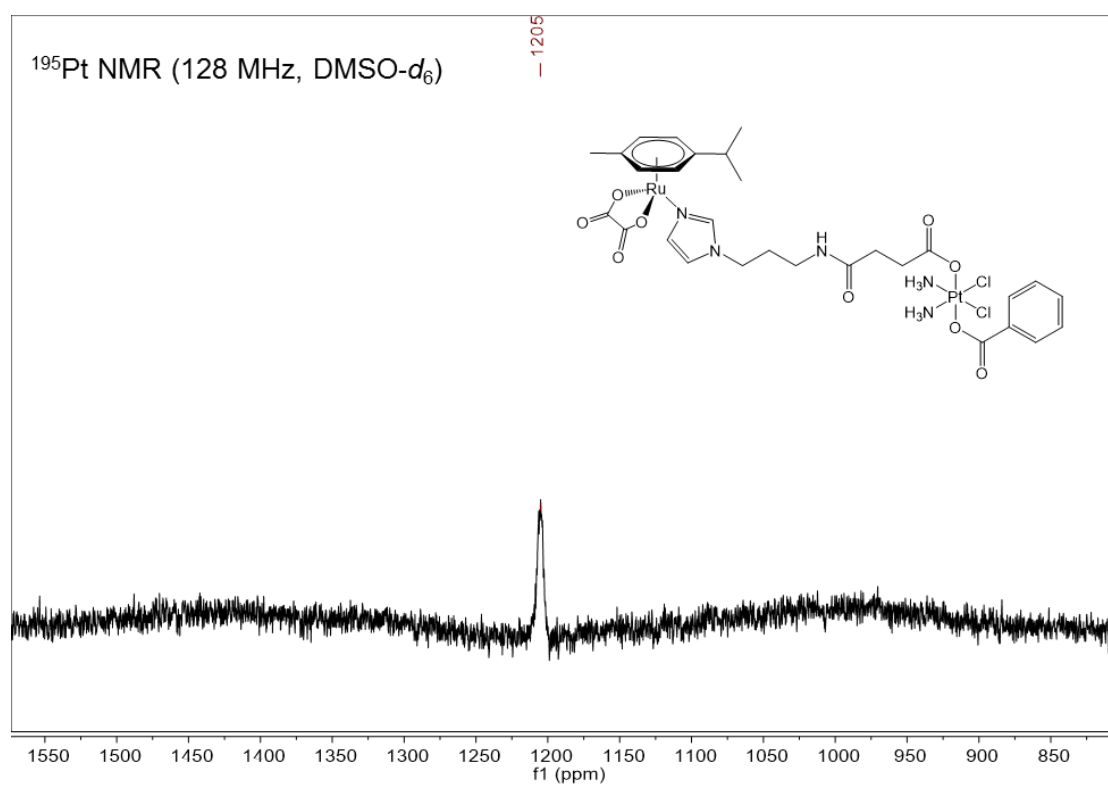


Figure S13. ^{195}Pt NMR of complex 5.

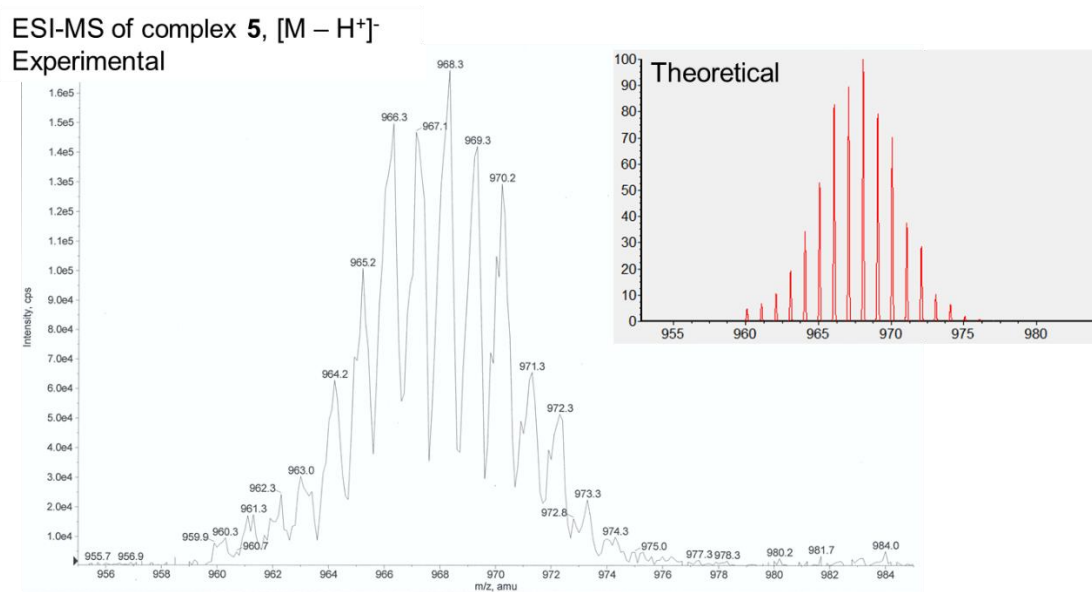


Figure S14. ESI-MS of complex **5**, negative mode in methanol. m/z $[M - H]^-$, calcd. 968.1, found 968.3.

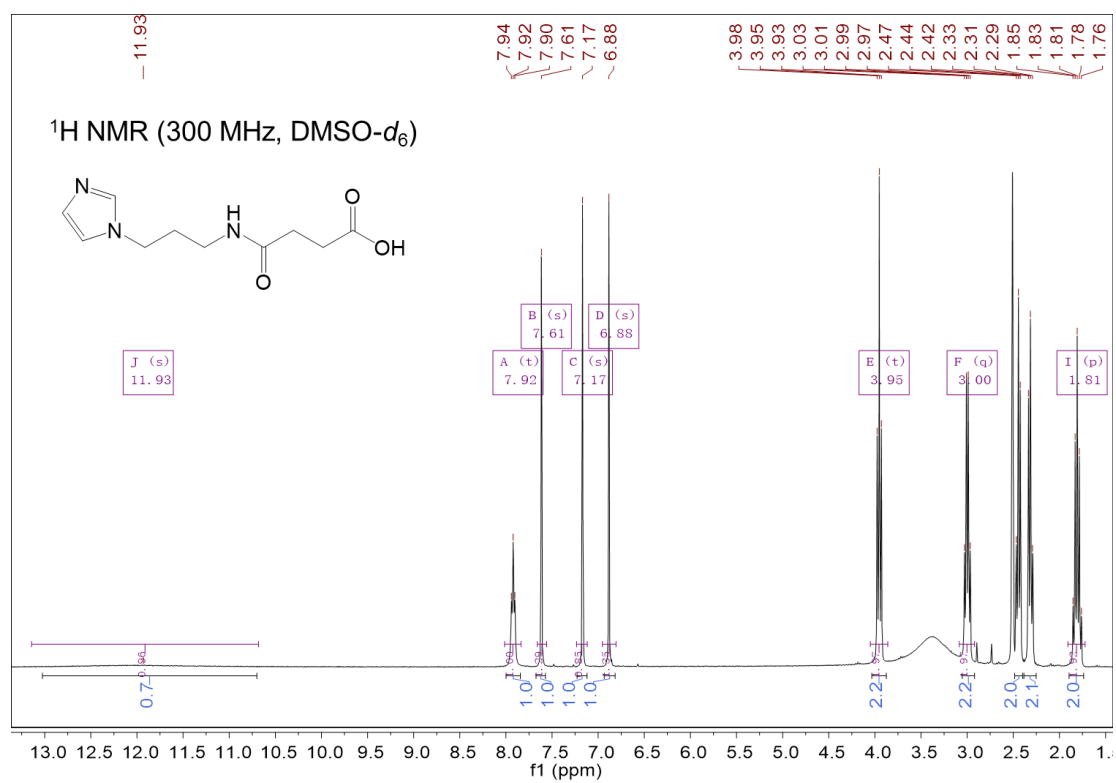


Figure S15. ¹H NMR of imidazole ligand.

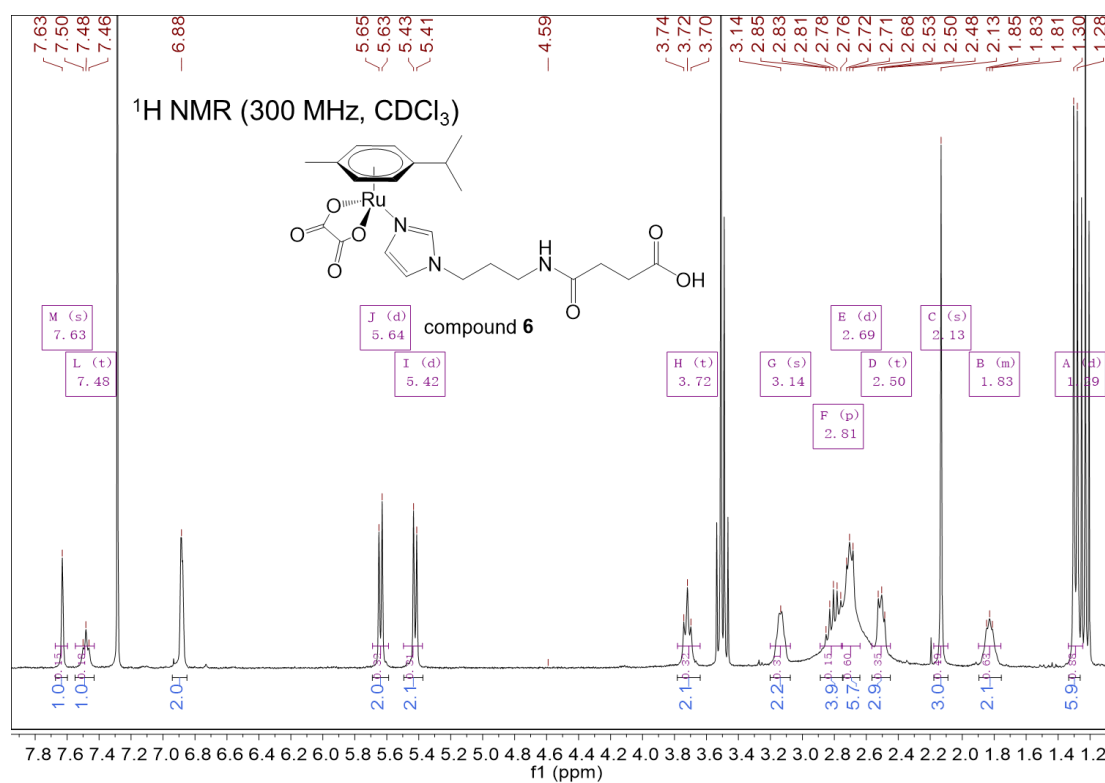


Figure S16. ¹H NMR of arene-Ru(II) compound **6**.

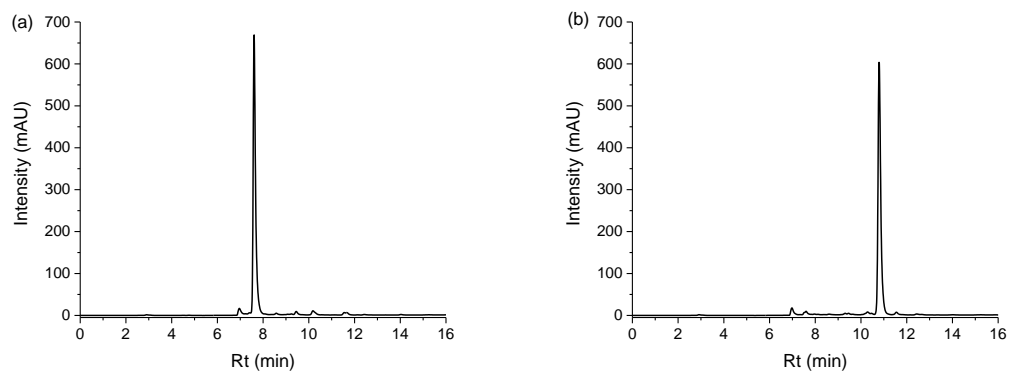


Figure S17. Purity of (a) compound **4** ($R_t = 7.61$ min), 95%; (b) complex **5** ($R_t = 10.80$ min), 96%, by HPLC@254 nm.

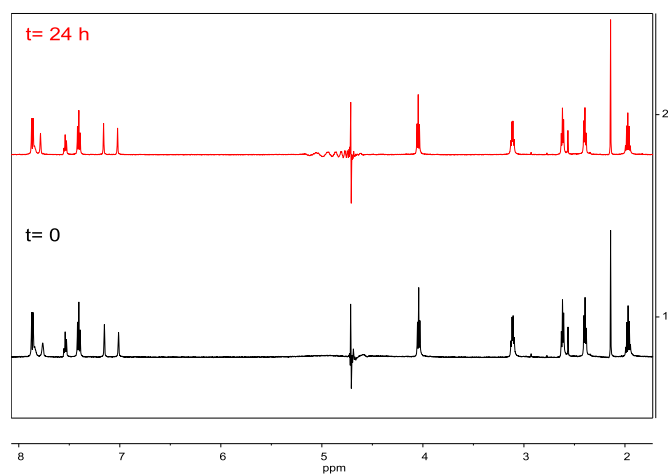


Figure S18. Stability of compound **4** (1.6 mM) in H₂O. Black line, t = 0; red line, t = 24 h.

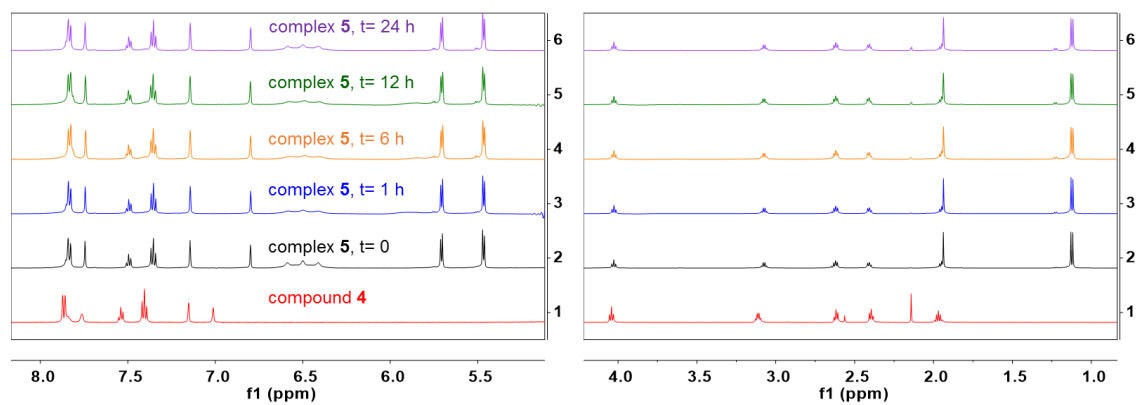


Figure S19. Stability of complex **5** (2 mM) in H₂O.

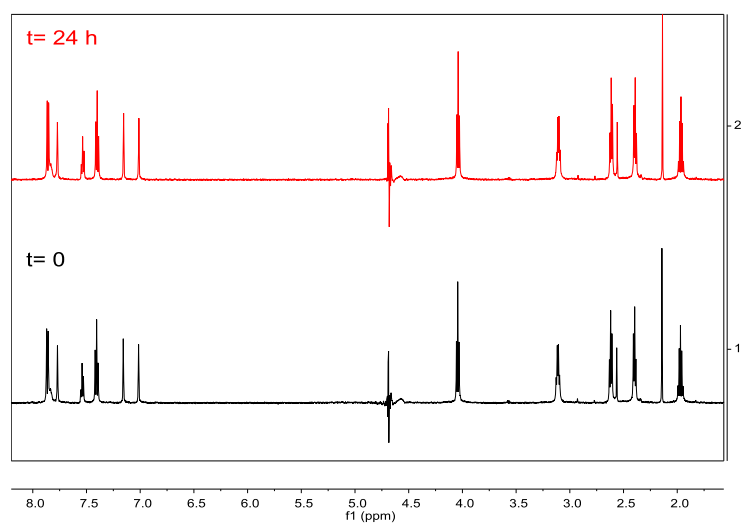


Figure S20. Stability of compound **4** (1.6 mM) in PBS. Black line, $t = 0$; red line, $t = 24$ h.

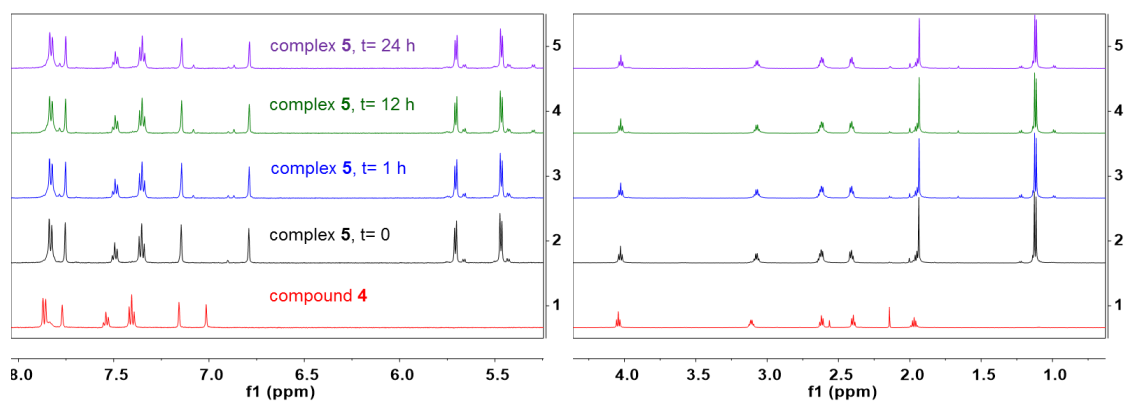


Figure S21. Stability of complex **5** (2 mM) in PBS.

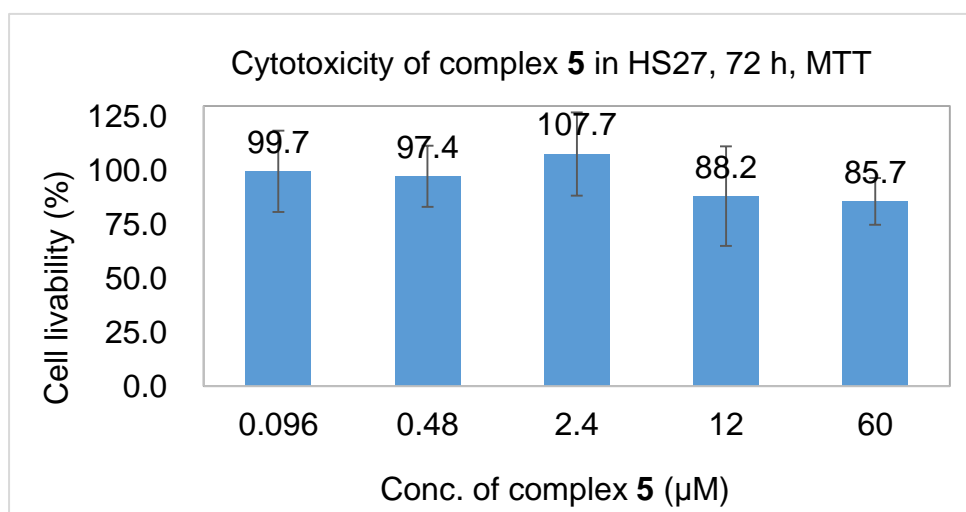


Figure S22. Cytotoxicity of complex **5** in Hs27 cells by MTT assay (72 h treatment).

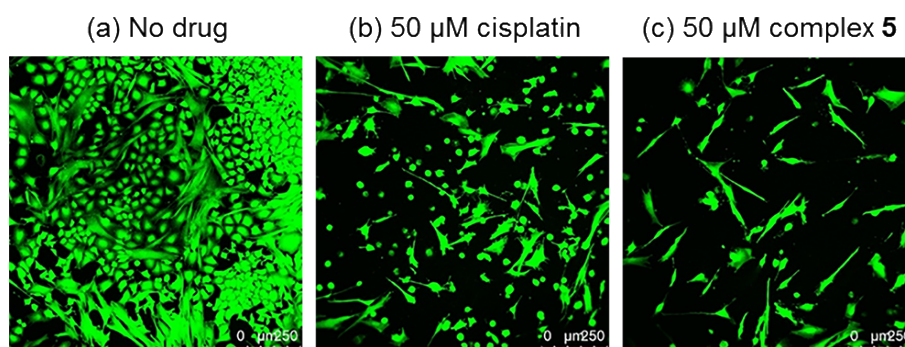


Figure S23. Cancer cell selectivity of complex **5** by co-culture of normal cells (MRC-5, long shape) and cancer cells (A549, round shape) with a 1:1 ratio. Mixed cells were exposed to cisplatin or complex **5** for 48 h. After treatment, cells were stained with calcein-AM and the remaining live cells showed a green color. Images were taken by an SPE confocal laser scanning microscope (CLSM) with 10X magnification. Scale bar, 250 μm .

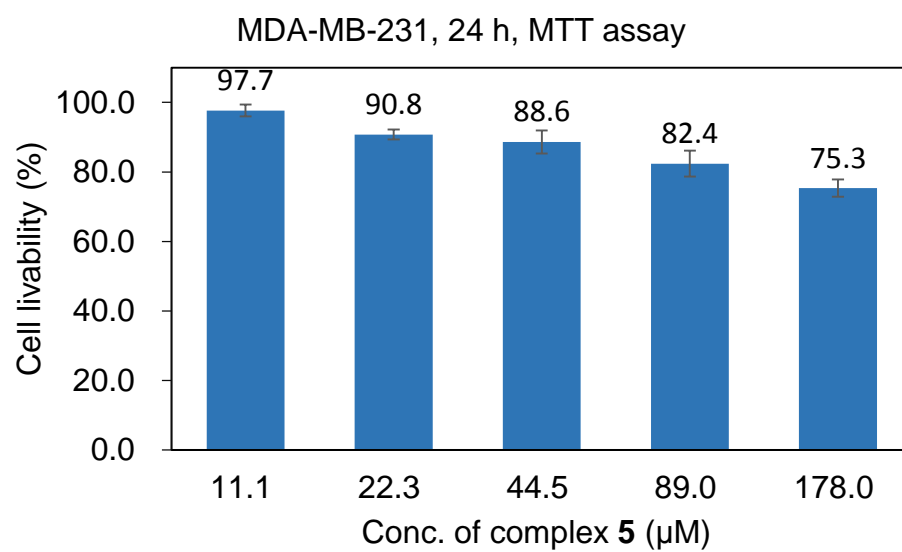


Figure S24. Cytotoxicity of complex **5** in MDA-MB-231 cells by MTT assay (24 h treatment).

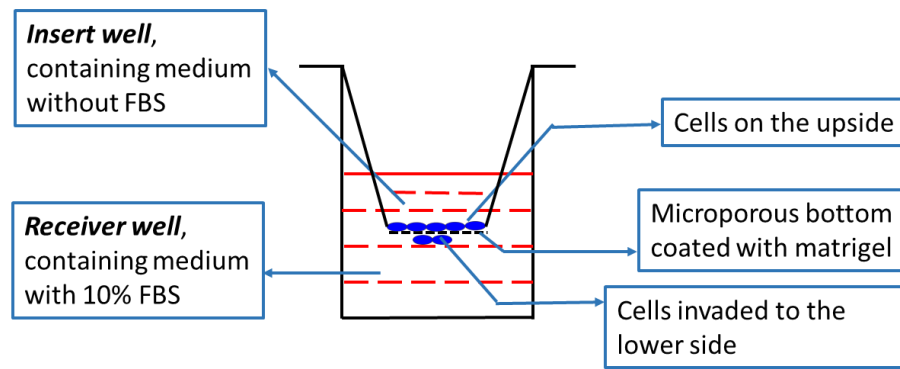


Figure S25. Brief illustration of transwell invasion assay.

Table S1. Wound closure ratios after 8 h or 24 h treatment of different concentrations of complex

5.

wound closure ratio (%)	no drug	75 μ M	100 μ M	125 μ M	150 μ M	175 μ M
8 h	31 \pm 14	11 \pm 6	15 \pm 8	12 \pm 2	12 \pm 5	8 \pm 3
24 h	76 \pm 15	43 \pm 11	32 \pm 7	26 \pm 11	25 \pm 7	25 \pm 4

Table S2. Toxicity assessments of cisplatin and complex **5** using zebrafish embryos. Survival rates of zebrafish embryos in the presence of (a) cisplatin and (c) complex **5** are shown, together with hatching rates of zebrafish embryos after the exposure to (b) cisplatin and (d) complex **5**. Data are collected from 4 replicates of 2 independent experiments.

(a) Survival rate after cisplatin treatment							
%	Control	15 μ M	30 μ M	45 μ M	60 μ M	75 μ M	90 μ M
0h	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
24h	98.3 \pm 2.9	95.0 \pm 2.9	93.3 \pm 0	91.7 \pm 5.5	93.3 \pm 4.7	91.7 \pm 5.5	93.3 \pm 4.7
48h	98.3 \pm 2.9	95.0 \pm 2.9	91.7 \pm 2.9	91.7 \pm 5.5	90.0 \pm 5.8	88.3 \pm 7.3	85.0 \pm 2.9
72h	98.3 \pm 2.9	93.3 \pm 0	90.0 \pm 3.3	90.0 \pm 5.8	88.3 \pm 2.9	83.3 \pm 7.5	81.7 \pm 2.9
96h	98.3 \pm 2.9	91.7 \pm 2.9	90.0 \pm 3.3	86.7 \pm 0	85.0 \pm 2.9	78.3 \pm 5.5	75.0 \pm 2.9
(b) Hatching rate after cisplatin treatment							
%	Control	15 μ M	30 μ M	45 μ M	60 μ M	75 μ M	90 μ M
0h	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24h	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48h	76.7 \pm 3.3	66.7 \pm 4.7	73.3 \pm 4.7	28.3 \pm 5.5	18.3 \pm 5.5	23.3 \pm 3.3	1.7 \pm 2.9
72h	95.0 \pm 2.9	76.7 \pm 3.3	78.3 \pm 2.9	35.0 \pm 5.5	25.0 \pm 2.9	25.0 \pm 2.9	6.7 \pm 6.7
96h	98.5 \pm 2.9	83.3 \pm 5.8	83.3 \pm 3.3	45.0 \pm 8.7	28.3 \pm 2.9	26.7 \pm 0	10.0 \pm 11.1
(c) Survival rate after complex 5 treatment							
%	Control	15 μ M	30 μ M	45 μ M	60 μ M	75 μ M	90 μ M
0h	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0	100 \pm 0
24h	98.3 \pm 2.9	96.7 \pm 3.3	95.0 \pm 2.9	95.0 \pm 5.5	93.3 \pm 4.7	93.3 \pm 0	91.7 \pm 7.3
48h	98.3 \pm 2.9	96.7 \pm 3.3	95.0 \pm 2.9	93.3 \pm 4.7	91.7 \pm 5.5	90.0 \pm 3.3	88.3 \pm 5.5
72h	96.7 \pm 2.9	96.7 \pm 3.3	93.3 \pm 0	90.0 \pm 2.9	93.3 \pm 5.8	90.0 \pm 2.9	86.7 \pm 5.5
96h	96.7 \pm 2.9	93.3 \pm 2.9	93.3 \pm 2.9	90.0 \pm 3.3	90.0 \pm 2.9	86.7 \pm 2.9	83.3 \pm 2.9
(d) Hatching rate after complex 5 treatment							
%	Control	15 μ M	30 μ M	45 μ M	60 μ M	75 μ M	90 μ M
0h	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24h	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48h	76.7 \pm 3.3	60.0 \pm 4.7	38.3 \pm 2.9	28.3 \pm 5.5	28.3 \pm 8.7	20.0 \pm 12.5	15.0 \pm 15.2
72h	95.0 \pm 2.9	90.0 \pm 3.3	56.7 \pm 5.8	53.3 \pm 4.7	48.3 \pm 5.5	35.0 \pm 8.7	25.0 \pm 12.8
96h	95.3 \pm 2.9	91.7 \pm 5.5	80.0 \pm 8.2	66.7 \pm 6.7	65.0 \pm 9.9	55.0 \pm 9.9	50.0 \pm 3.3