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Ruthenium-arene complexes with NSAIDs: Synthesis, characterization and bioactivity

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Supplementary material

S1. NMR spectra of synthesized complexes

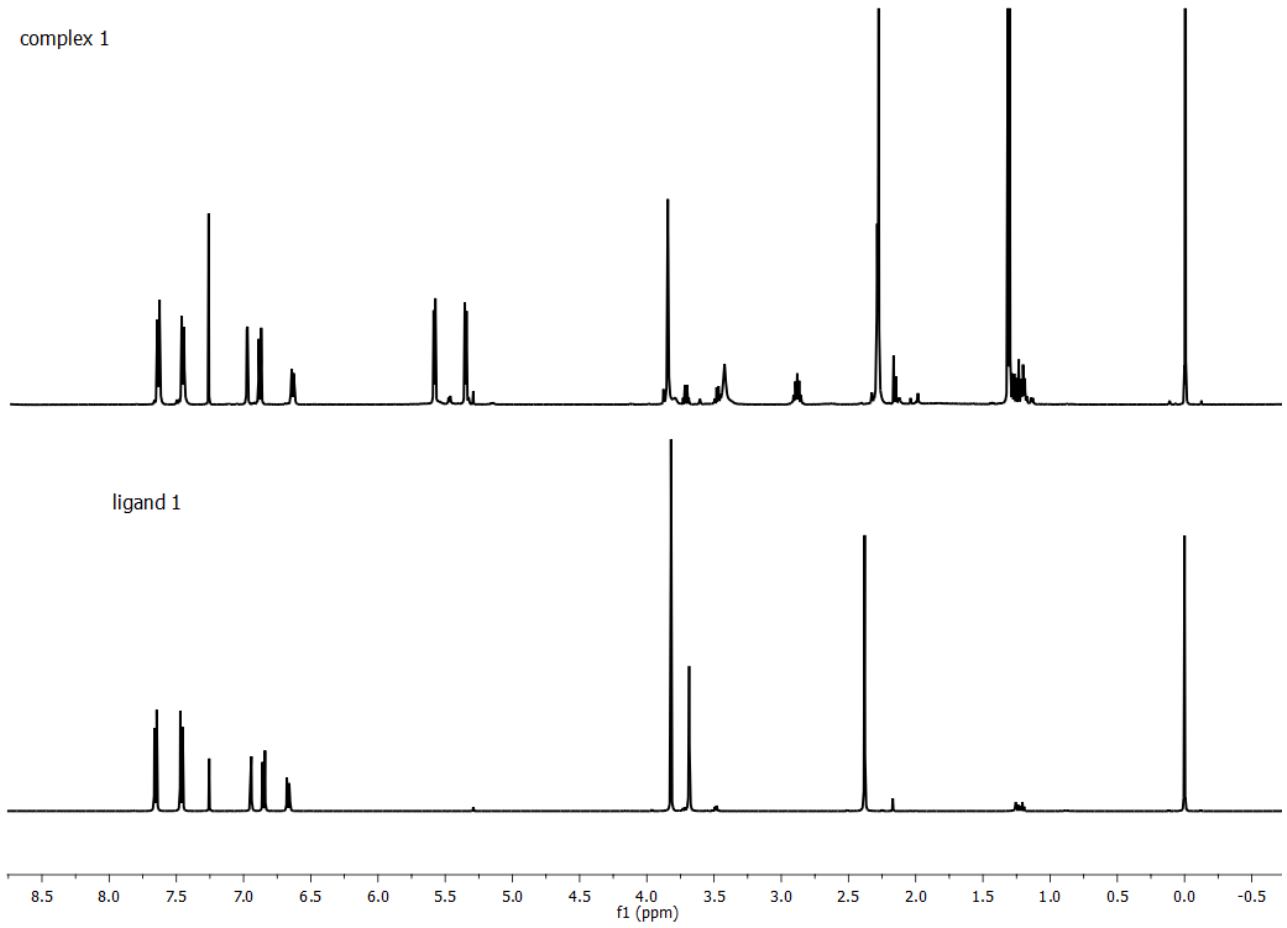

Figure S1. Parallel ^{1}H NMR spectra of ligand1 and complex 1

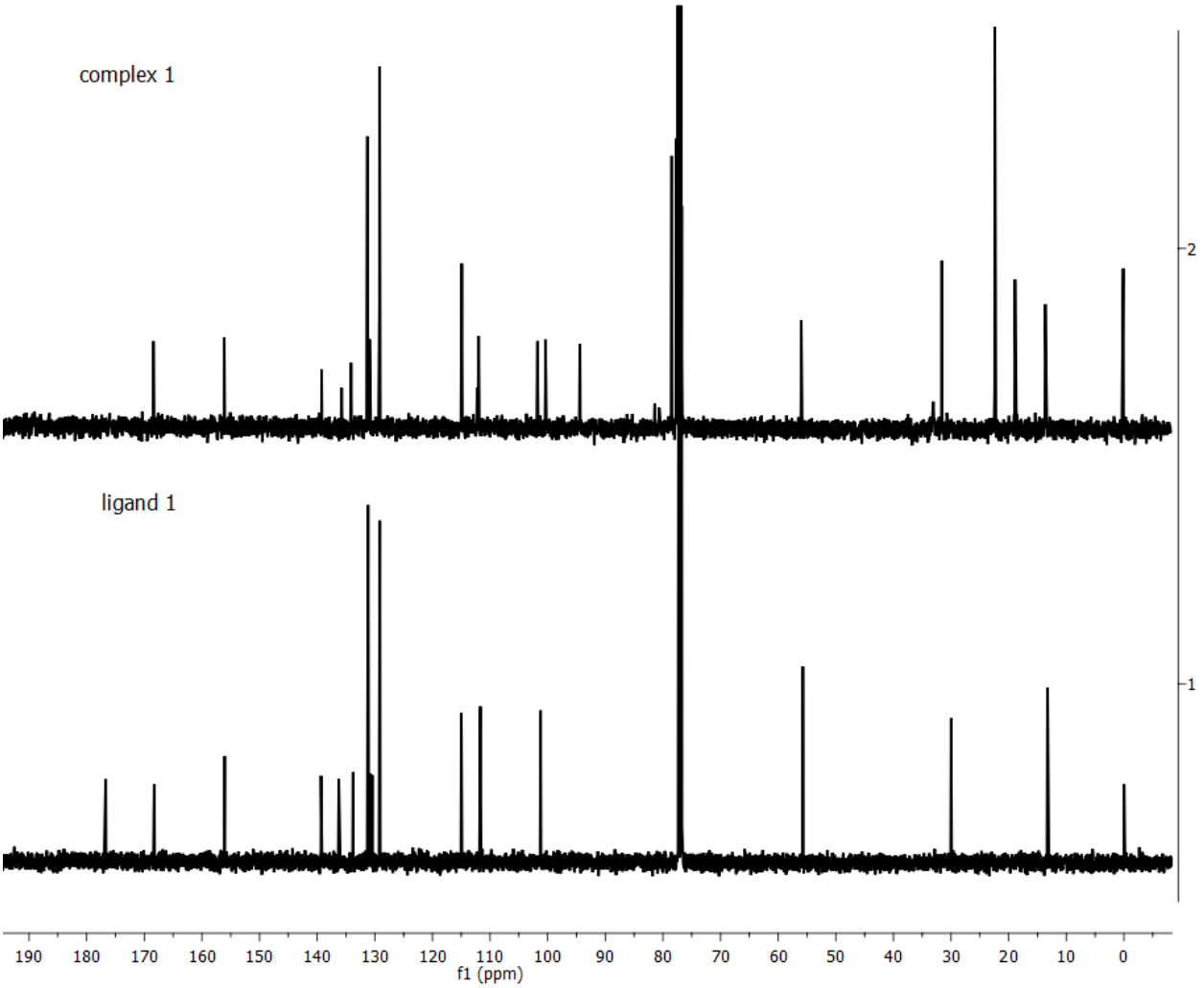

Figure S2. Parallel ^{13}C NMR spectra of ligand 1 and complex 1

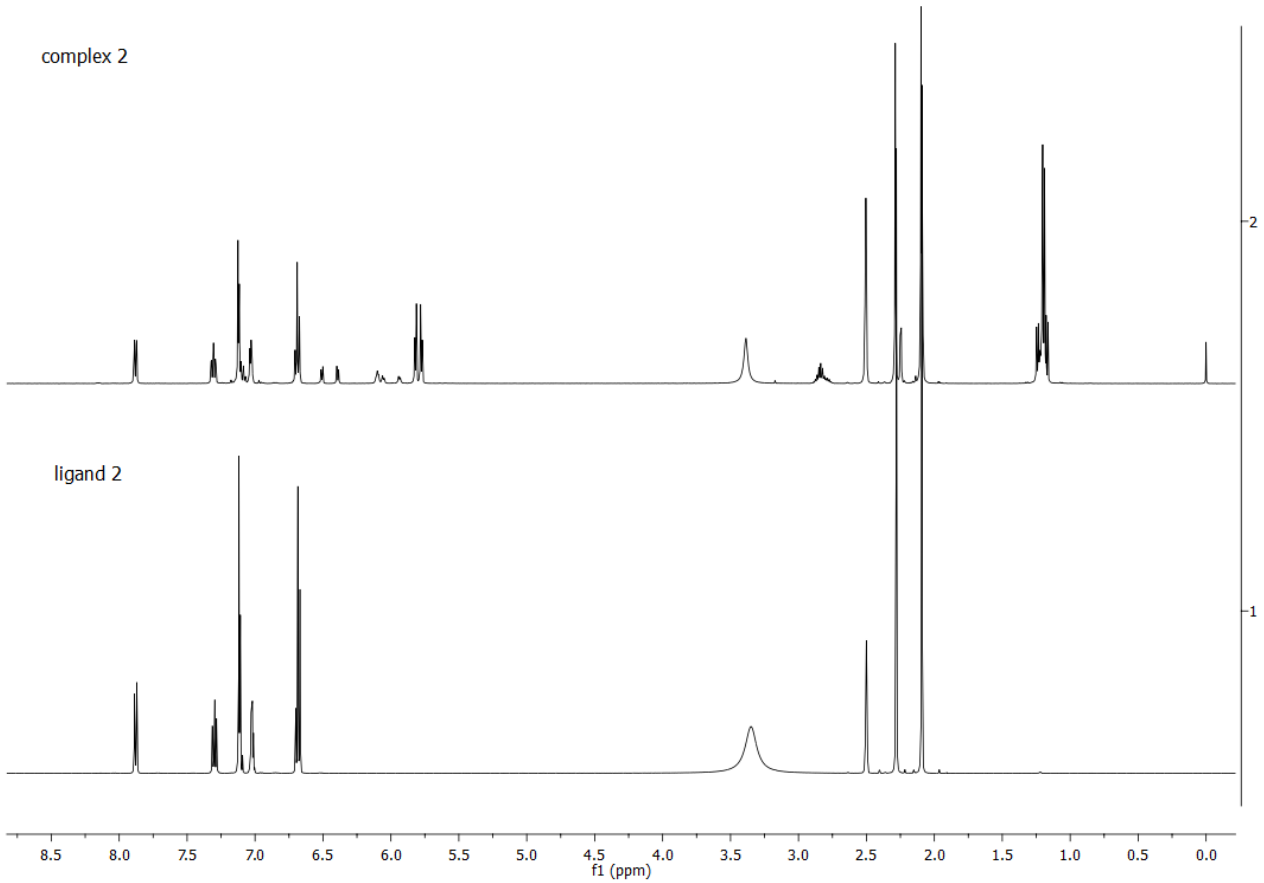

Figure S3. Parallel ^{1}H NMR spectra of ligand 2 and complex 2

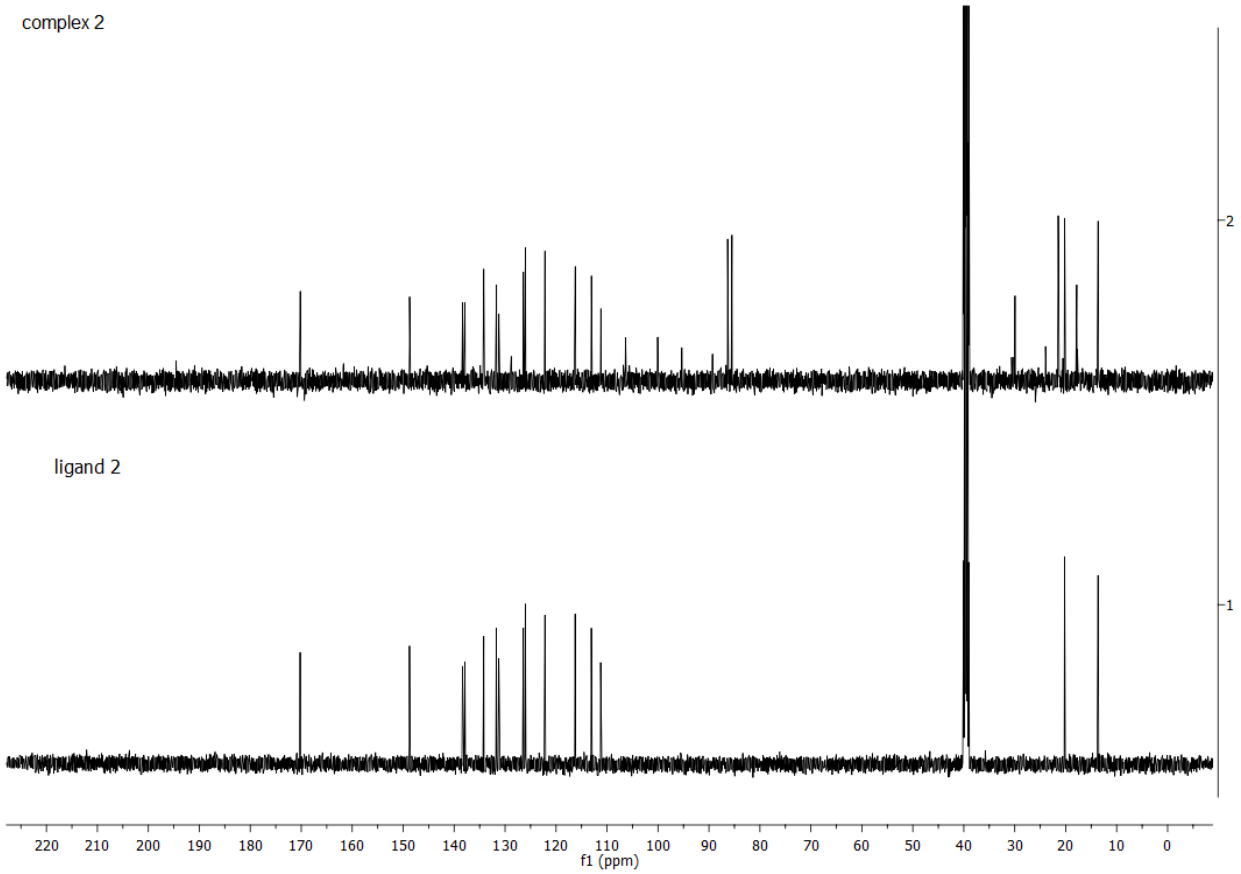

Figure S4. Parallel ^{13}C NMR spectra of ligand 2 and complex 2

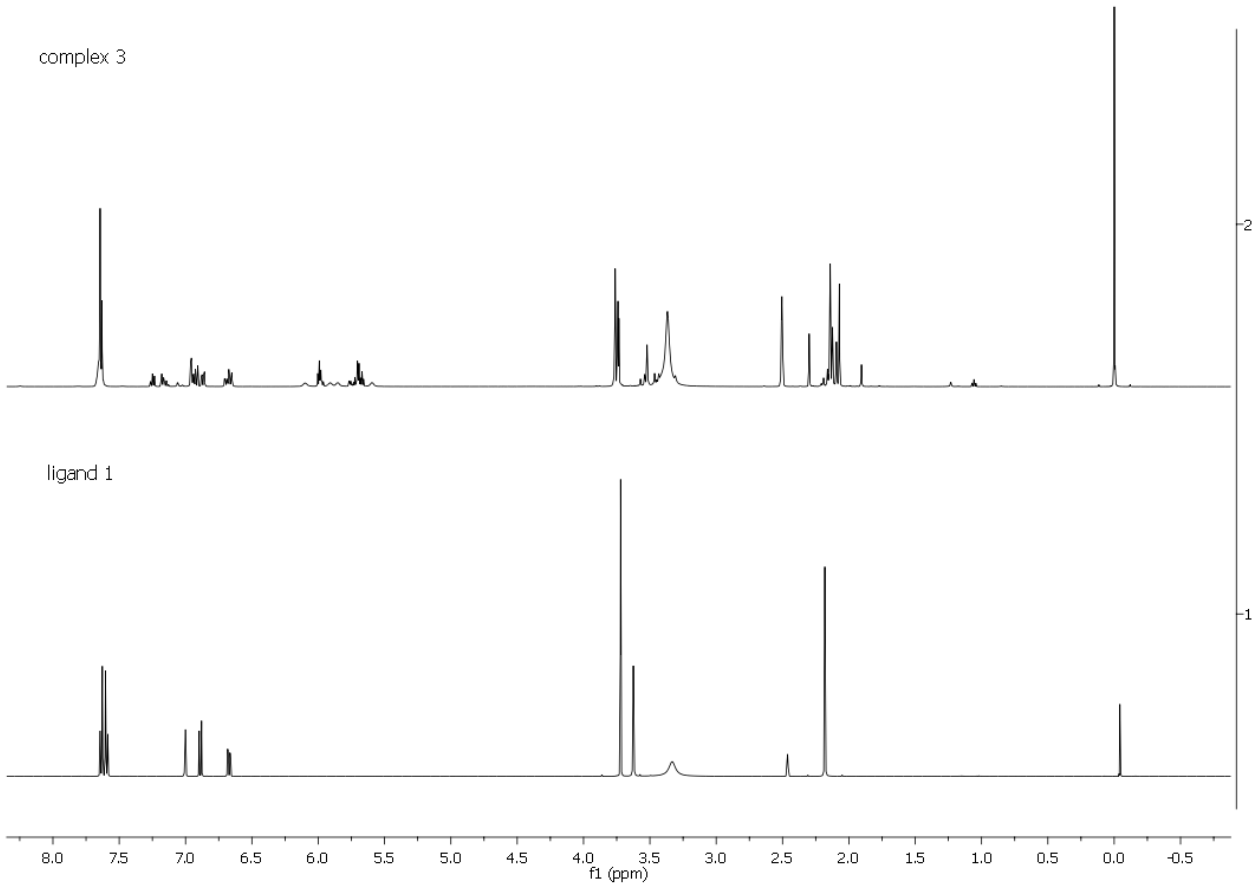

Figure S5. Parallel ^{1}H NMR spectra of ligand1 and complex 3

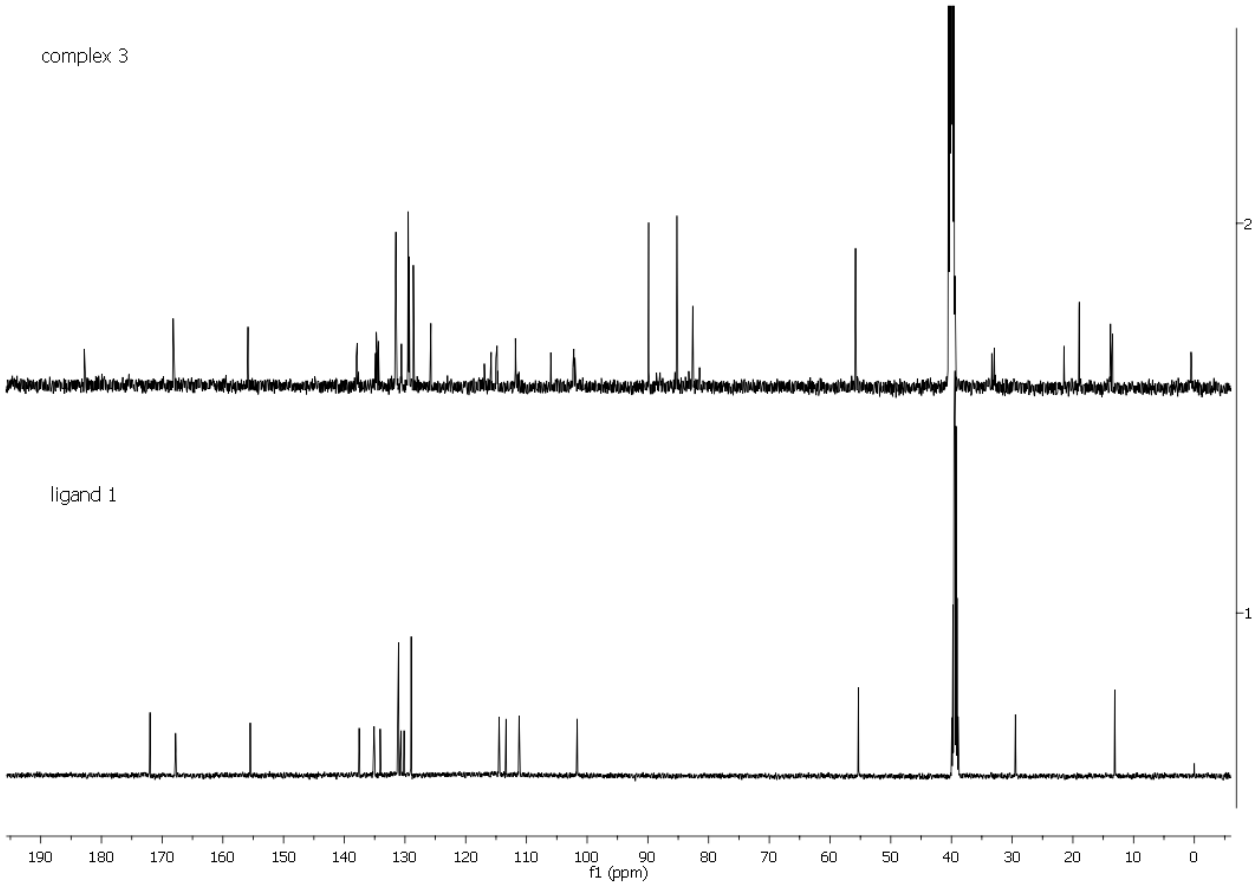

Figure S6. Parallel ^{13}C NMR spectra of ligand 1 and complex 3

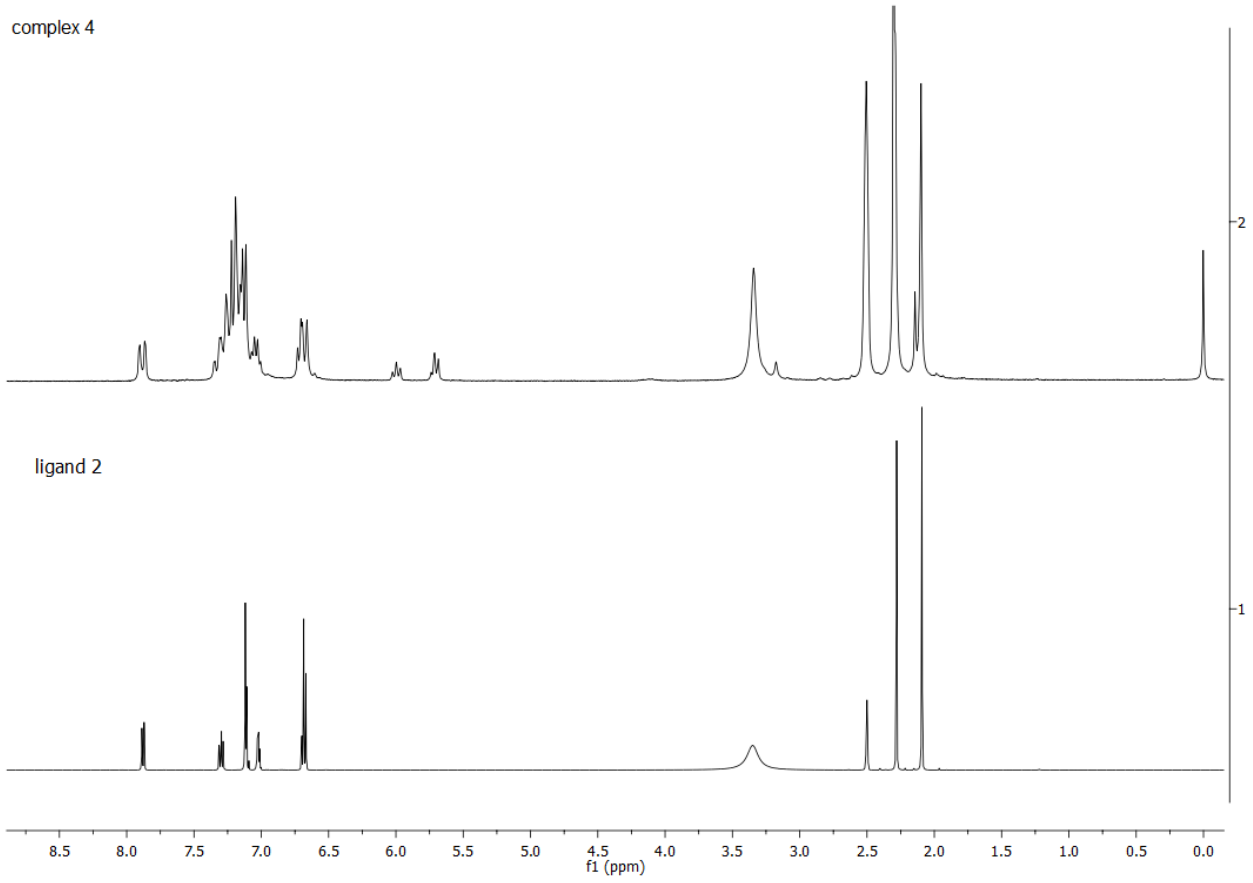

Figure S7. Parallel ^{1}H NMR spectra of ligand 2 and complex 4

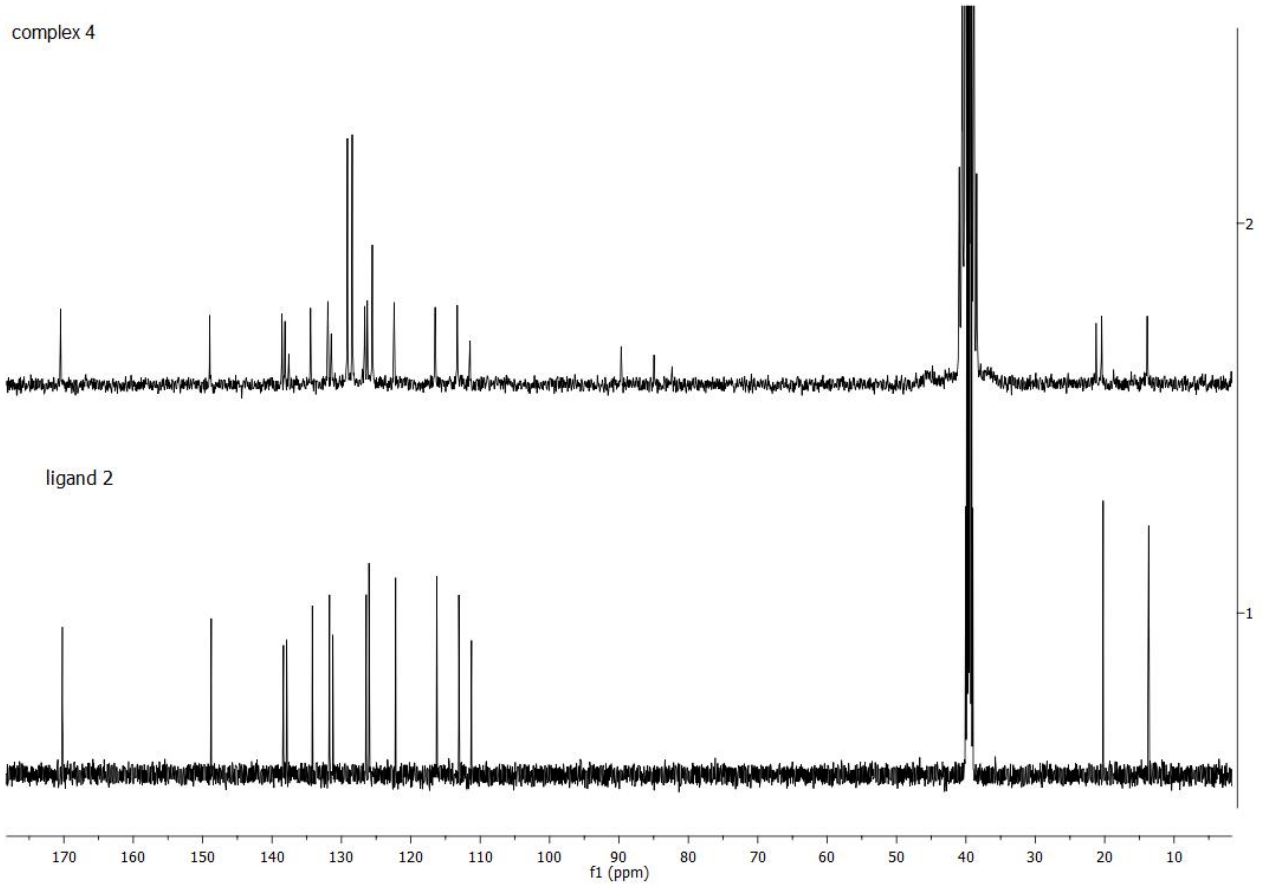

Figure S8. Parallel ^{13}C NMR spectra of ligand 2 and complex 4

S2. MTT assay

Table 1. IC_{50} [μM] values obtained after 72 h of continuous drug action.

Compound	K562	A549	MDA-MB-231	MRC-5	*SI $_{K562}$	*SI $_{A549}$	*SI$_{MDA-MB-231}$
1	11.9±4.4	45.5±2.7	22±3.6	39.6±3.7	3.33	0.87	1.80
2	96.4±2	145.1±6.4	153±1.2	222.6±23.9	2.31	1.53	1.45
3	13.2±6.2	31.7±1.15	26±1.7	42±1.3	3.18	1.32	1.62
4	133±7	142.4±9.3	121.4±1.8	275.7±14.5	2.07	1.94	2.27
Hindo	155.9±11.4	161.5±13.9	244.7±17.8	230.5±17.8	1.48	1.43	0.94
Hmef	143.9±4.1	217.3±46.7	237.9±18.8	>300	>2.08	>1.38	>1.26
CDDP	10.3±1.2	13.6±1.8	15.9±2.1	9.3±0.9	0.90	0.68	0.58

* IC_{50} [μM] values are presented as the mean ± SEM of three independent experiments. > 300 denotes that IC_{50} was not obtained in the range of concentrations tested up to 300 μM.

*SI-selectivity index for tested complexes, ligands and cisplatin, in tumor cell lines (K562, A549 and MDA-MB-231), related to non-tumor MRC-5 cell line: SI_{K562} (IC_{50} MRC-5/IC_{50} K562), SI_{A549} (IC_{50} MRC-5/IC_{50} A549), $SI_{MDA-MB-231}$(IC_{50} MRC-5/IC_{50} MDA-MB-231). SI values for tested complexes and ligands were obviously higher than for cisplatin, particularly in MDA-MB-231 and K562.

S3. Interaction with biomolecules

S3-1. Interaction with serum albumins

The extent of the inner-filter effect can be roughly estimated with the following formula:

$$I_{corr} = I_{meas} \times 10^{\frac{\varepsilon(\lambda_{exc})cd}{2}} \times 10^{\frac{\varepsilon(\lambda_{em})cd}{2}} \qquad \text{(eq. S1)}$$

where I_{corr} = corrected intensity, I_{meas} = the measured intensity, c = the concentration of the quencher, d = the cuvette (1 cm), $\varepsilon(\lambda_{exc})$ and $\varepsilon(\lambda_{em})$ = the ε of the quencher at the excitation and the emission wavelength, respectively, as calculated from the UV-vis spectra of the complexes.[1]

The Stern-Volmer and Scatchard graphs are used in order to study the interaction of a quencher with serum albumins. According to Stern-Volmer quenching equation: [2]

$$\frac{Io}{I} = 1 + k_q \tau_0 [Q] = 1 + K_{SV}[Q] \qquad \text{(eq. S2)}$$

where Io = the initial tryptophan fluorescence intensity of SA, I = the tryptophan fluorescence intensity of SA after the addition of the quencher (i.e. complexes **1**-**4**), k_q = the quenching constant, K_{SV} = the Stern-Volmer constant, τ_o = the average lifetime of SA without the quencher, [Q] = the concentration of the quencher) K_{SV} (in M^{-1}) can be obtained by the slope of the diagram Io/I versus [Q], and subsequently the quenching constant (k_q, in $M^{-1}s^{-1}$) is calculated from eq. S3, with $\tau_o = 10^{-8}$ s as fluorescence lifetime of tryptophan in SA,

$$K_{SV} = k_q \tau_o \qquad \text{(eq. S3)}$$

From the Scatchard equation:[3]

$$\frac{\Delta I / Io}{[Q]} = nK - K\frac{\Delta I}{Io} \qquad \text{(eq. S4)}$$

where n is the number of binding sites per albumin and K is the SA-binding constant, K (in M^{-1}) is calculated from the slope in plots (ΔI/Io)/[Q] versus ΔI/Io and n is given by the ratio of y intercept to the slope.[3]

S3-2. Interaction with CT DNA

The DNA-binding constant (K_b, in M^{-1}) can be obtained by monitoring the changes in the absorbance at the corresponding λ_{max} with increasing concentrations of CT DNA and it is given by the ratio of slope to the y intercept in plots [DNA]/(ε_A-ε_f) versus [DNA], according to the Wolfe-Shimer equation:[4]

$$\frac{[DNA]}{(\varepsilon_A - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \qquad \text{(eq. S5)}$$

where [DNA] is the concentration of DNA in base pairs, $\varepsilon_A = A_{obsd}/[compound]$, ε_f = the extinction coefficient for the free compound and ε_b = the extinction coefficient for the compound in the fully bound form.

S3-3. Competitive studies with EB

The Stern-Volmer constant (K_{SV}, in M^{-1}) is used to evaluate the quenching efficiency for each compound according to the Stern-Volmer equation (eq. S2),[2] where Io and I are the emission intensities of the EB-DNA solution in the absence and the presence of the quencher, respectively, [Q] is the concentration of the quencher (i.e. complexes **1-4**), τ_o = the average lifetime of the emitting system without the quencher and k_q = the quenching constant. K_{SV} may be obtained from the Stern-Volmer plots by the slope of the diagram Io/I versus [Q]. Taking τ_o = 23 ns as the fluorescence lifetime of the EB-DNA system,[5] the quenching constants (k_q, in $M^{-1}s^{-1}$) of the compounds can be determined according to eq. (S3).

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Table S2. The BSA and HSA binding constants and parameters (K_{sv}, k_q, K, n) for complexes **1-4.**

Compound	**Ksv (M^{-1})**	**k_q ($M^{-1}s^{-1}$)**	**K (M^{-1})**	**n**
BSA				
K[Ru(η^6-*p*-cymene)(indo)Cl_2], **1**	1.25(±0.05)×10^5	1.25(±0.05)×10^{13}	4.49(±0.30)×10^5	0.68
(NH_4)[Ru(η^6-*p*-cymene)(mef)Cl_2], **2**	1.70(±0.08)×10^5	1.70(±0.08)×10^{13}	3.63(±0.15)×10^5	0.86
K[Ru(η^6-*p*-toluene)(indo)Cl_2], **3**	4.85(±0.11)×10^4	4.85(±0.11)×10^{12}	5.30(±0.18)×10^4	0.96
(NH_4)[Ru(η^6-*p*-toluene)(mef)Cl_2], **4**	1.30(±0.04)×10^5	1.30(±0.04)×10^{13}	2.63(±0.10)×10^5	0.84
HSA				
K[Ru(η^6-*p*-cymene)(indo)Cl_2], **1**	6.10(±0.29)×10^4	6.10(±0.29)×10^{12}	2.15(±0.08)×10^5	0.57
(NH_4)[Ru(η^6-*p*-cymene)(mef)Cl_2], **2**	5.46(±0.19)×10^4	5.46(±0.19)×10^{12}	9.79(±0.34)×10^4	0.78
K[Ru(η^6-*p*-toluene)(indo)Cl_2], **3**	2.04(±0.13)×10^4	2.04(±0.13)×10^{12}	9.44(±0.40)×10^4	0.31
(NH_4)[Ru(η^6-*p*-toluene)(mef)Cl_2], **4**	4.32(±0.29)×104	4.32(±0.29)×1012	4.24(±0.12)×10^5	0.37

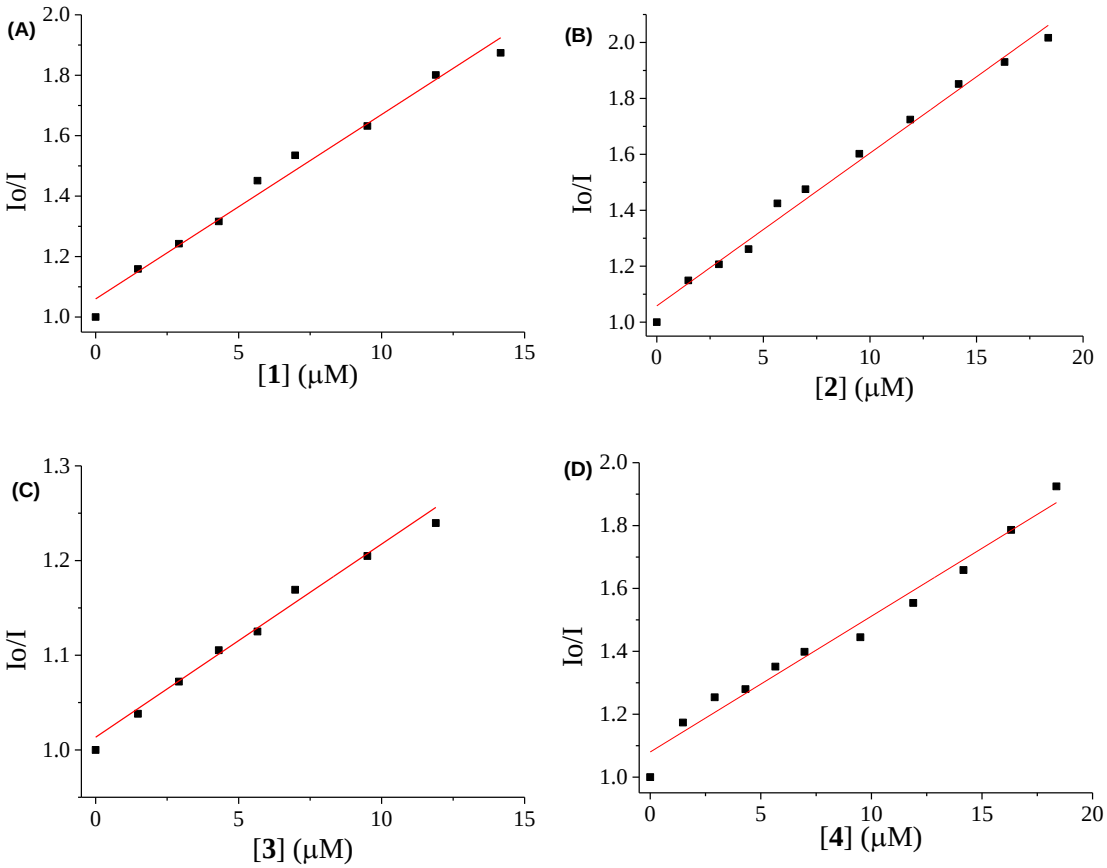

Figure S9. Stern-Volmer quenching plot of HSA for complexes (A)-(D) **1**-**4**, respectively.

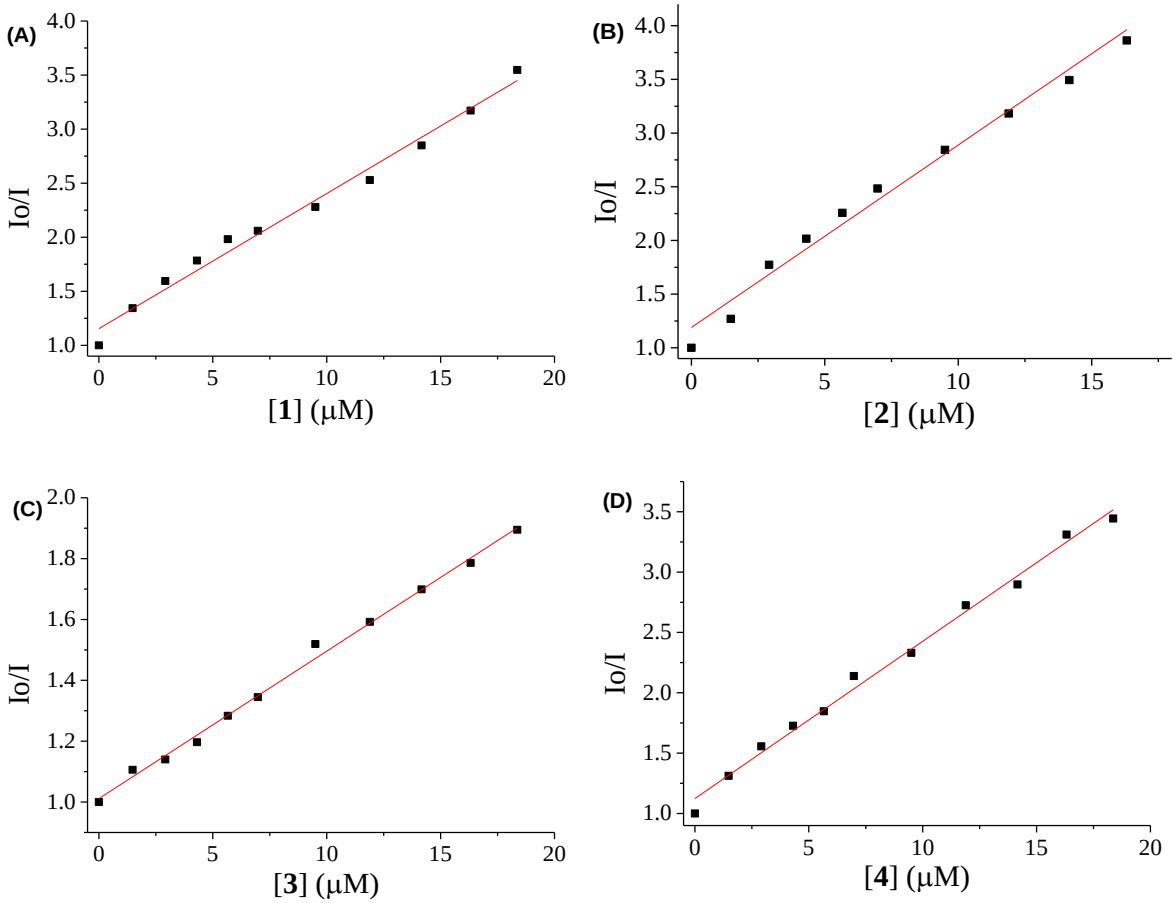

Figure S10. Stern-Volmer quenching plot of BSA for complexes (A)-(D) **1**-**4**, respectively.

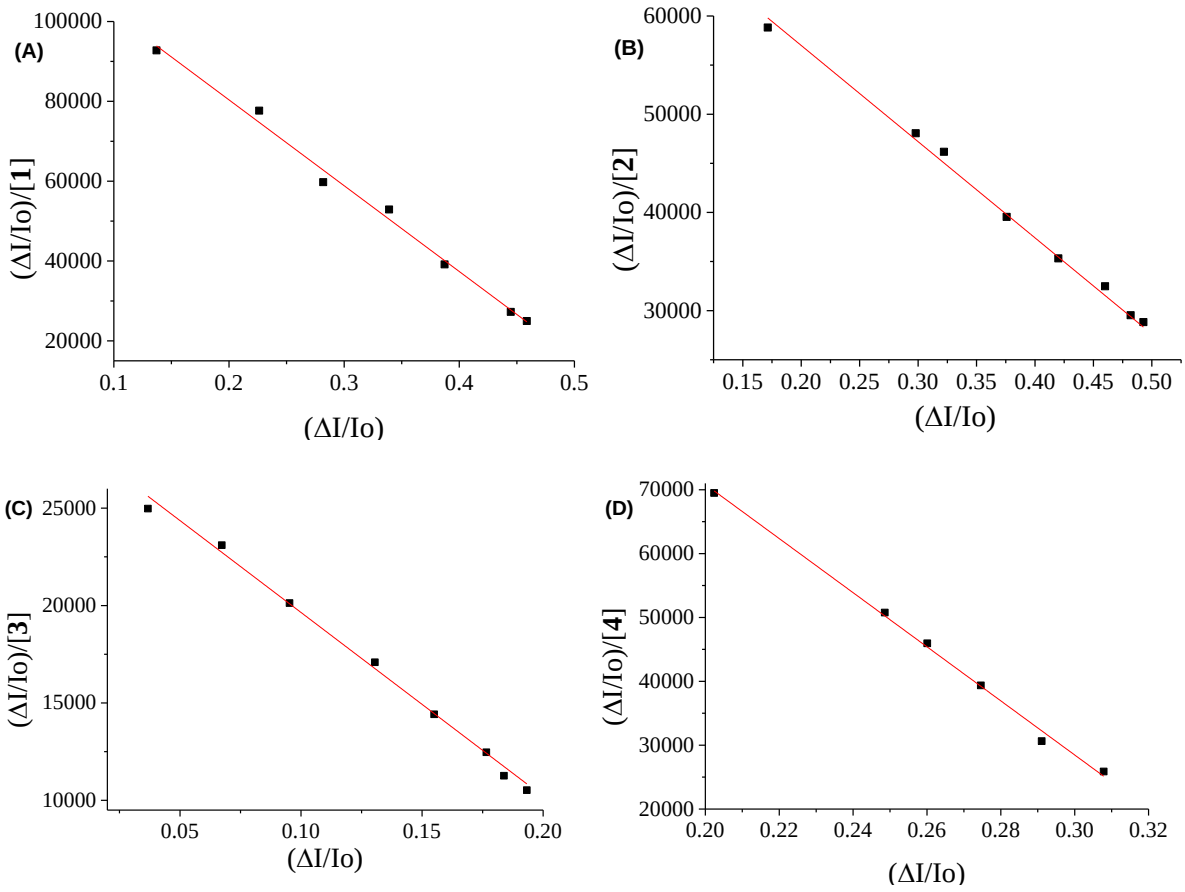

Figure S11. Scatchard plot of HSA for complexes (A)-(D) **1**-**4**, respectively.

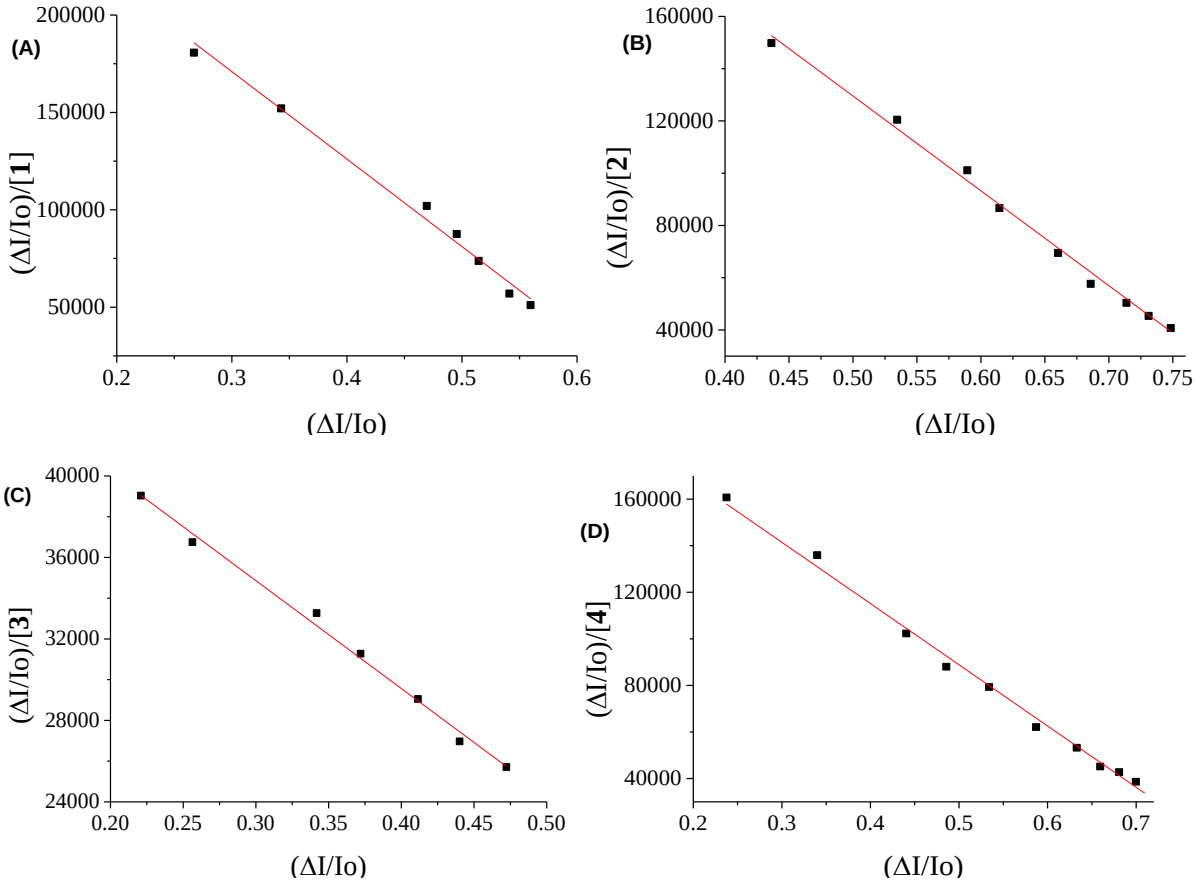

Figure S12. Scatchard plot of BSA for complexes (A)-(D) **1-4**, respectively.

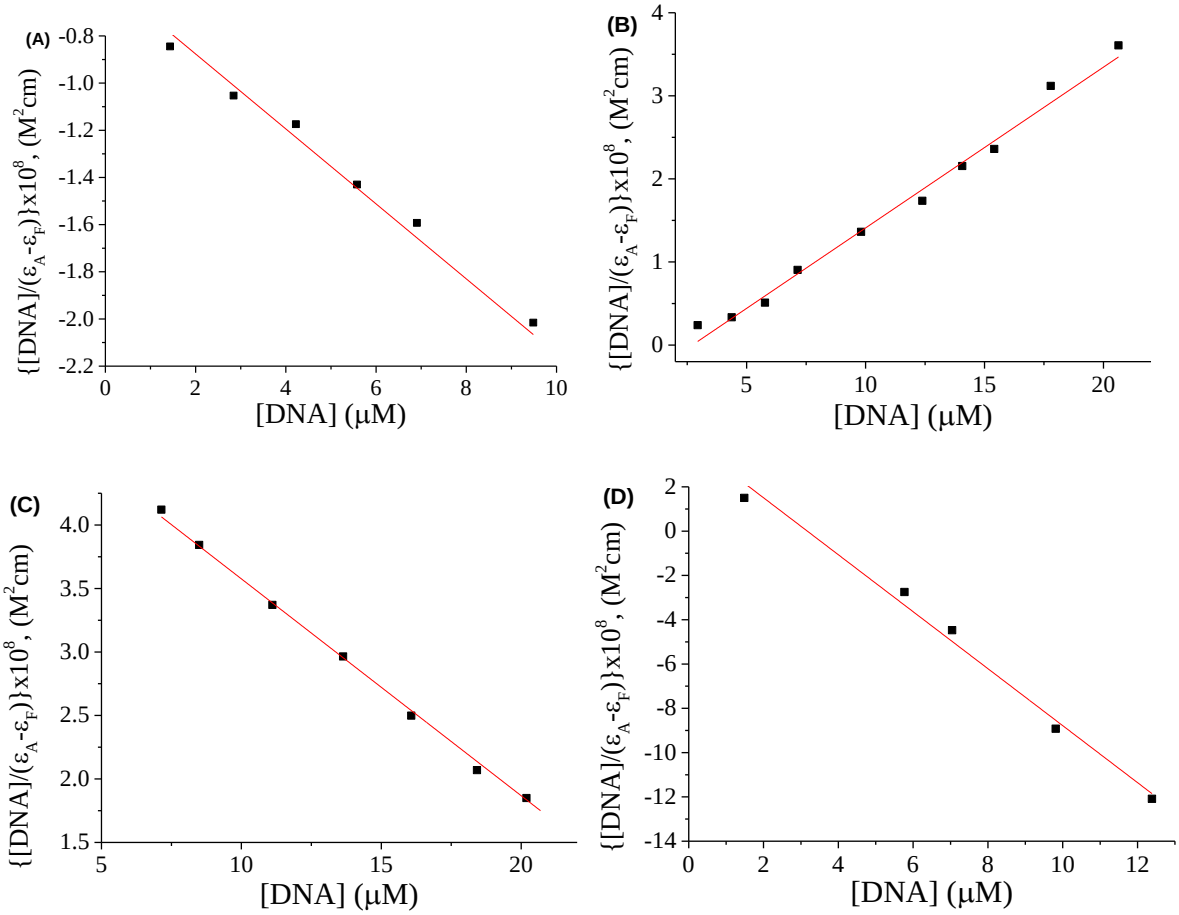

Figure S13. Plot of [DNA]/(ε_A-ε_f) vs [DNA] for complexes (A)-(D) **1**-**4**, respectively.

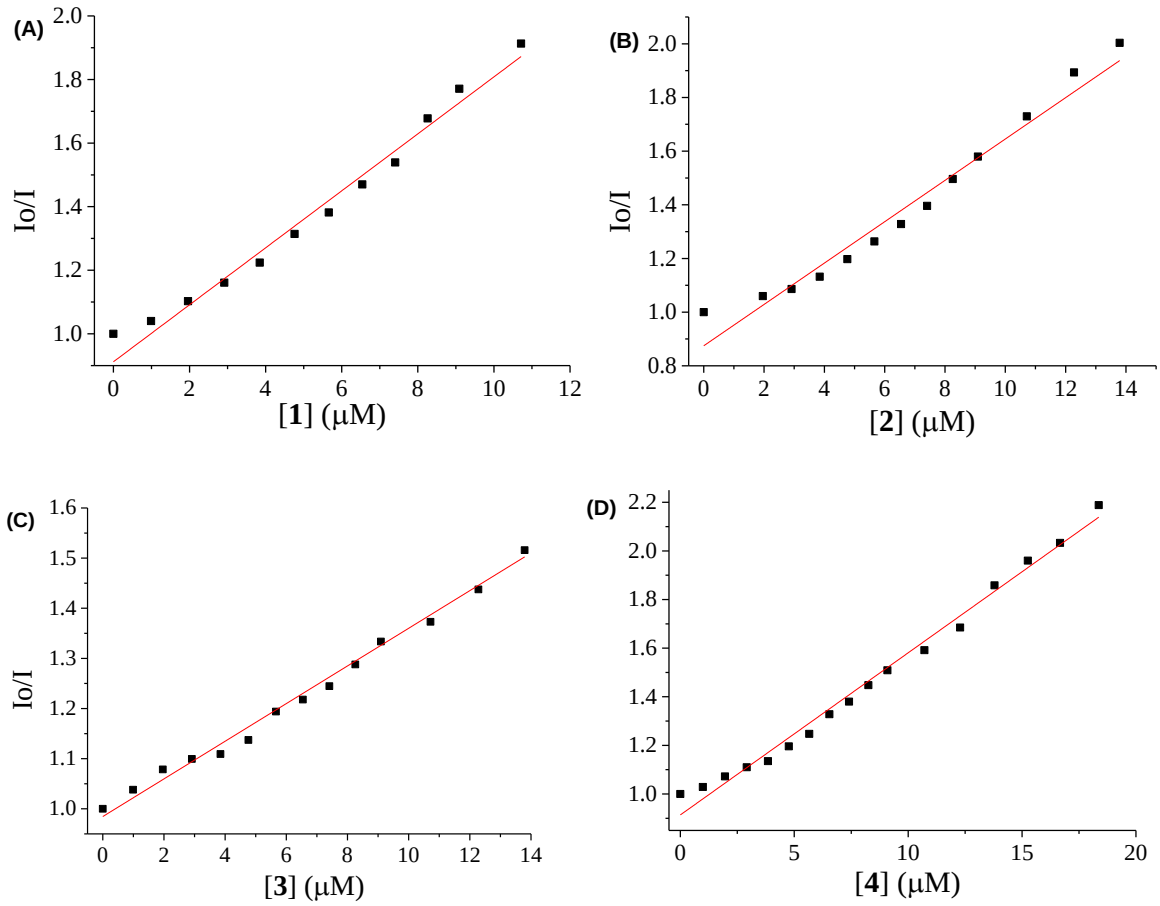

Figure S14. Stern-Volmer quenching plot of EB-DNA fluorescence for complexes (A)-(D) **1-4**, respectively.