

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

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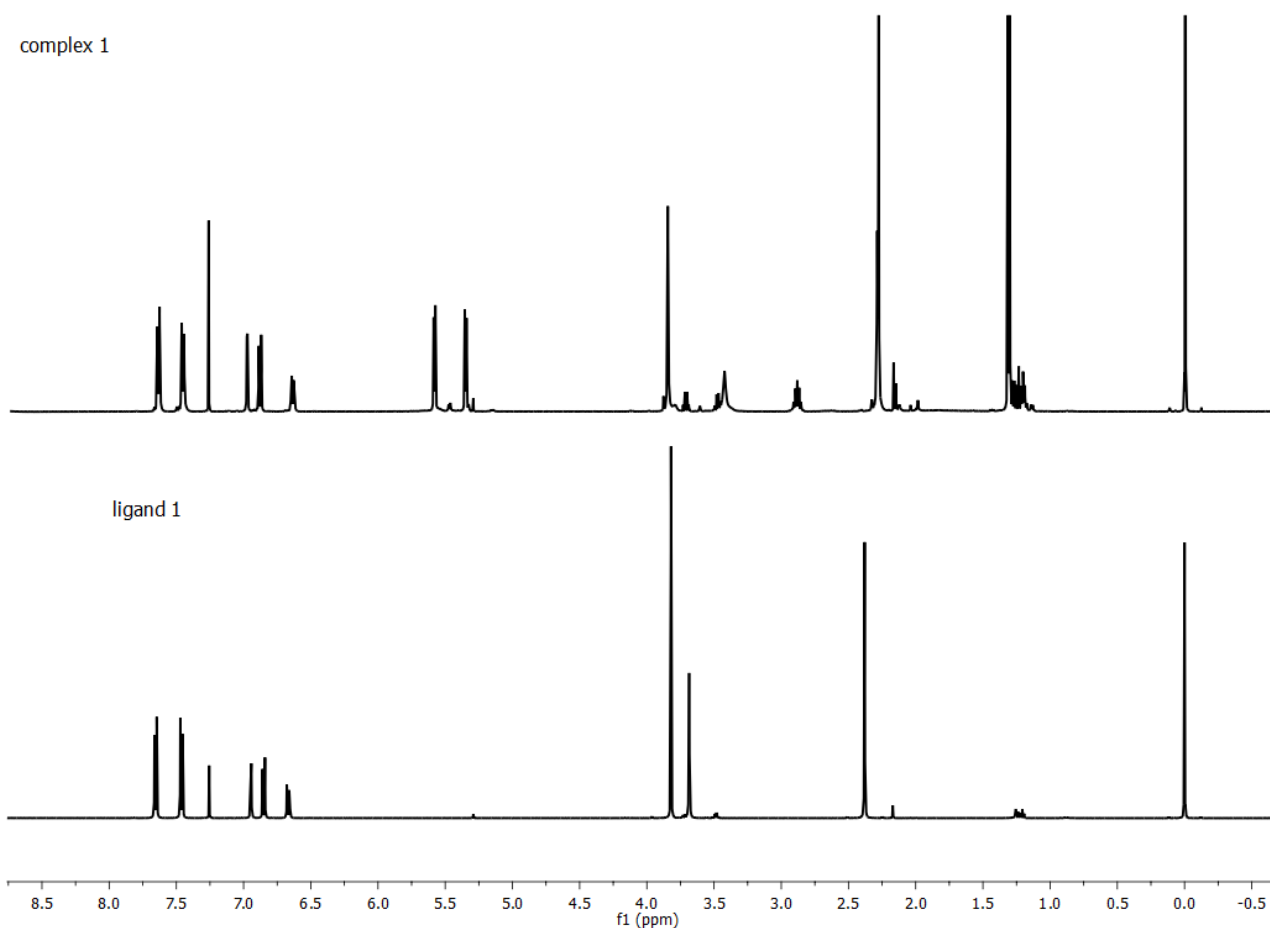
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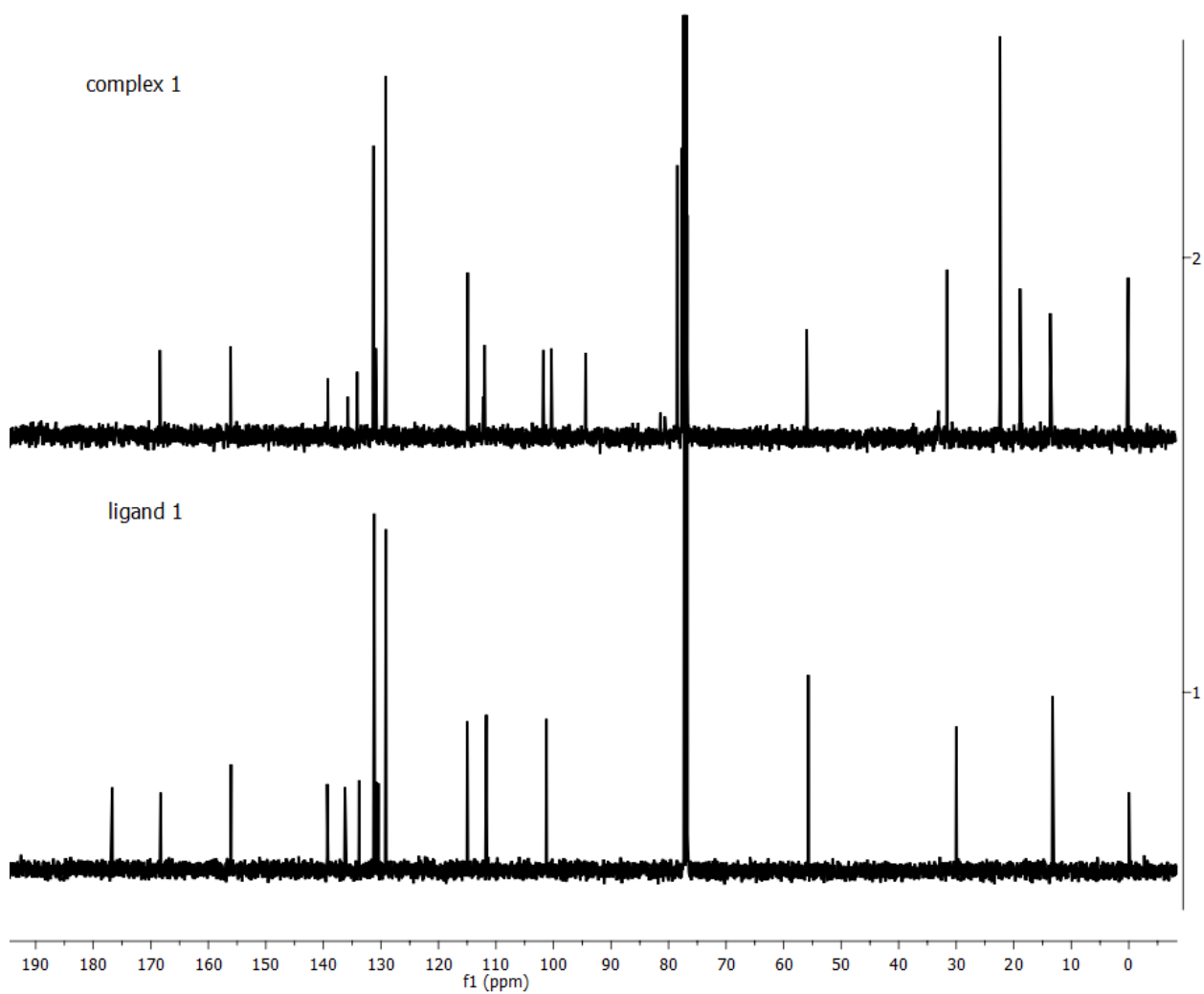
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### Supplementary material

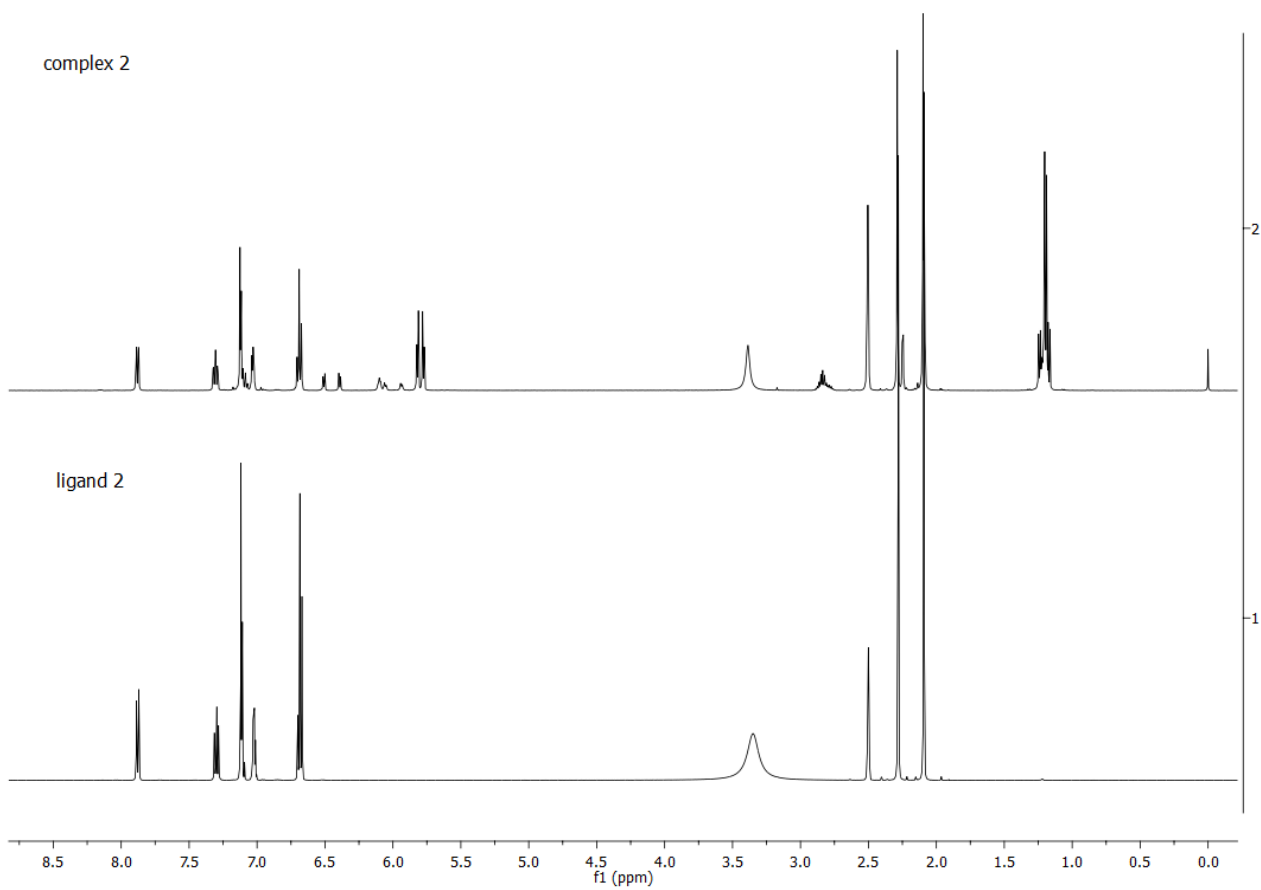
#### S1. NMR spectra of synthesized complexes



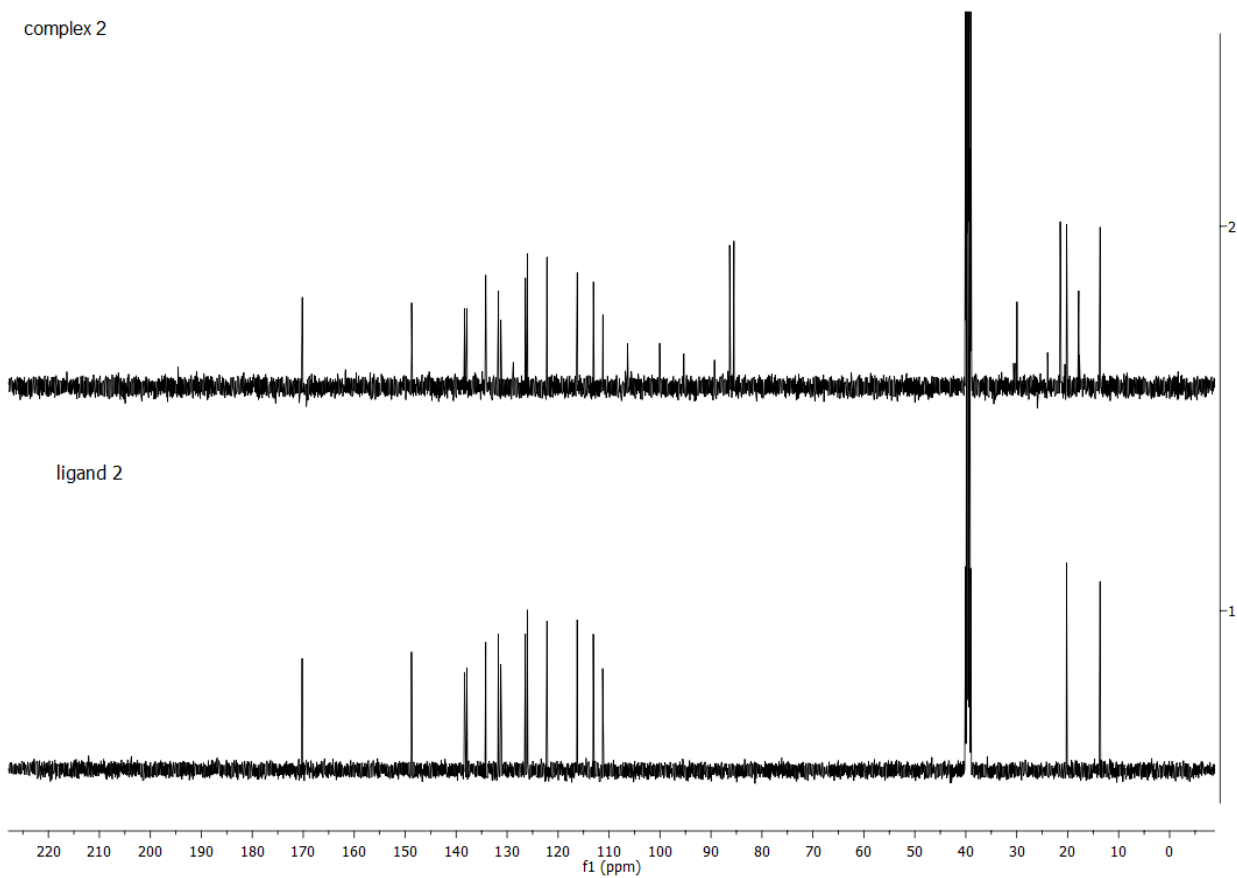
**Figure S1.** Parallel  $^1\text{H}$  NMR spectra of ligand1 and complex 1



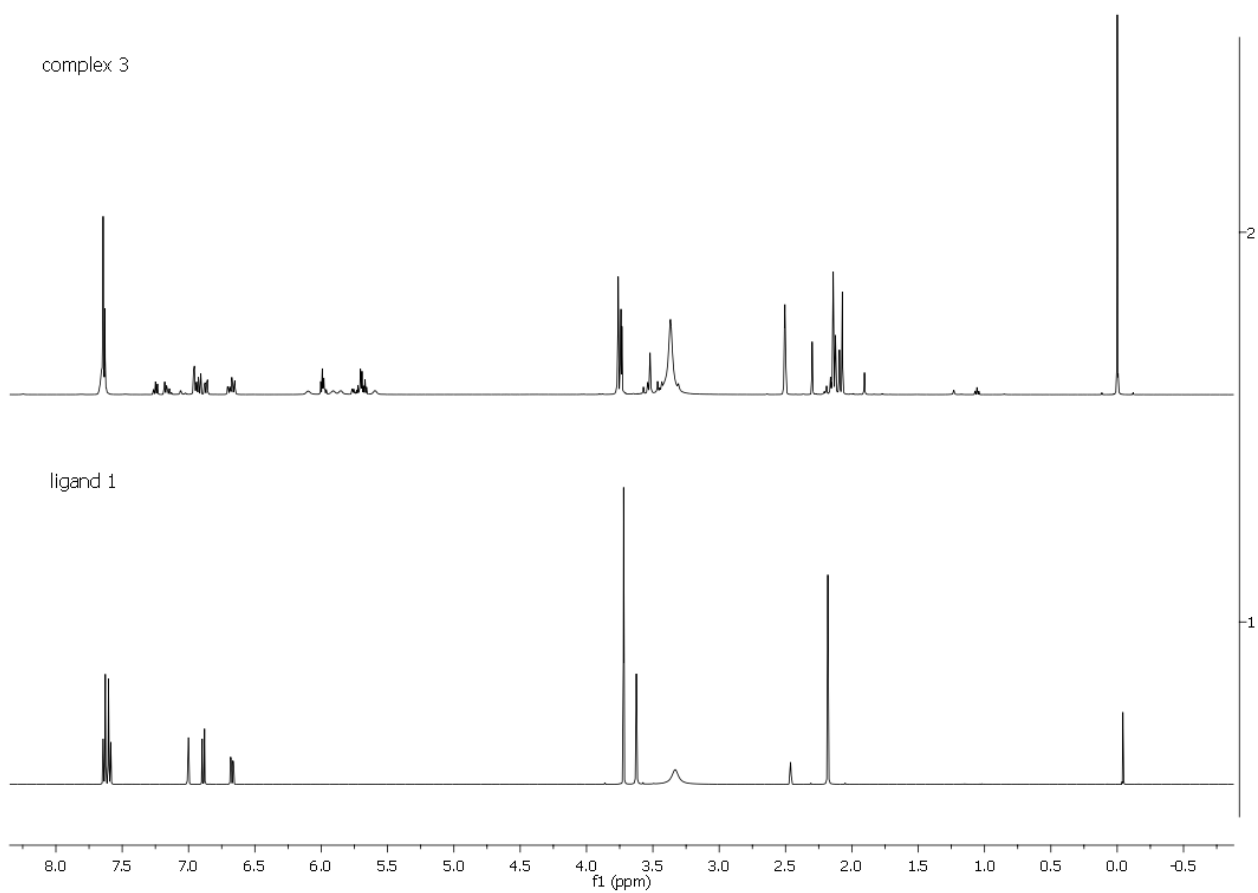
**Figure S2.** Parallel  $^{13}\text{C}$  NMR spectra of ligand 1 and complex 1



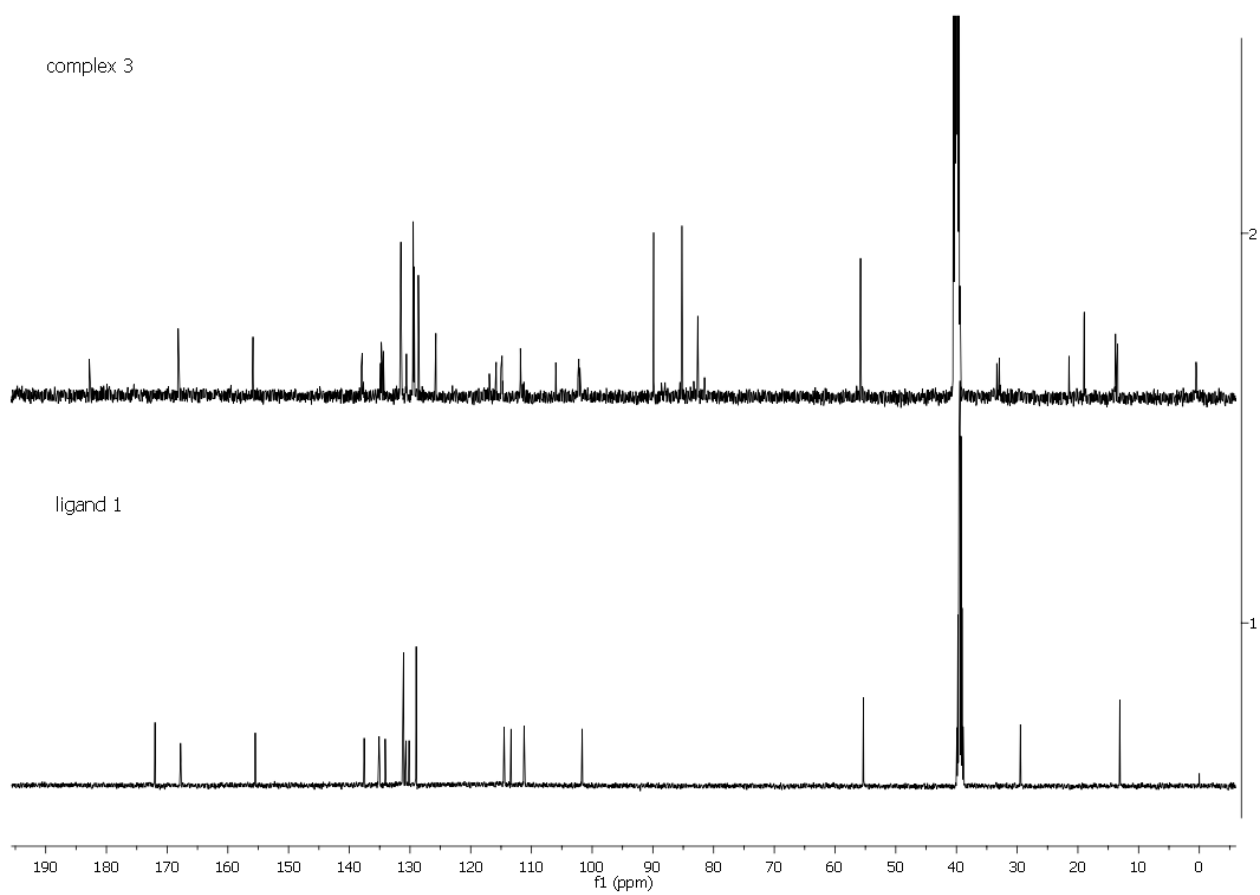
**Figure S3.** Parallel <sup>1</sup>H NMR spectra of ligand 2 and complex 2



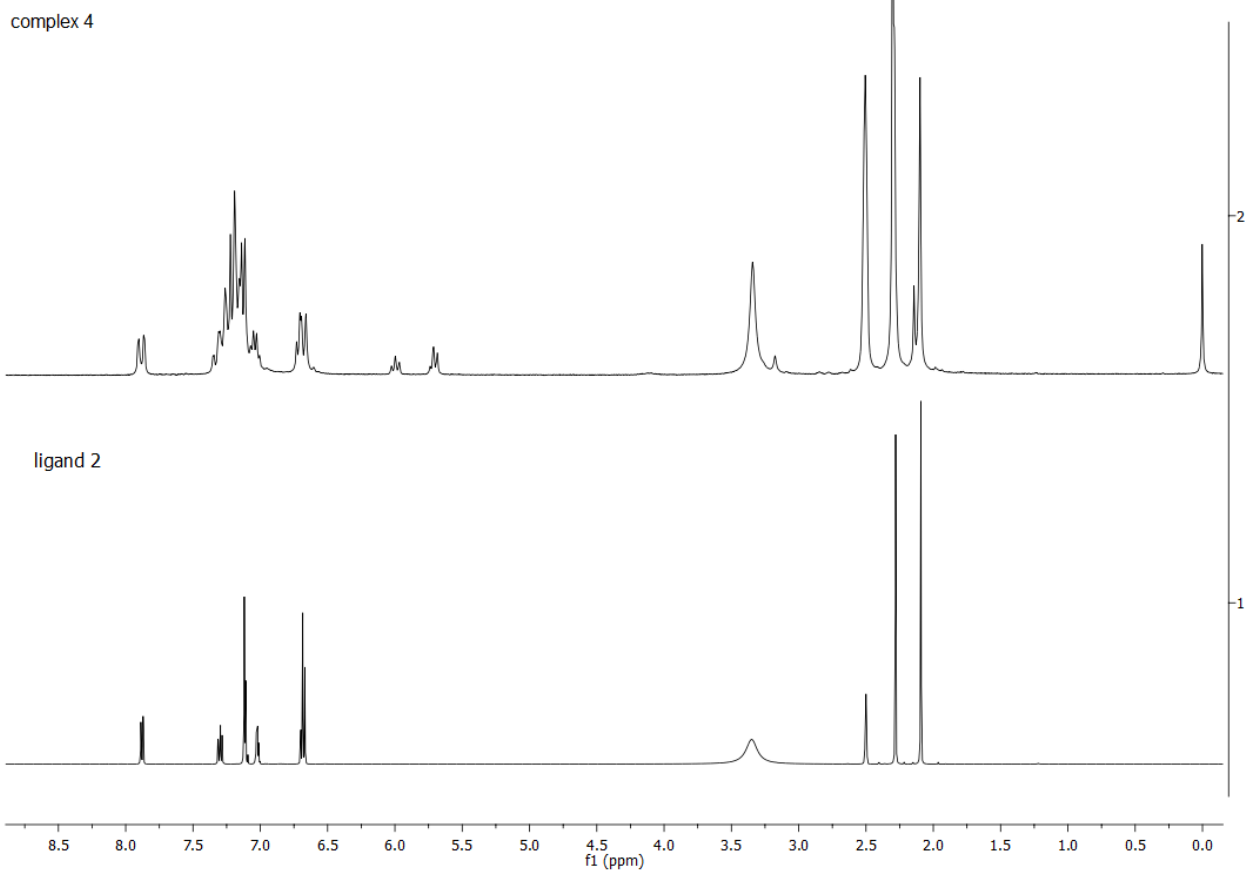
**Figure S4.** Parallel <sup>13</sup>C NMR spectra of ligand 2 and complex 2



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

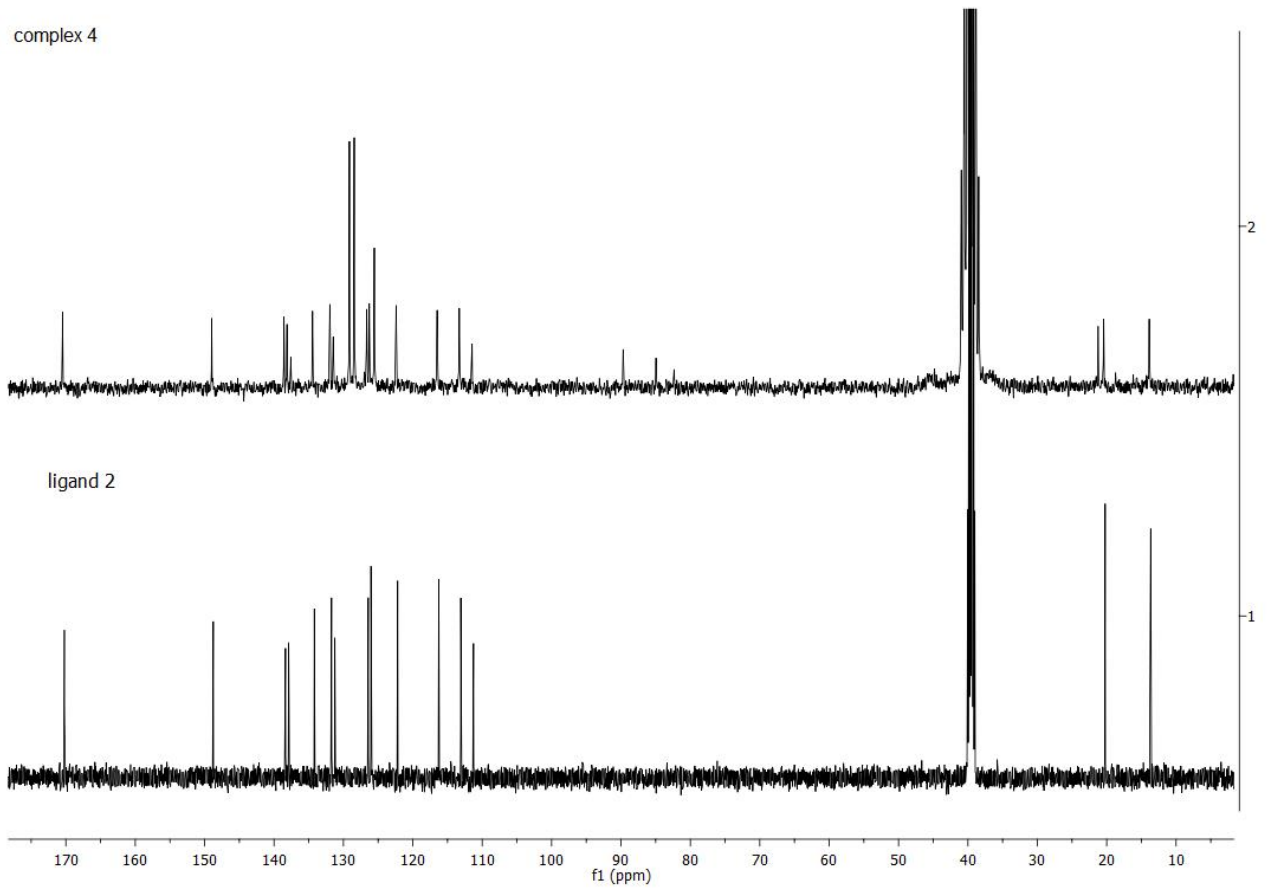


**Figure S6.** Parallel  $^{13}\text{C}$  NMR spectra of ligand 1 and complex 3



**Figure S7.** Parallel <sup>1</sup>H NMR spectra of ligand 2 and complex 4

complex 4



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

## S2. MTT assay

**Table 1.** IC<sub>50</sub> [μM] values obtained after 72 h of continuous drug action.

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

\* IC<sub>50</sub> [μM] values are presented as the mean ± SEM of three independent experiments. > 300 denotes that IC<sub>50</sub> 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<sub>K562</sub> (IC<sub>50</sub> MRC-5/IC<sub>50</sub> K562), SI<sub>A549</sub> (IC<sub>50</sub> MRC-5/IC<sub>50</sub> A549), SI<sub>MDA-MB-231</sub> (IC<sub>50</sub> MRC-5/IC<sub>50</sub> 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:

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

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

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:<sup>2</sup>

$$\frac{I_0}{I} = 1 + k_q \tau_0 [Q] = 1 + K_{\text{SV}} [Q] \quad (\text{eq. S2})$$

where  $I_0$  = 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,  $K_{\text{SV}}$  = the Stern-Volmer constant,  $\tau_0$  = the average lifetime of SA without the quencher,  $[Q]$  = the concentration of the quencher)  $K_{\text{SV}}$  (in  $M^{-1}$ ) can be obtained by the slope of the diagram  $I_0/I$  versus  $[Q]$ , and subsequently the quenching constant ( $k_q$ , in  $M^{-1}s^{-1}$ ) is calculated from eq. S3, with  $\tau_0 = 10^{-8}$  s as fluorescence lifetime of tryptophan in SA,

$$K_{\text{SV}} = k_q \tau_0 \quad (\text{eq. S3})$$

From the Scatchard equation:<sup>3</sup>

$$\frac{\Delta I/I_0}{[Q]} = nK - K \frac{\Delta I}{I_0} \quad (\text{eq. S4})$$

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  $(\Delta I/I_0)/[Q]$  versus  $\Delta I/I_0$  and  $n$  is given by the ratio of y intercept to the slope.<sup>3</sup>

#### 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  $\lambda_{\text{max}}$  with increasing concentrations of CT DNA and it is given by the ratio of slope to the y intercept in plots  $[DNA]/(\varepsilon_A - \varepsilon_f)$  versus  $[DNA]$ , according to the Wolfe-Shimer equation:<sup>4</sup>

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

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

### S3-3. Competitive studies with EB

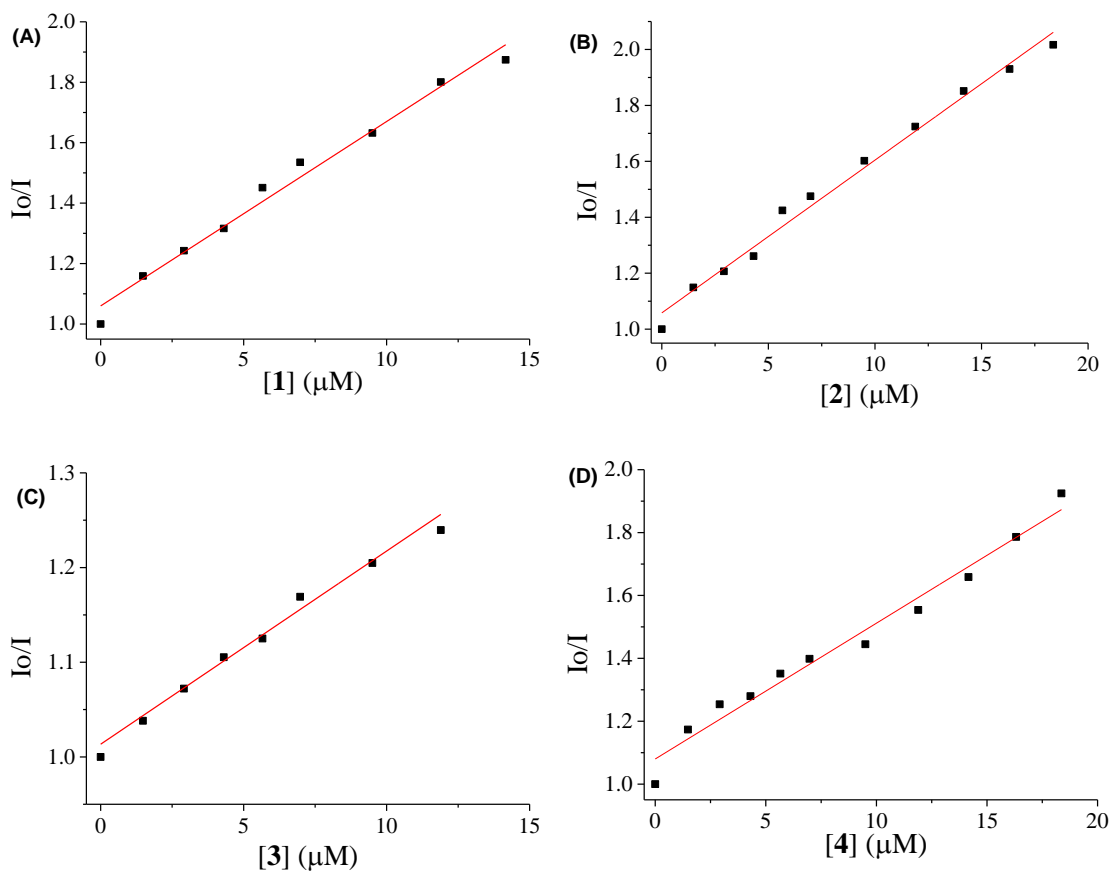
The Stern-Volmer constant ( $K_{SV}$ , in  $M^{-1}$ ) is used to evaluate the quenching efficiency for each compound according to the Stern-Volmer equation (eq. S2),<sup>2</sup> where  $I_0$  and  $I$  are the emission intensities of the EB-DNA solution in the absence and the presence of the quencher, respectively,  $[Q]$  is the concentration of the quencher (i.e. complexes **1-4**),  $\tau_0$  = 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  $I_0/I$  versus  $[Q]$ . Taking  $\tau_0 = 23$  ns as the fluorescence lifetime of the EB-DNA system,<sup>5</sup> the quenching constants ( $k_q$ , in  $M^{-1}s^{-1}$ ) of the compounds can be determined according to eq. (S3).

### References

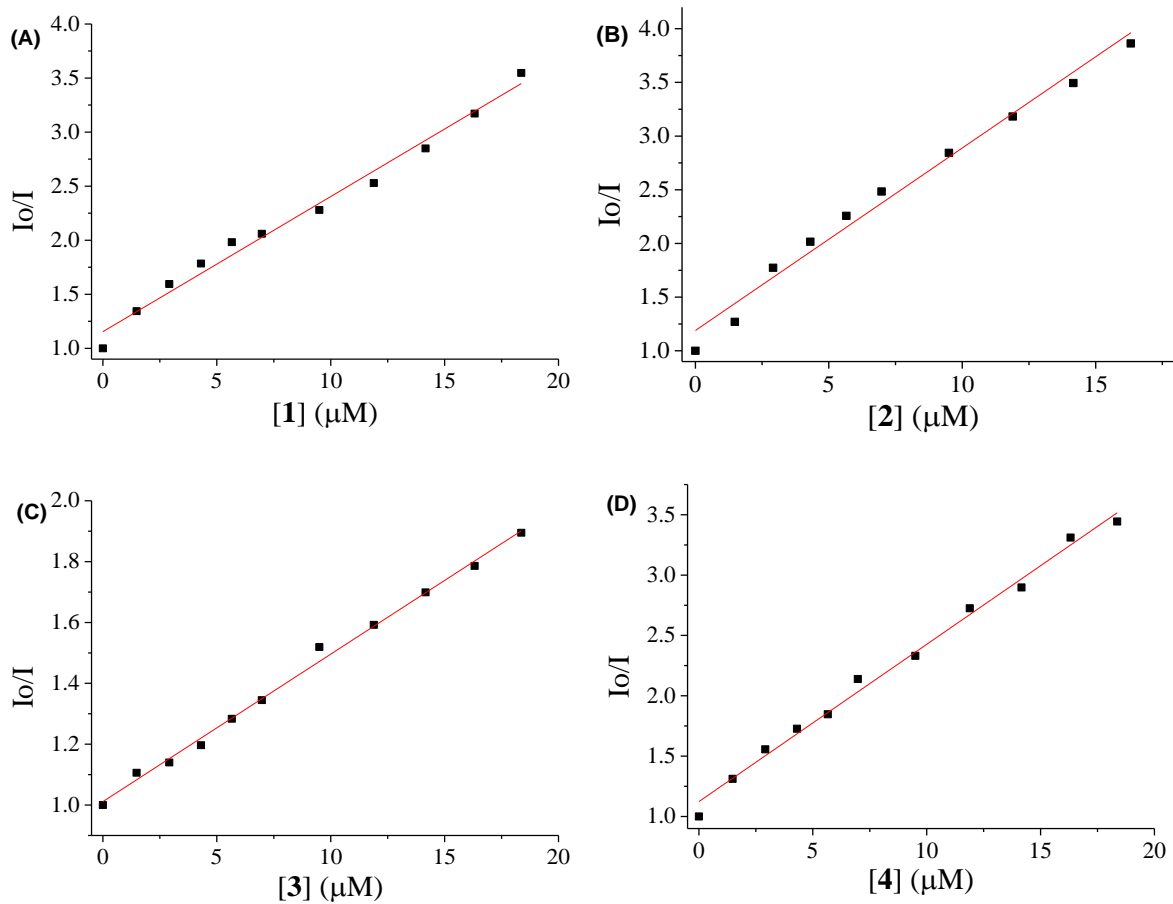
- 1 L. Stella, A.L. Capodilupo and M. Bietti, *Chem. Commun.*, 2008, 4744.
- 2 J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, third ed., Plenum Press, New York, 2006.
- 3 Y. Wang, H. Zhang, G. Zhang, W. Tao and S. Tang, *J. Luminescence*, 2007, **126**, 211.
- 4 A. Wolfe, G. Shimer and T. Meehan, *Biochemistry*, 1987, **26**, 6392.
- 5 D.P. Heller and C.L. Greenstock, *Biophys. Chem.*, 1994, **50**, 305.

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

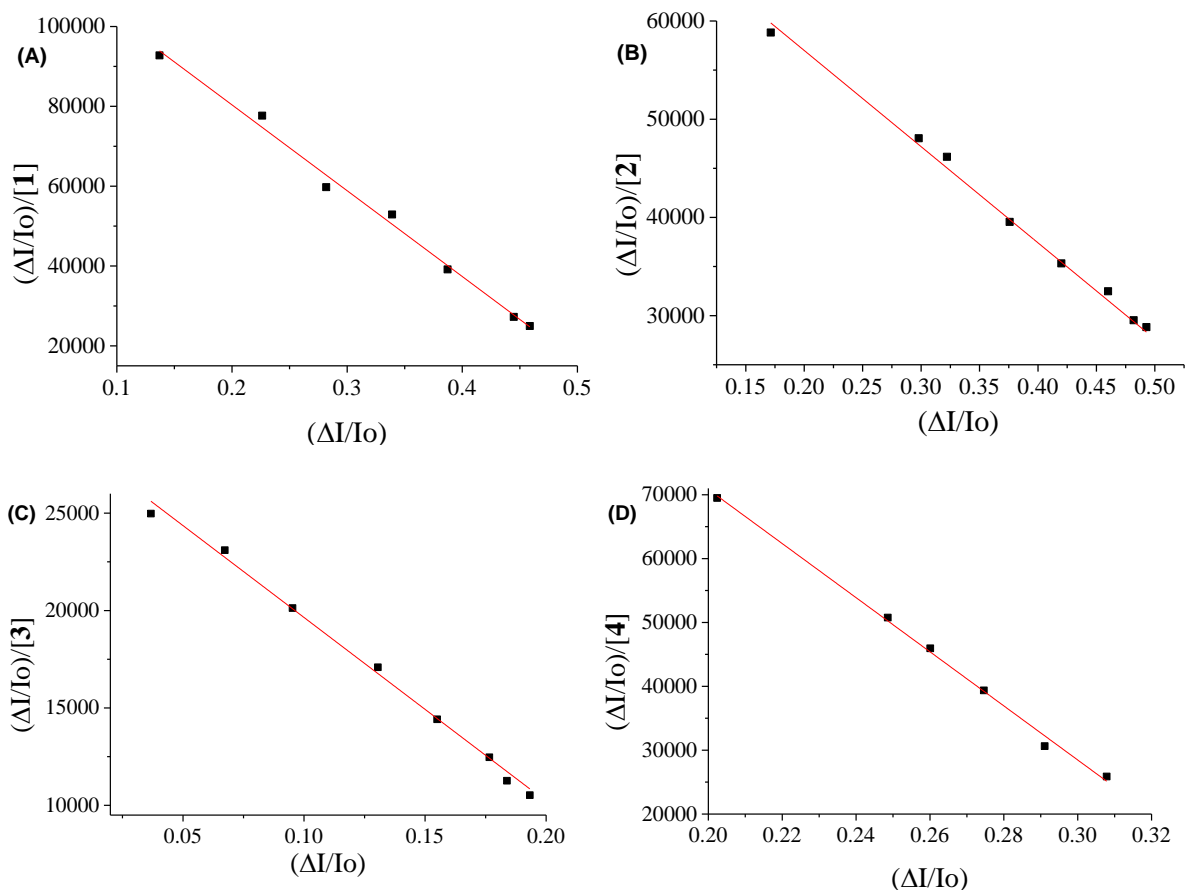
Compound	$K_{sv}$ ( $M^{-1}$ )	$k_q$ ( $M^{-1}s^{-1}$ )	$K$ ( $M^{-1}$ )	$n$
BSA				
$K[Ru(\eta^6\text{-}p\text{-cymene})(\text{indo})Cl_2]$ , <b>1</b>	$1.25(\pm 0.05) \times 10^5$	$1.25(\pm 0.05) \times 10^{13}$	$4.49(\pm 0.30) \times 10^5$	0.68
$(NH_4)[Ru(\eta^6\text{-}p\text{-cymene})(\text{mef})Cl_2]$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



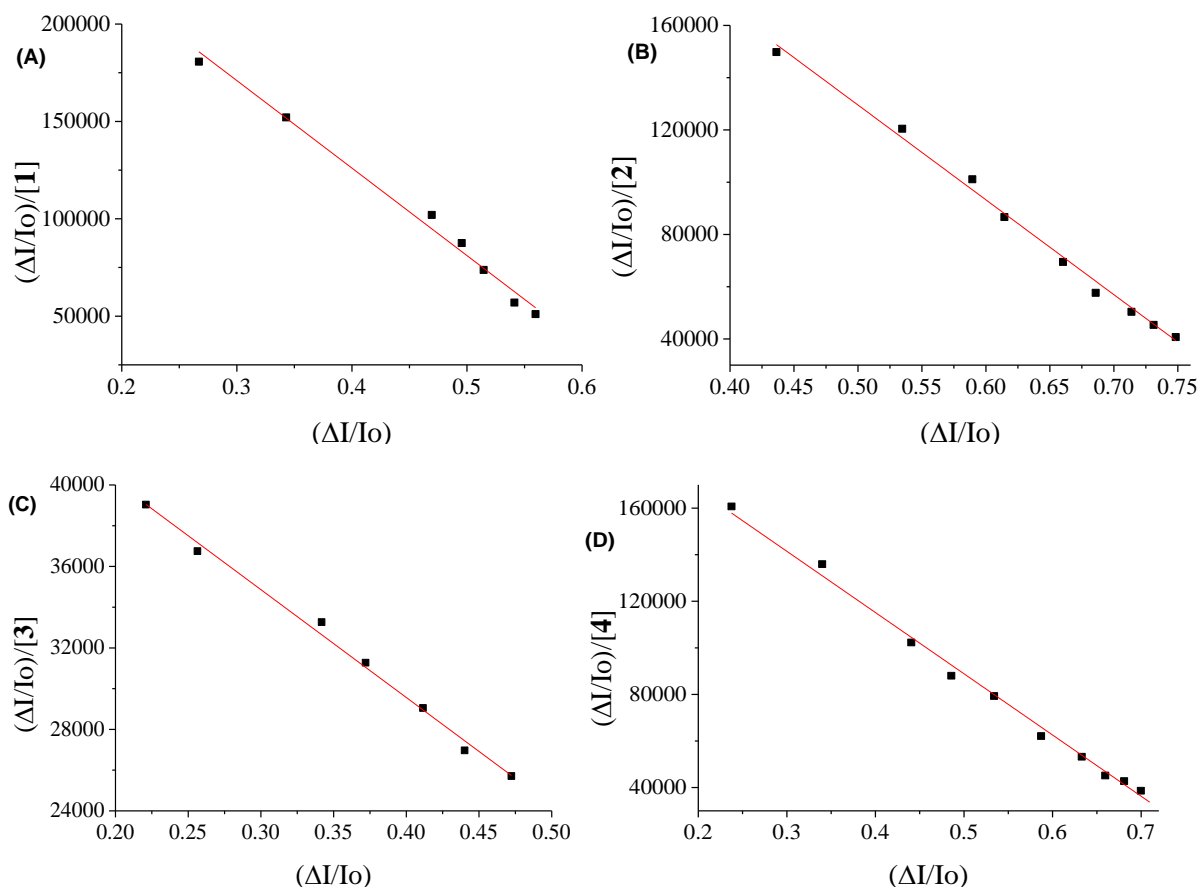
**Figure S9.** Stern-Volmer quenching plot of HSA for complexes (A)-(D) **1-4**, respectively.



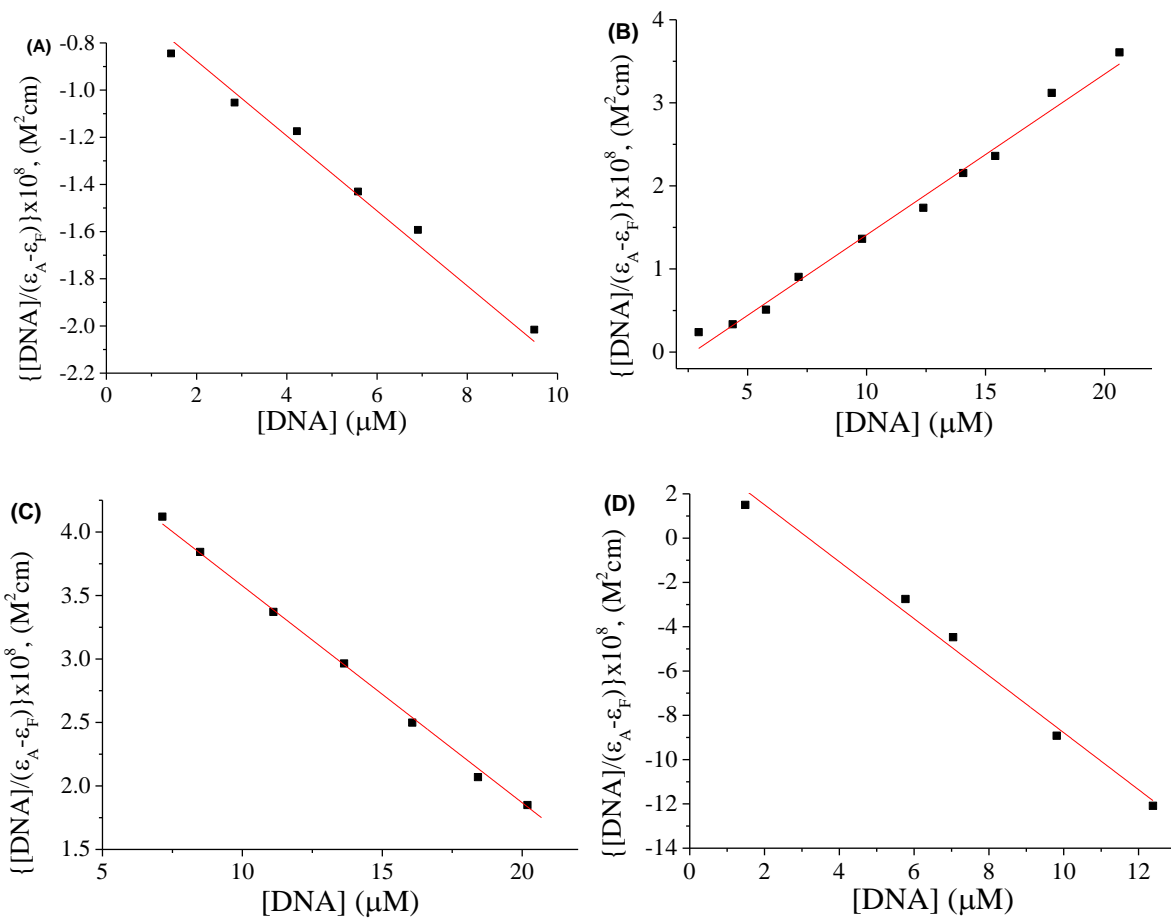
**Figure S10.** Stern-Volmer quenching plot of BSA for complexes (A)-(D) **1-4**, respectively.



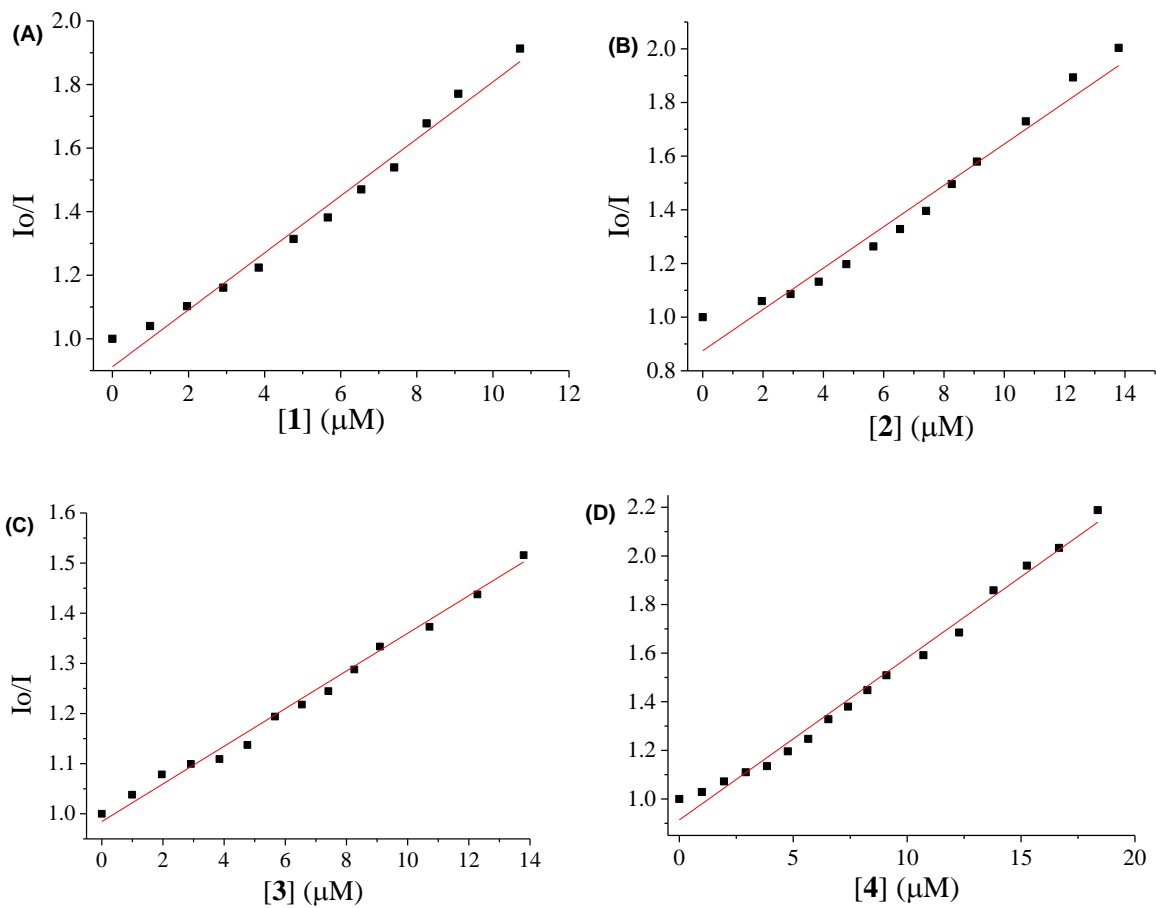
**Figure S11.** Scatchard plot of HSA for complexes (A)-(D) 1-4, respectively.



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



**Figure S13.** Plot of  $[DNA]/(\epsilon_A - \epsilon_f)$  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.