

## Supplementary Information

### Highly Active Copper(I) Complexes of Aroylthiourea Ligands Against Cancer Cells – Synthetic and Biological Studies

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### **MTT Assay**

The all cell lines were cultured and maintained in standard Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum. The cells were stored at 37 °C under a humidified condition with 5% CO<sub>2</sub> and 95% air. Briefly, cells were seeded in 96-well plates (2×10<sup>4</sup>/well) and incubated with fresh DMEM at 37 °C under 5% CO<sub>2</sub> and 95% air for 24 h. After the cells were washed twice with phosphate-buffered saline (PBS), the medium was changed to serially diluted test samples in DMEM with a control and blank in each test plate. After 24 h of incubation with each test compound in DMEM, the cells were washed twice with PBS and replaced with 100 μL of DMEM containing a MTT solution. After 3-4 h of incubation, the absorbance at 570 nm was measured (ELISA Reader, BioRad). Cell viability was calculated from the mean values of data from three wells by using the following equation:

$$\text{Cell viability (\%)} = [\text{Abs}_{(\text{Test sample})} - \text{Abs}_{(\text{Blank})}] / [\text{Abs}_{(\text{Control})} - \text{Abs}_{(\text{Blank})}] \times 100 \%$$

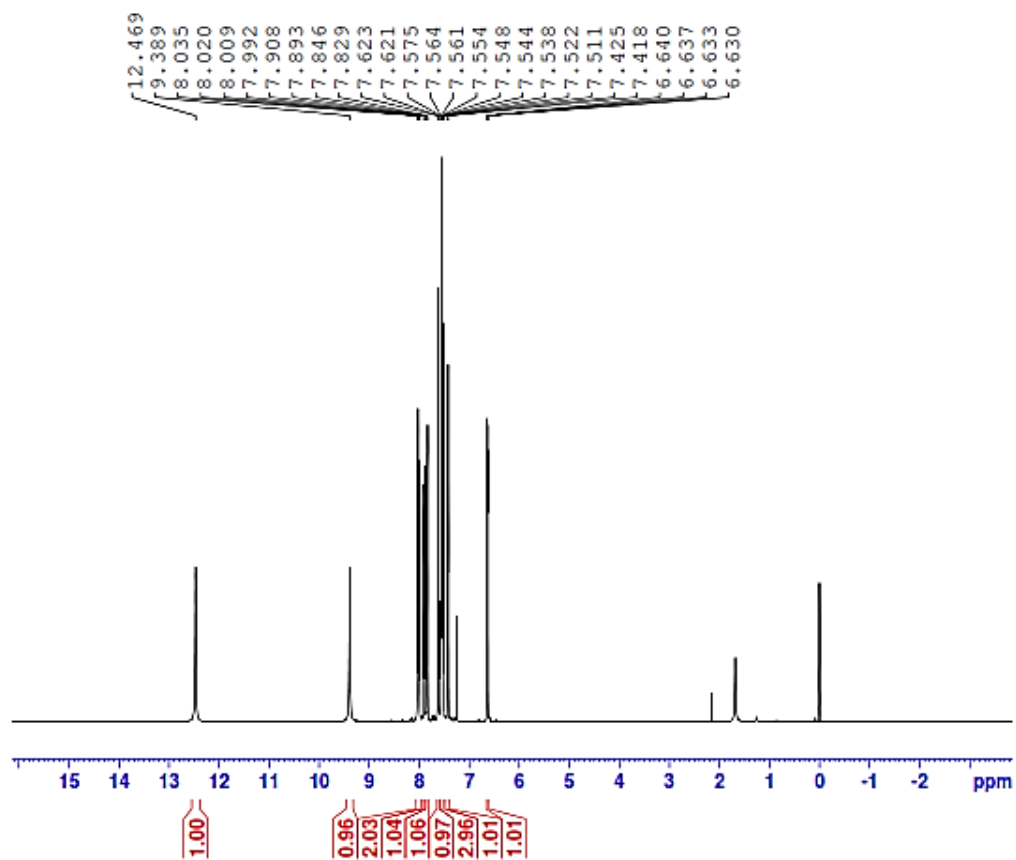


Fig. S1  $^1\text{H}$  NMR spectrum of L4.

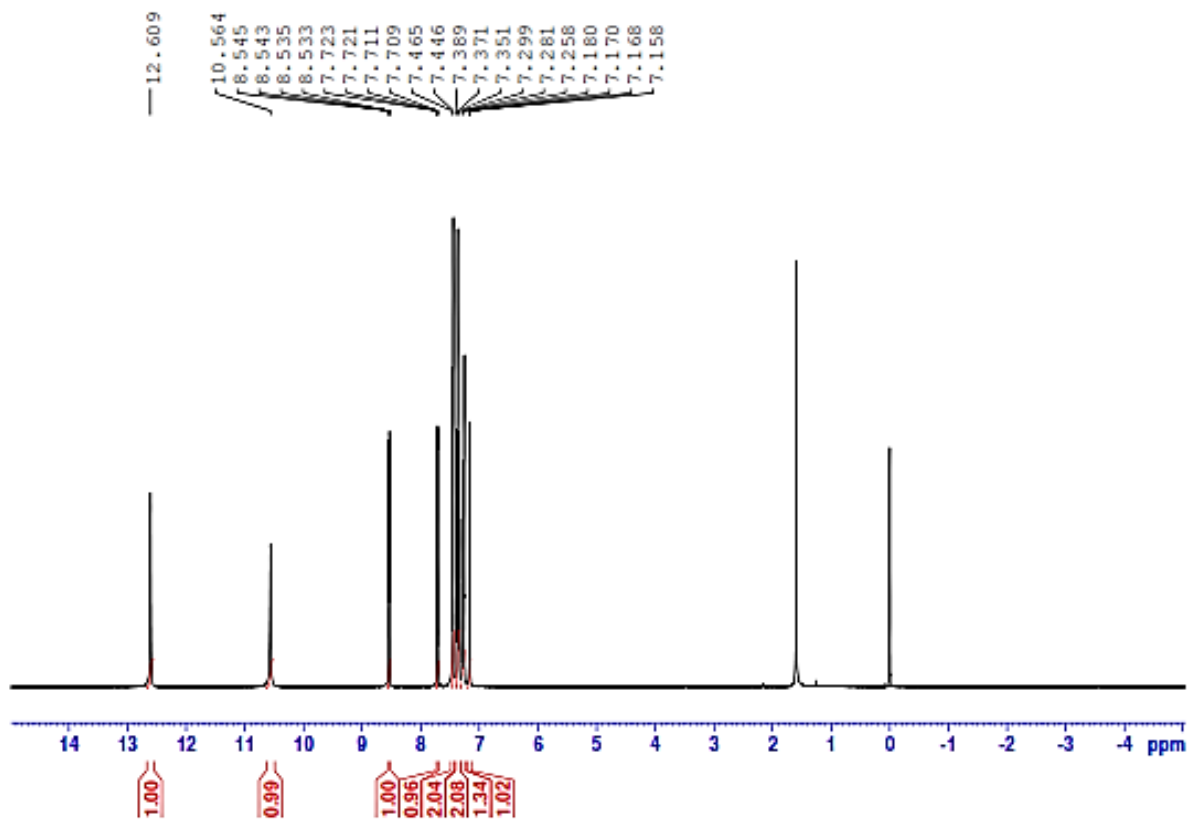


Fig. S2  $^1\text{H}$  NMR spectrum of **1**.

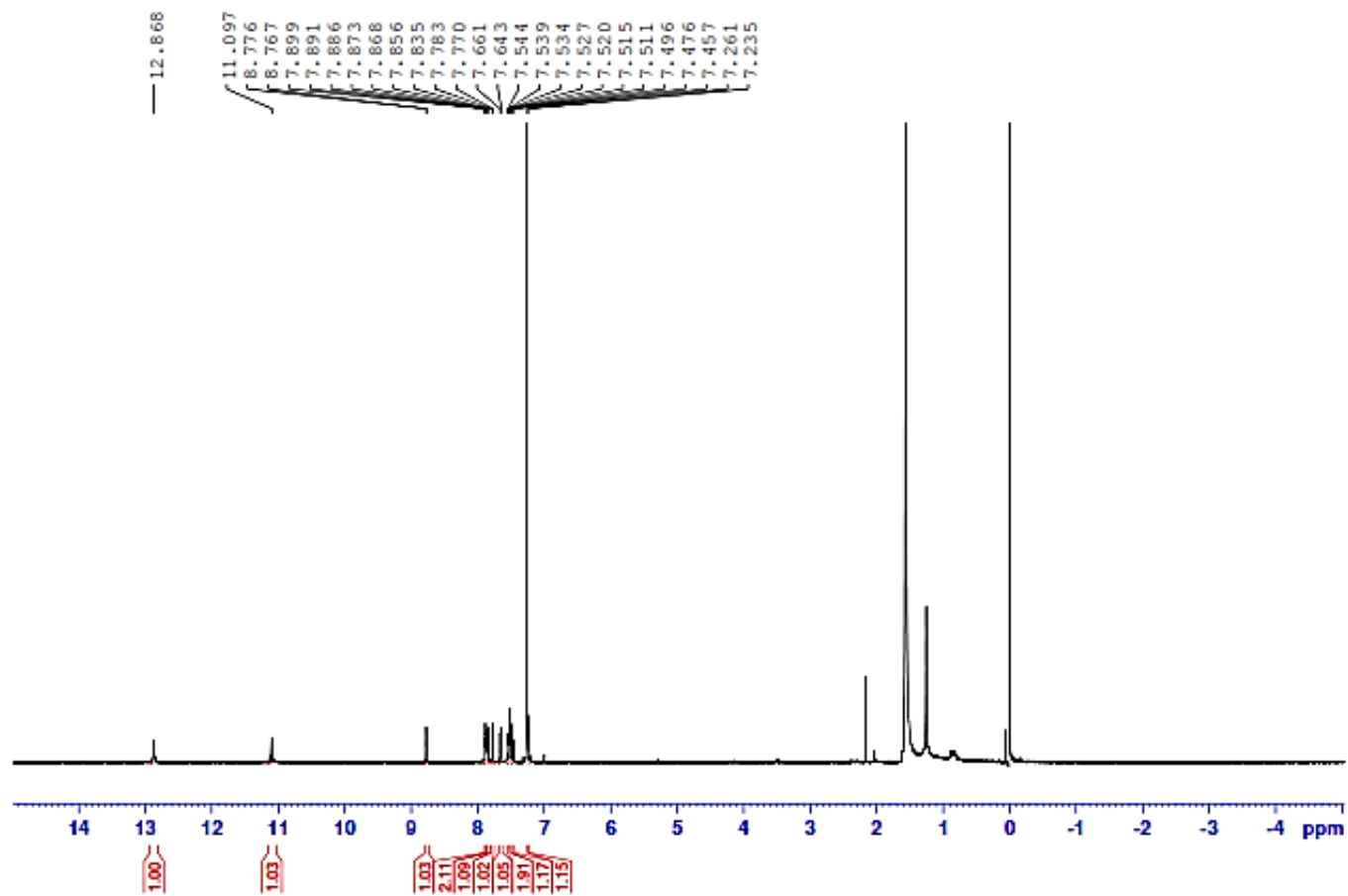


Fig. S3  $^1\text{H}$  NMR spectrum of 2.

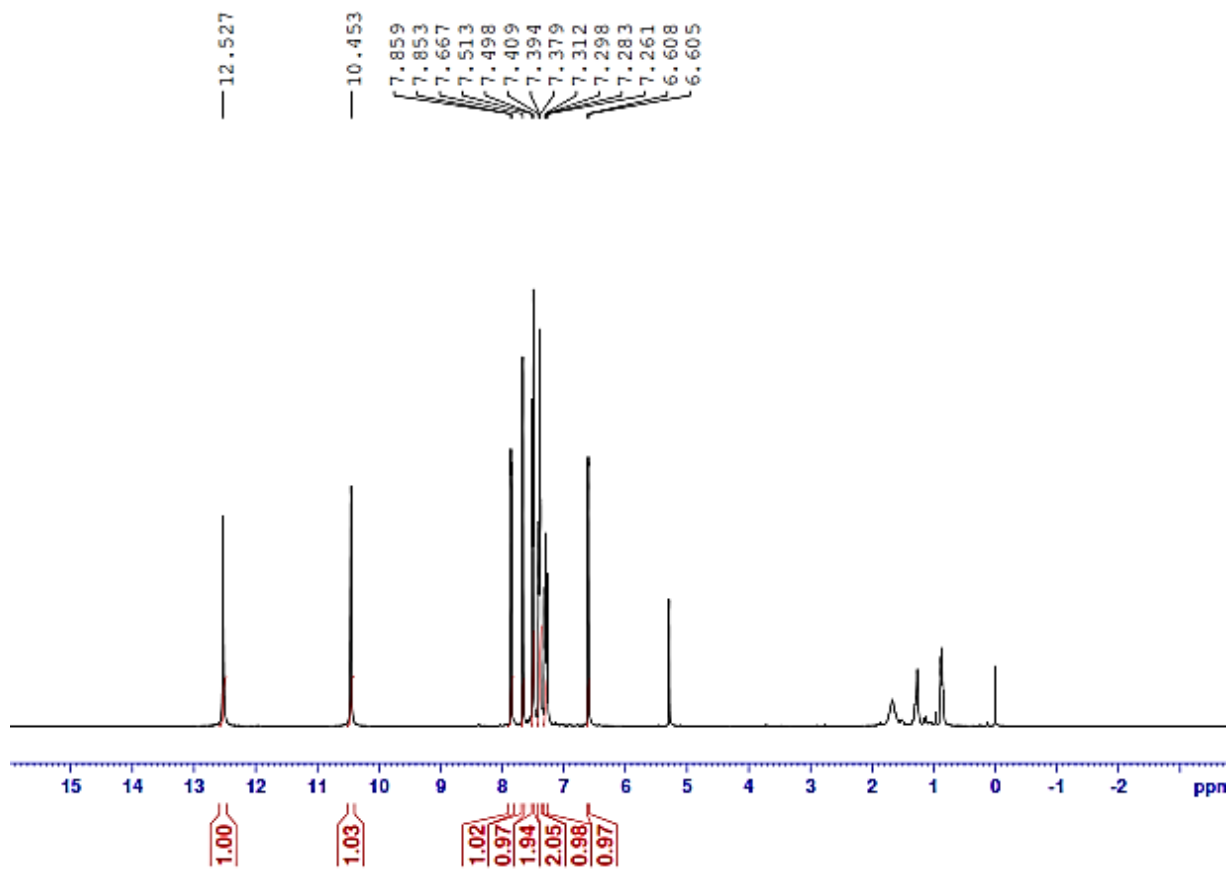


Fig. S4  $^1\text{H}$  NMR spectrum of 3.

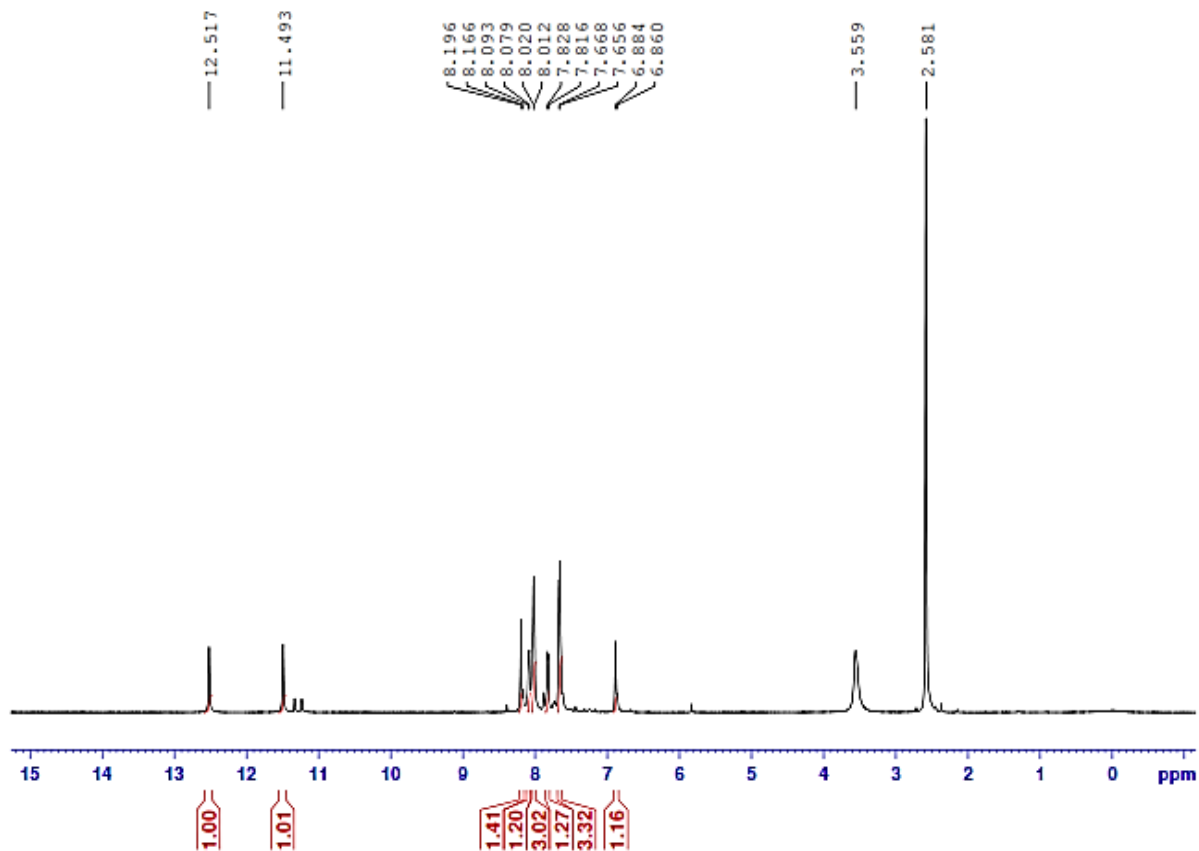


Fig. S5  $^1\text{H}$  NMR spectrum of **4**.

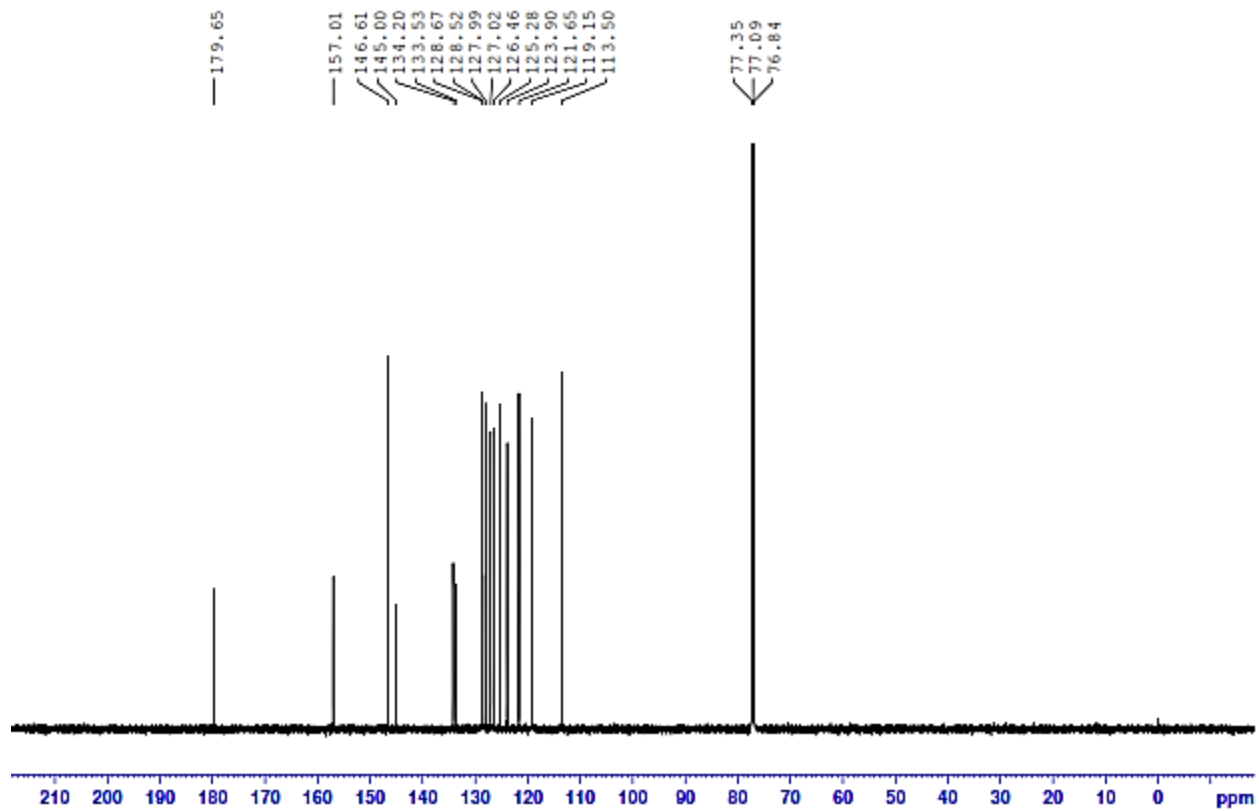


Fig. S6  $^{13}\text{C}$  NMR spectrum of L4.



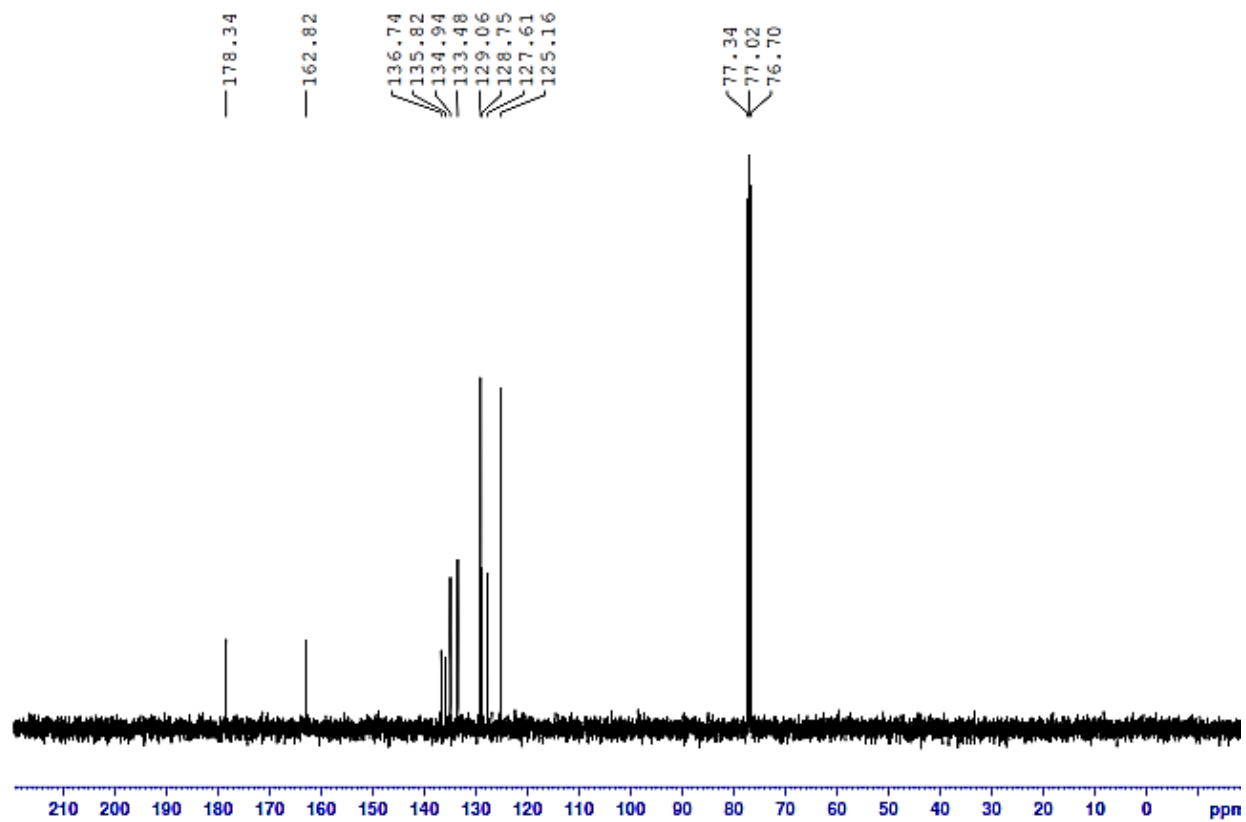


Fig. S7  $^{13}\text{C}$  NMR spectrum of **1**.

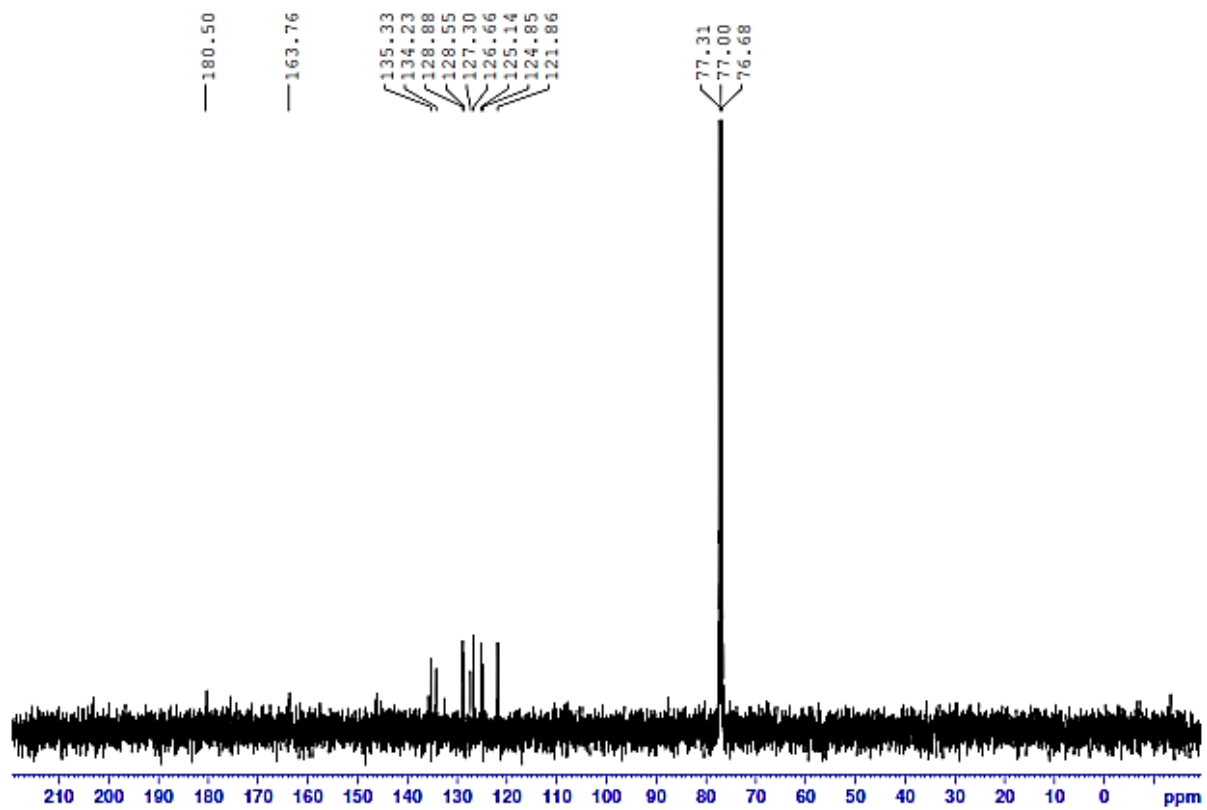
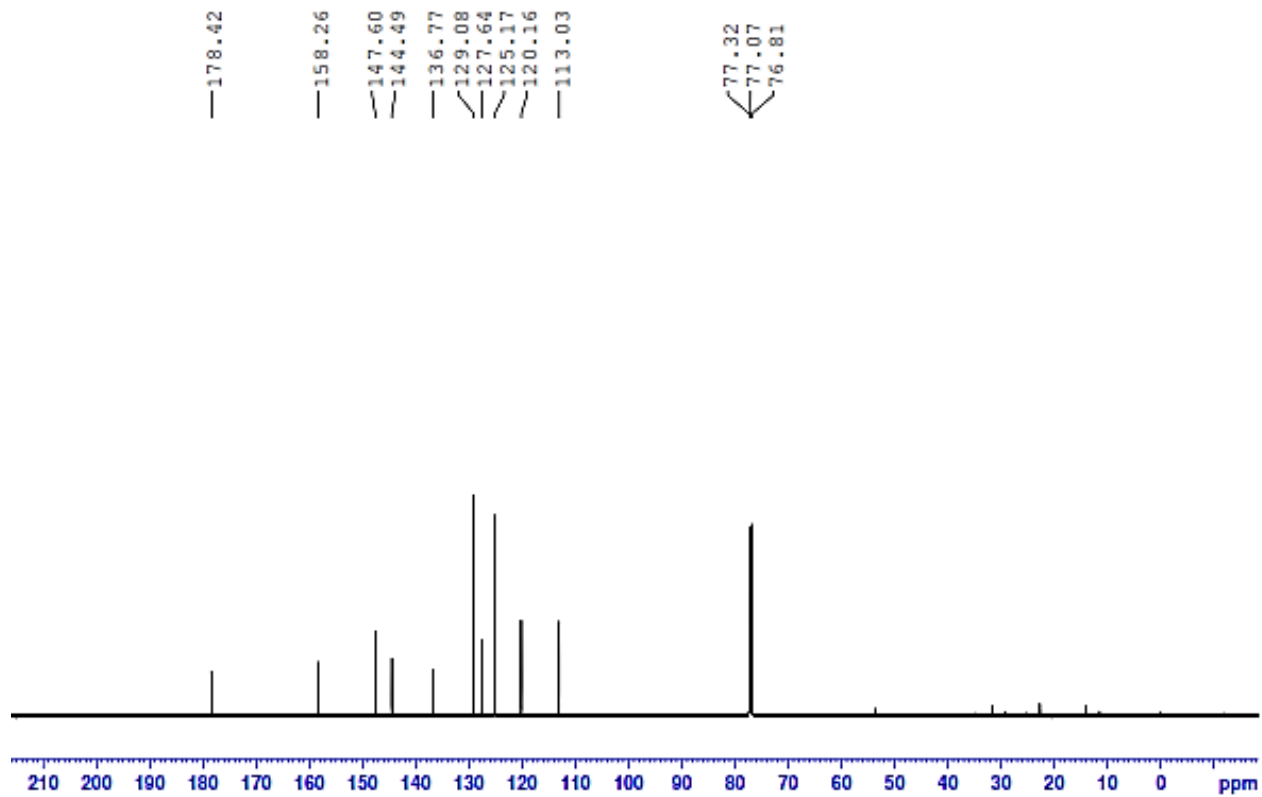
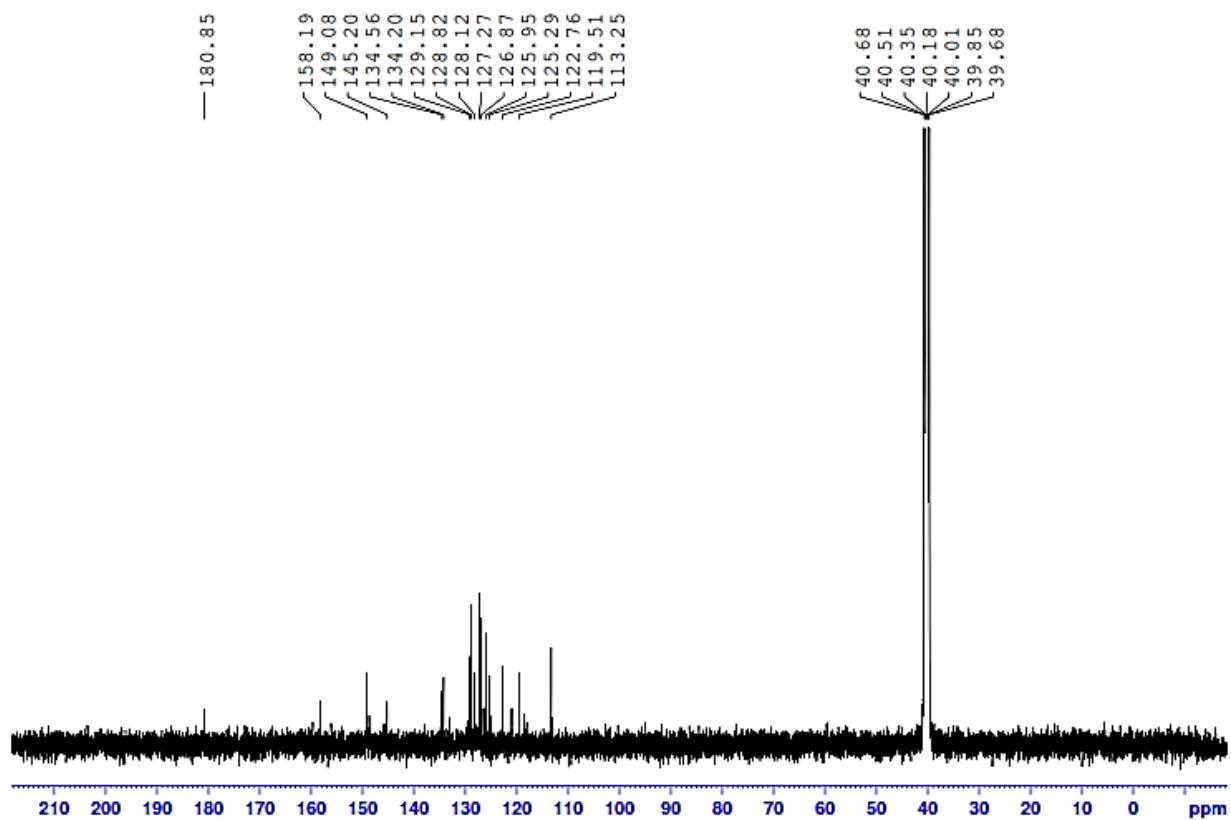


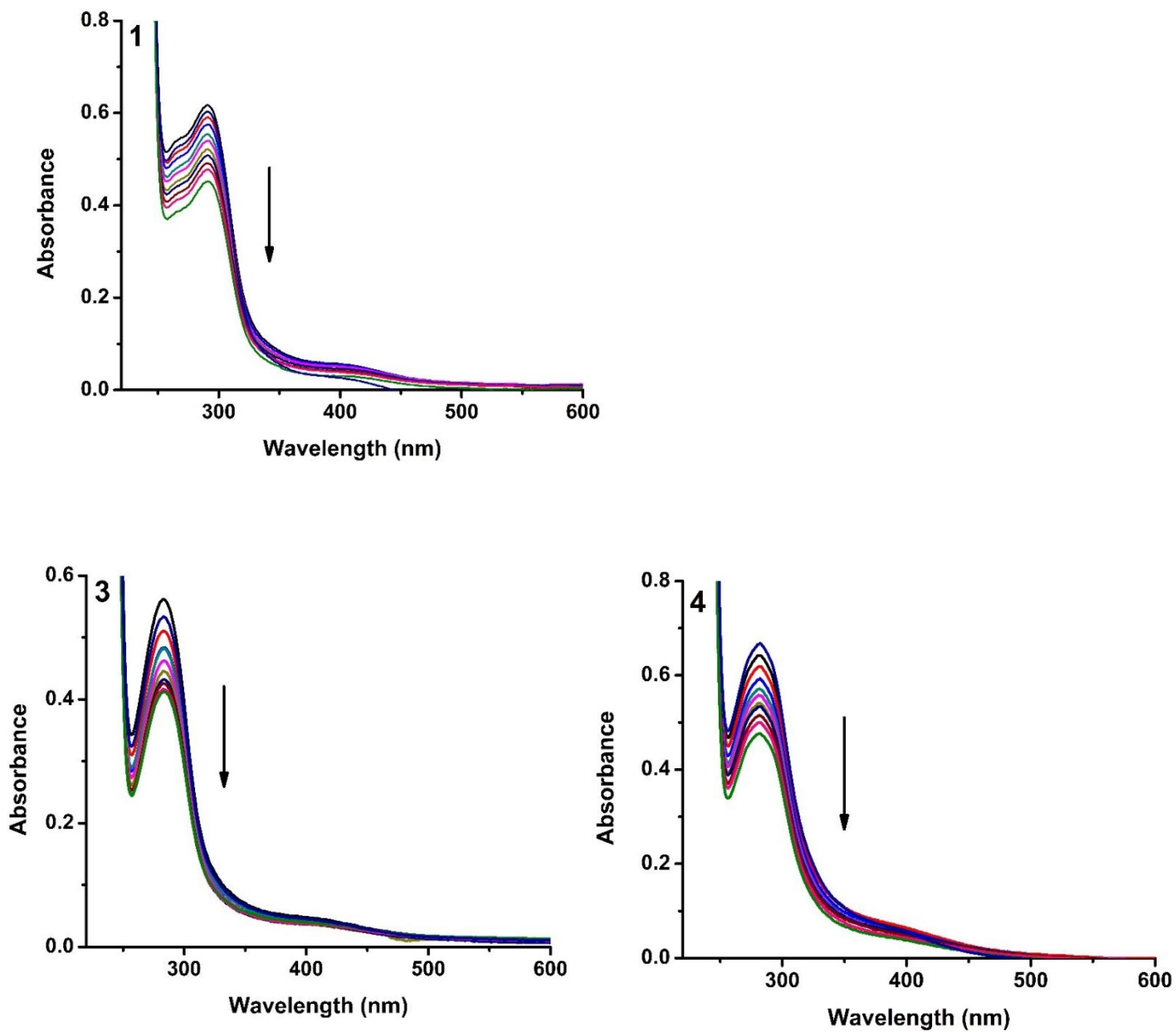
Fig. S8  $^{13}\text{C}$  NMR spectrum of **2**.



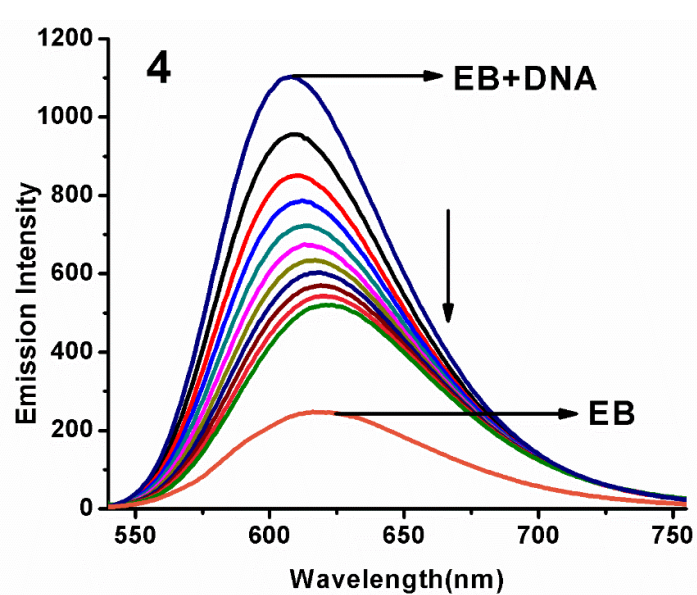
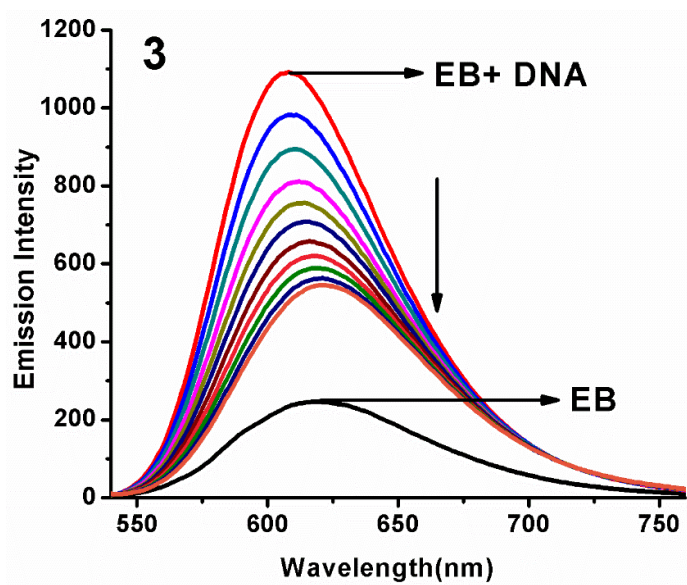
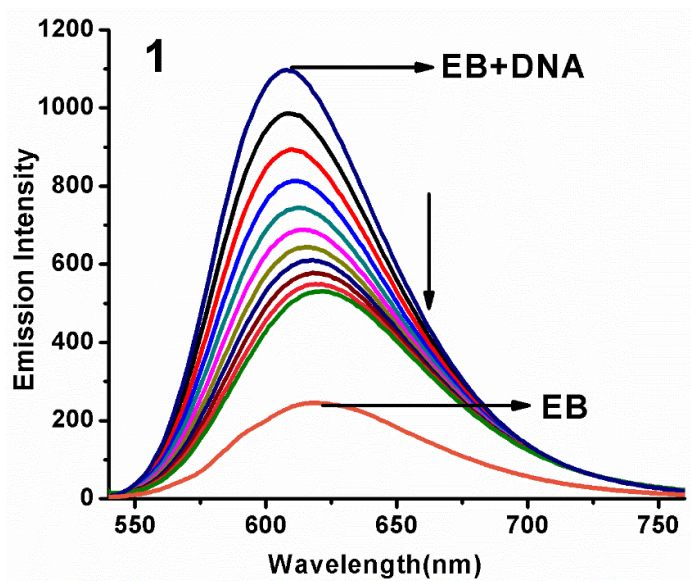
**Fig. S9**  $^{13}\text{C}$  NMR spectrum of **3**.



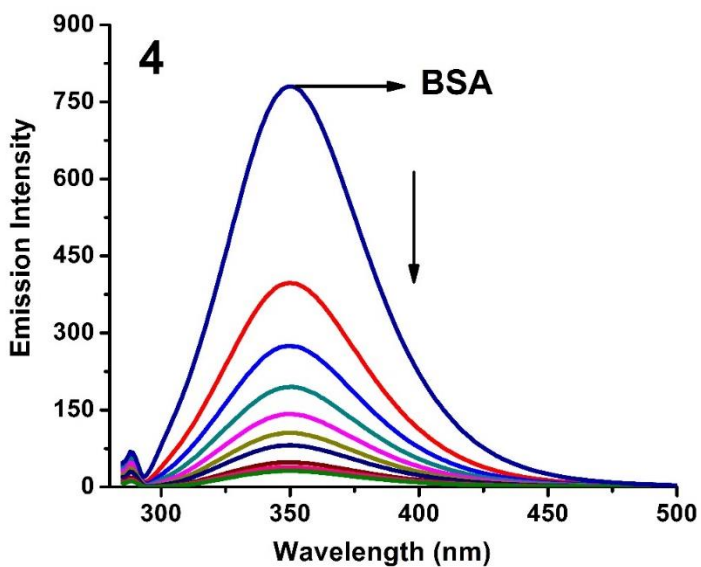
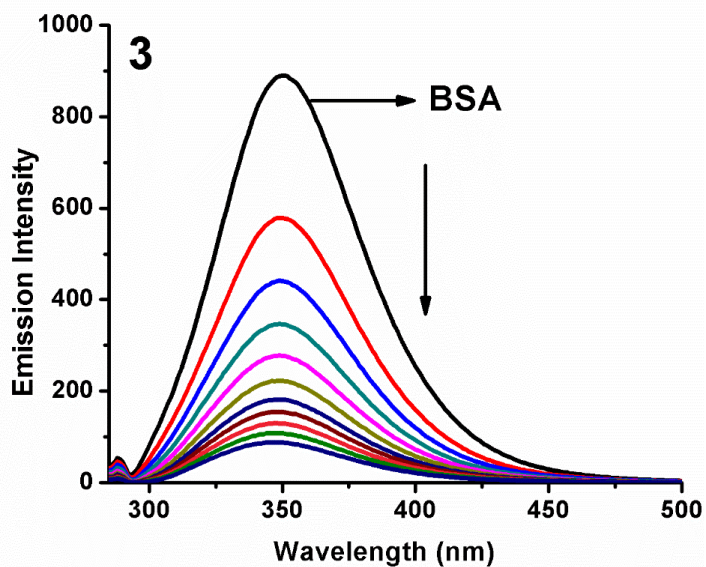
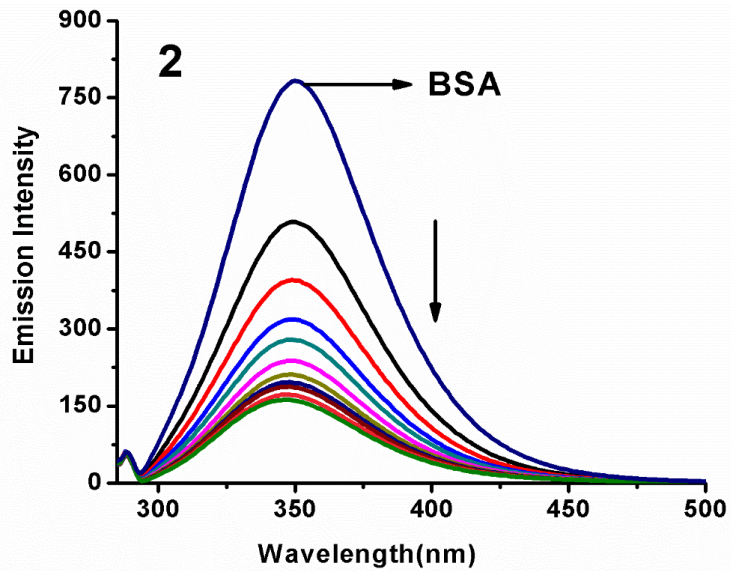
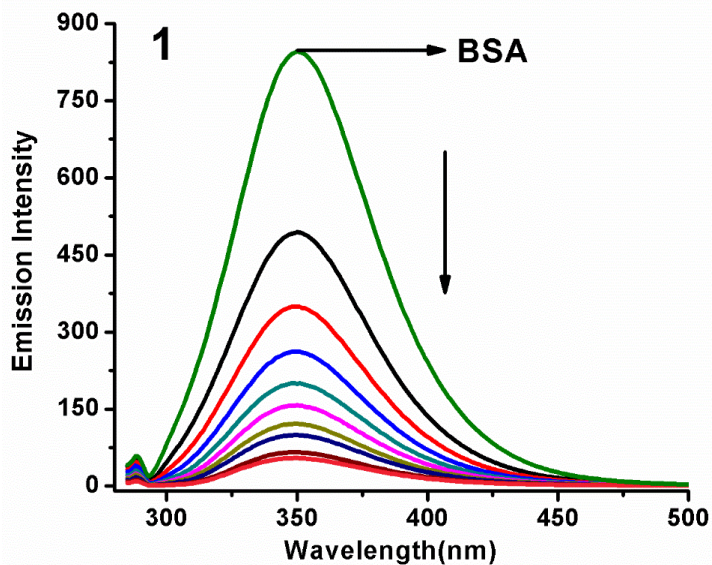
**Fig. S10**  $^{13}\text{C}$  NMR spectrum of **4**.



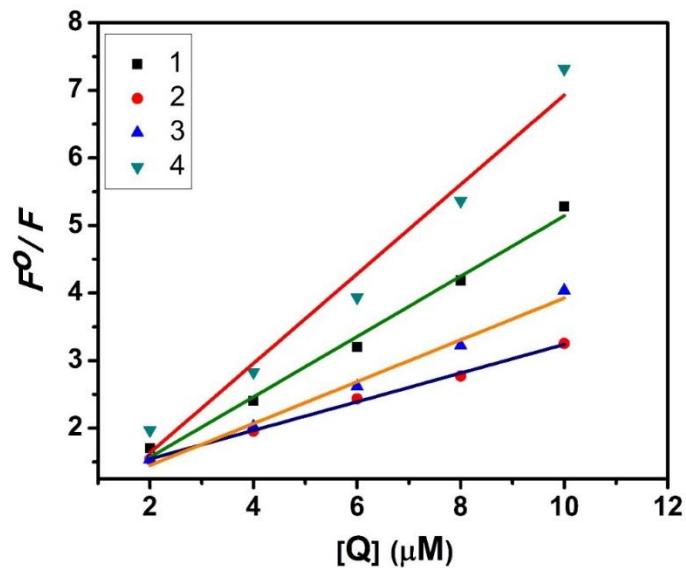
**Fig. S11** Absorption spectra of complexes (**1**, **3** and **4**) in Tris-HCl buffer upon addition of CT DNA. [Complex] =  $1.0 \times 10^{-5}$  M, [DNA] = 0-50  $\mu$ M. Arrow shows that the absorption intensities decrease upon increasing DNA concentration.



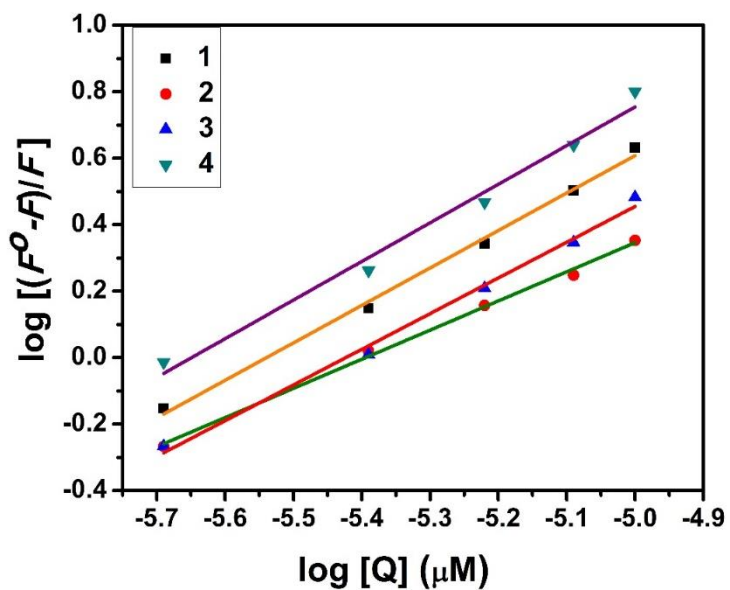
**Fig. S12** Fluorescence quenching curves of EB bound to DNA in the presence of **1**, **3** and **4**. [DNA] = 5  $\mu$ M, [EB] = 5  $\mu$ M and [complex] = 0-50  $\mu$ M.



**Fig. S13** Fluorescence quenching curves of BSA in the absence and presence of **1-4**. [BSA] = 1  $\mu$ M and [complex] = 0-50  $\mu$ M.

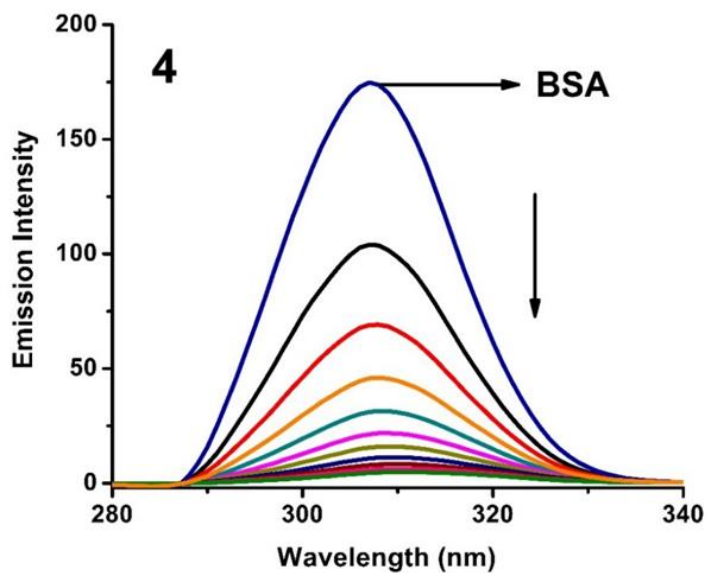
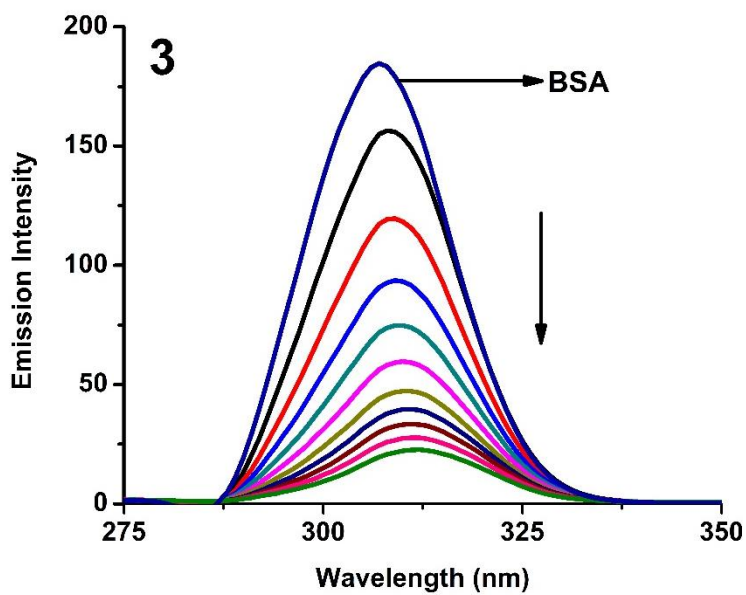
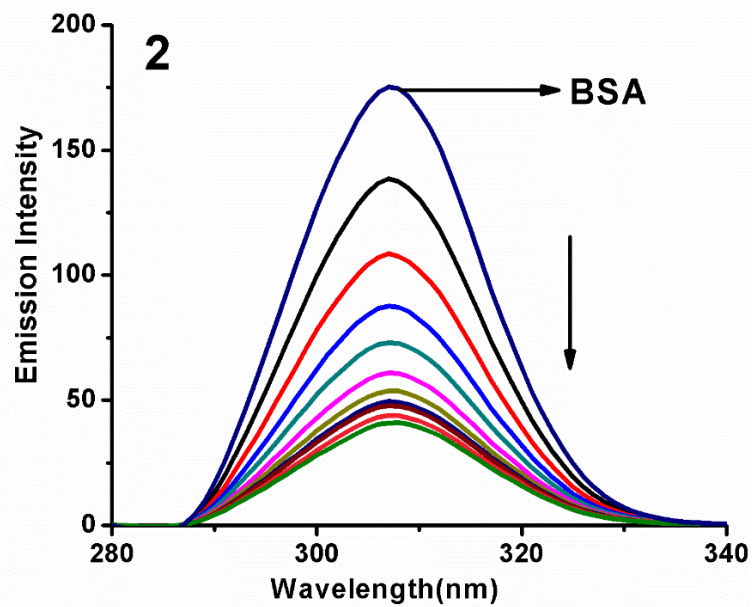
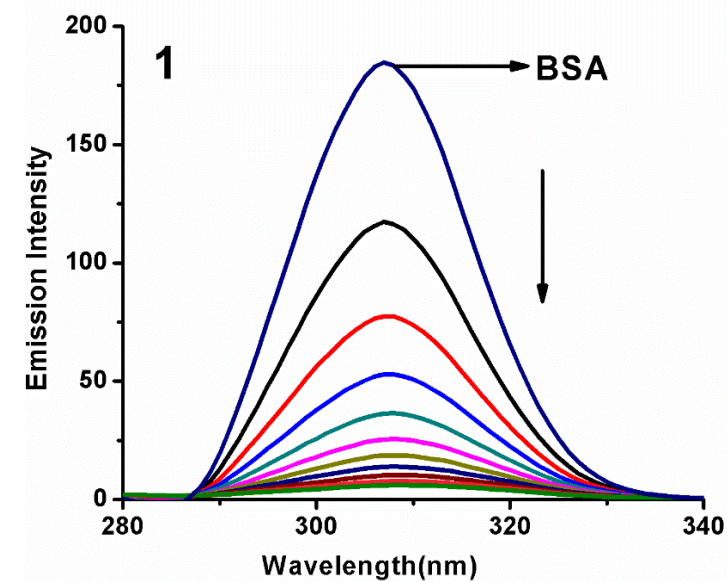


**Fig. S14** Stern-Volmer plots of the fluorescence titrations of the complexes with BSA.

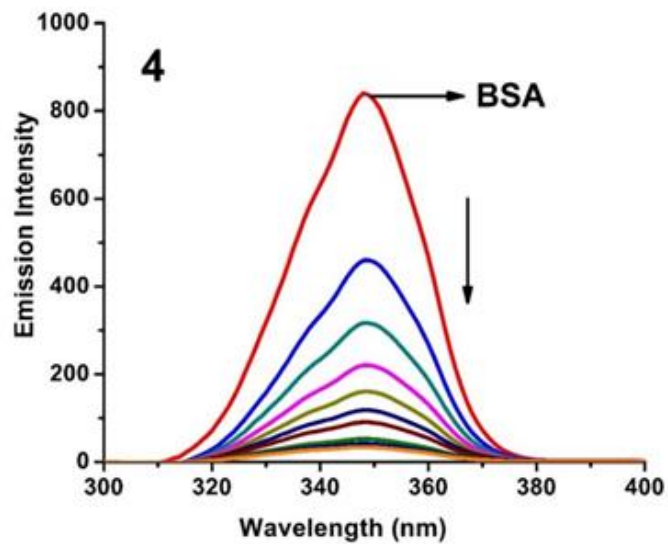
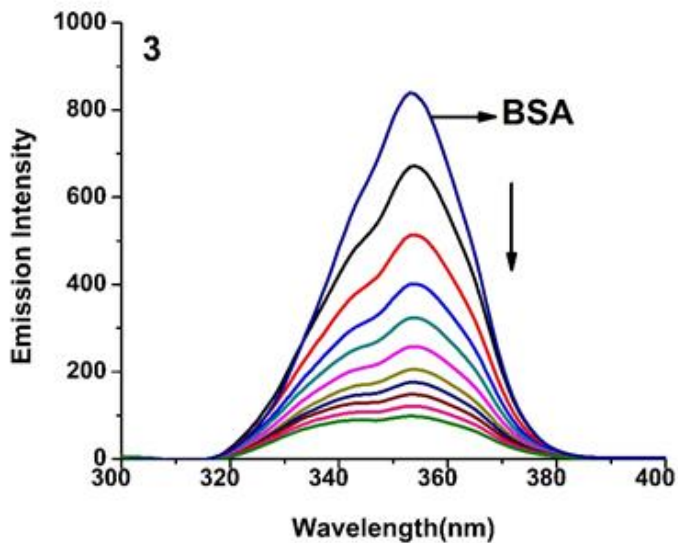
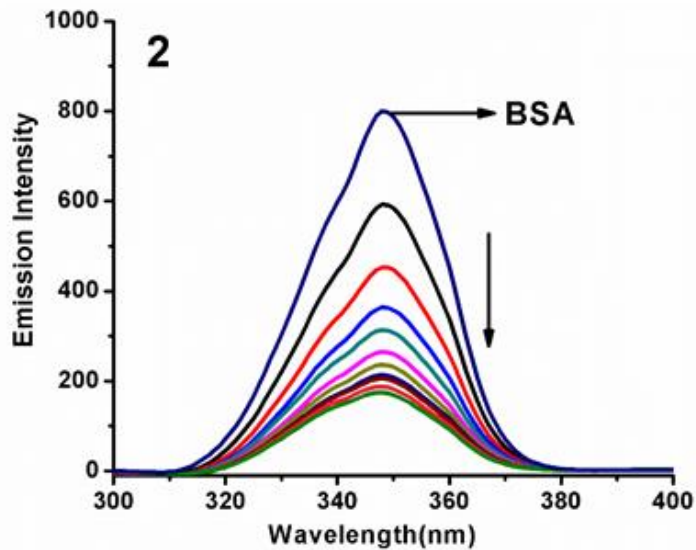
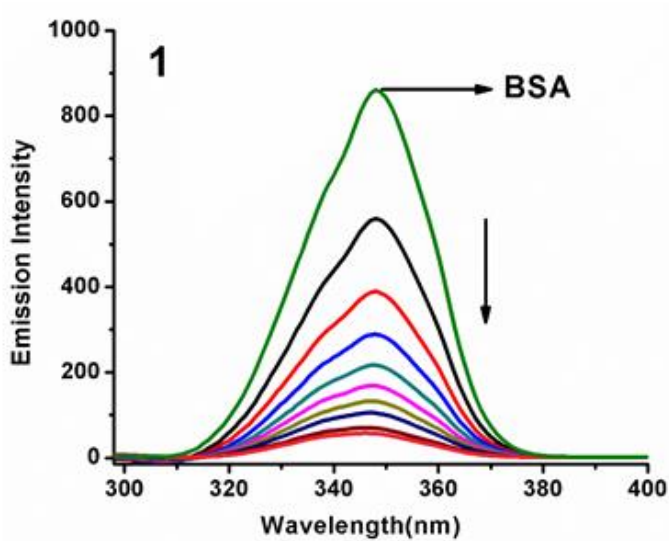


**Fig. S15** Scatchard plots of the fluorescence titrations of the complexes with BSA.

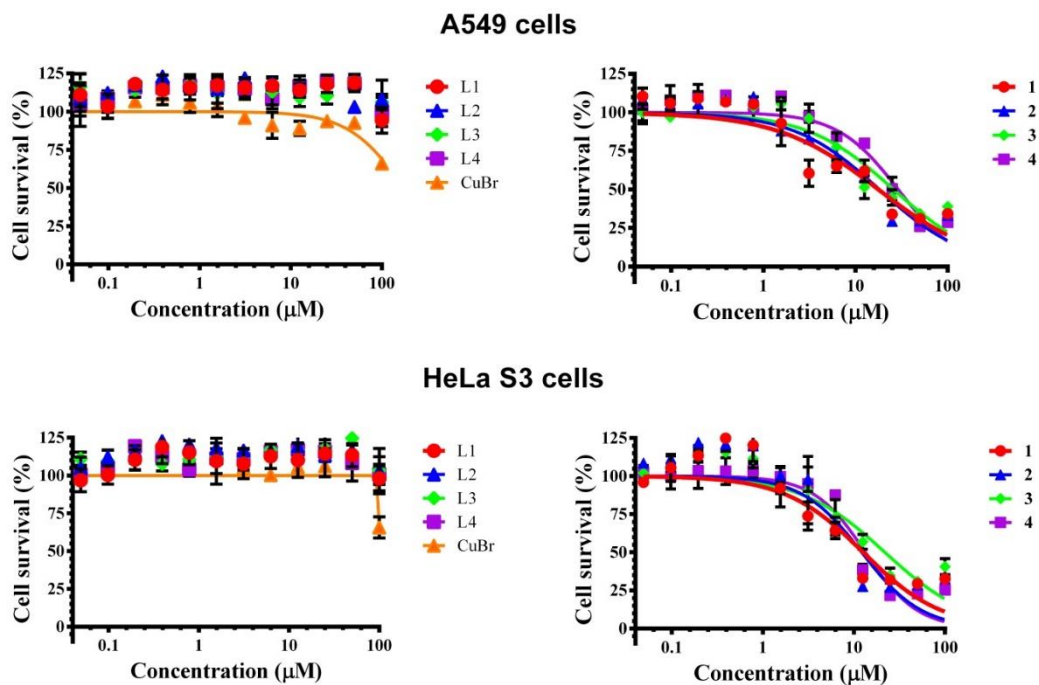




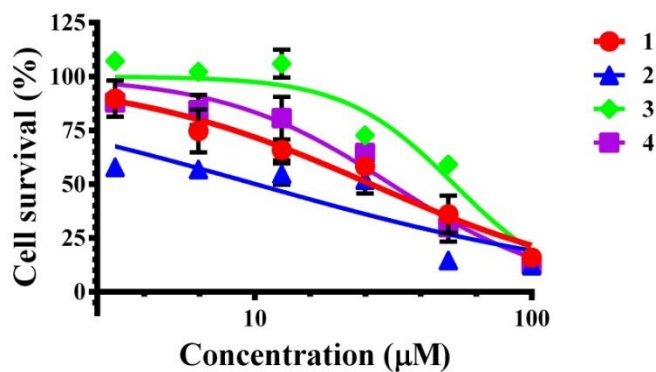
**Fig. S16** Synchronous spectra of BSA (1  $\mu\text{M}$ ) as a function of concentration of 1-4 (0-50  $\mu\text{M}$ ) with  $\Delta\lambda = 15$  nm.



**Fig. S17** Synchronous spectra of BSA (1  $\mu\text{M}$ ) as a function of concentration of **1-4** (0-50  $\mu\text{M}$ ) with  $\Delta\lambda = 60$  nm.



**Fig. S18** Cytotoxic effects of the compounds against A549 and HeLa S3 cells. The experiment was conducted for 24 h and data was calculated by  $SD \pm \text{mean}$  with three independent experiments.



**Fig. S19** Cytotoxic effects of the complexes against normal human cell IMR90. The experiment was conducted for 24 h and data was calculated by  $SD \pm \text{mean}$  with three independent experiments.