Supplementary Information

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

Kumaramangalam Jeyalakshmi, a,b Jebiti Haribabu, a Chandrasekar Balachandran, c Eswaramoorthi Narmatha, a Nattamai S. P. Bhuvanesh, d Shin Aoki, c Suresh Awale c and Ramasamy Karvembu a

a Department of Chemistry, National Institute of Technology, Tiruchirappalli 620015, India
b Department of Chemistry, M. Kumarasamy College of Engineering, Karur 639113, India
c Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda 278-8510 Japan
d Department of Chemistry, Texas A & M University, College Station, TX 77842, USA
e Division of Natural Drug Discovery, Department of Translational Research, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
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% CO₂ and 95% air. Briefly, cells were seeded in 96-well plates (2×10⁴/well) and incubated with fresh DMEM at 37 °C under 5% CO₂ 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:

\[
\text{Cell viability (\%)} = \frac{\text{Abs (Test sample)} - \text{Abs (Blank)}}{\text{Abs (Control)} - \text{Abs (Blank)}} \times 100 \%
\]
Fig. S1 $^1$H NMR spectrum of L4.
Fig. S2 $^1$H NMR spectrum of 1.
Fig. S3 $^1$H NMR spectrum of 2.
Fig. S4 $^1$H NMR spectrum of 3.
Fig. S5 $^1$H NMR spectrum of 4.
Fig. S6 $^{13}$C NMR spectrum of L4.
Fig. S7 $^{13}$C NMR spectrum of 1.
Fig. S8 $^{13}$C NMR spectrum of 2.
Fig. S9 $^{13}$C NMR spectrum of 3.
Fig. S10 $^{13}$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] = 1.0×10⁻⁵ M, [DNA] = 0-50 µM. 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 µM, [EB] = 5 µM and [complex] = 0-50 µM.
Fig. S13 Fluorescence quenching curves of BSA in the absence and presence of 1-4. [BSA] = 1 μM and [complex] = 0-50 μM.
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 µM) as a function of concentration of 1-4 (0-50 µM) with Δλ = 15 nm.
Fig. S17 Synchronous spectra of BSA (1 µM) as a function of concentration of 1-4 (0-50 µM) with Δλ = 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±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±mean with three independent experiments.