Supplementary Material

Mechanistic insights of a novel chromone–appended Cu(II) anticancer drug entity: *In vitro* binding profile with DNA/RNA substrates and cytotoxic activity against MCF-7 and HepG2 cancer cells

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Abbreviations:

DMEM = Dulbecco's modified eagle's medium

FBS = fetal bovine serum

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

DCFH-DA= 2,7-dichlorodihydrofluorescein diacetate

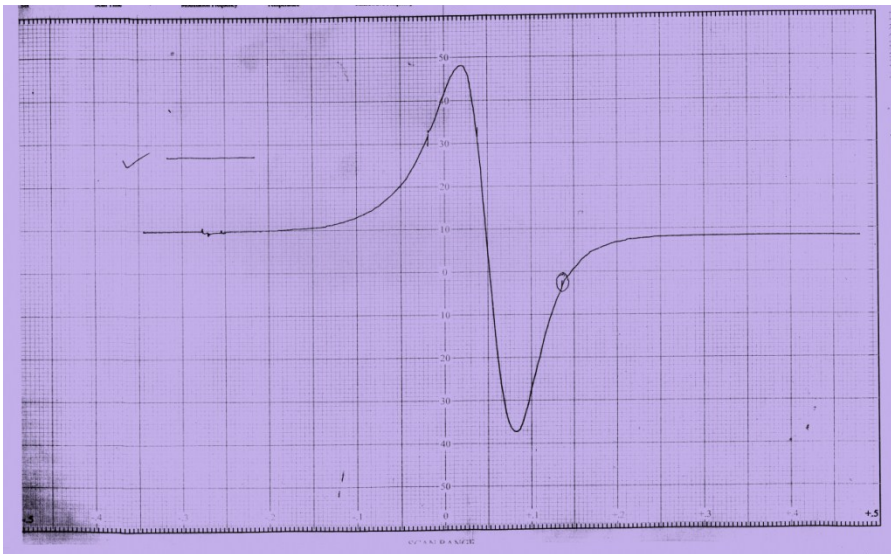
EDTA = ethylenediaminotetracetic acid

DTNB = 5,5'-dithionitrobenzoic acid

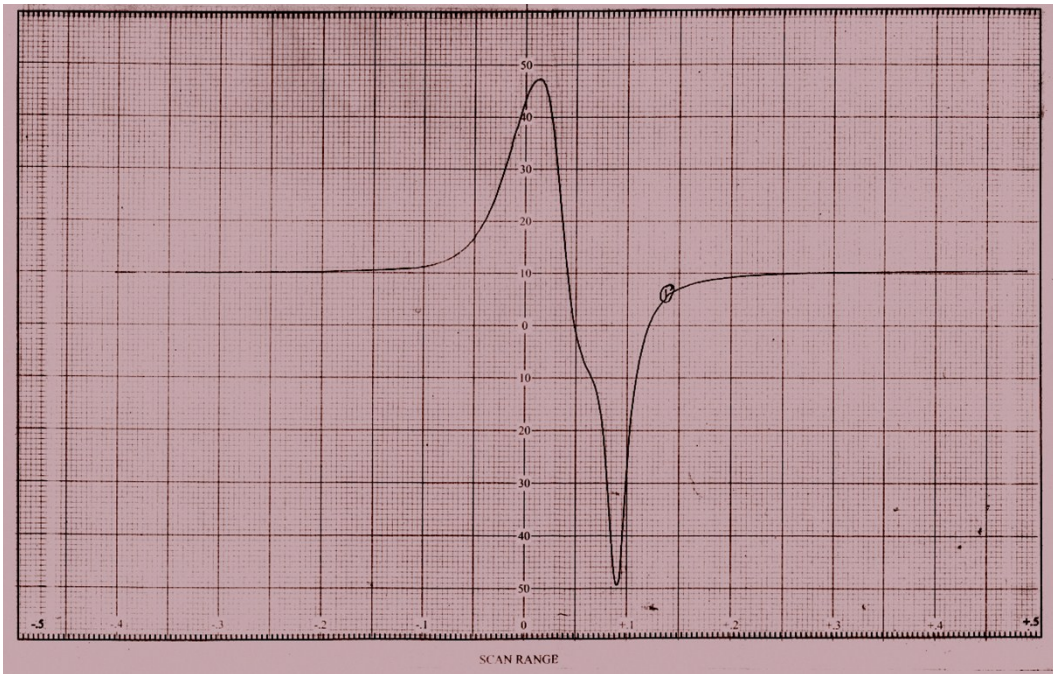
TBARS = thiobarbituric acid–reactive substances

TBA = thiobarbituric acid

Figures



(a)



(b)

Fig.S1 X–band EPR spectrum of complex **1** at (a) RT and (b) LNT.

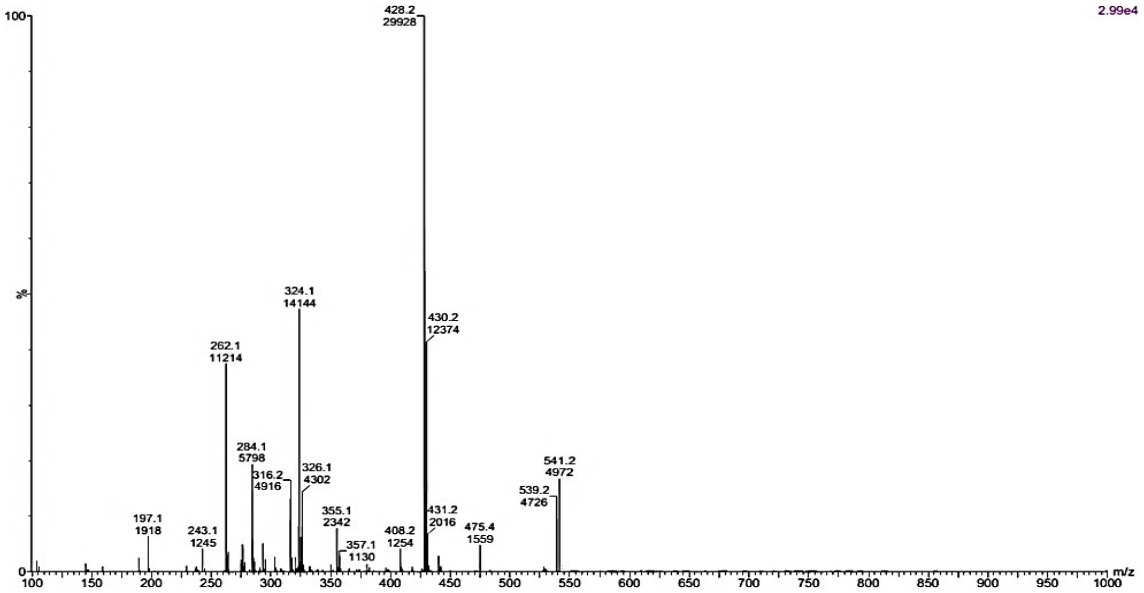


Fig. S2 ESI mass spectrum of complex **1**

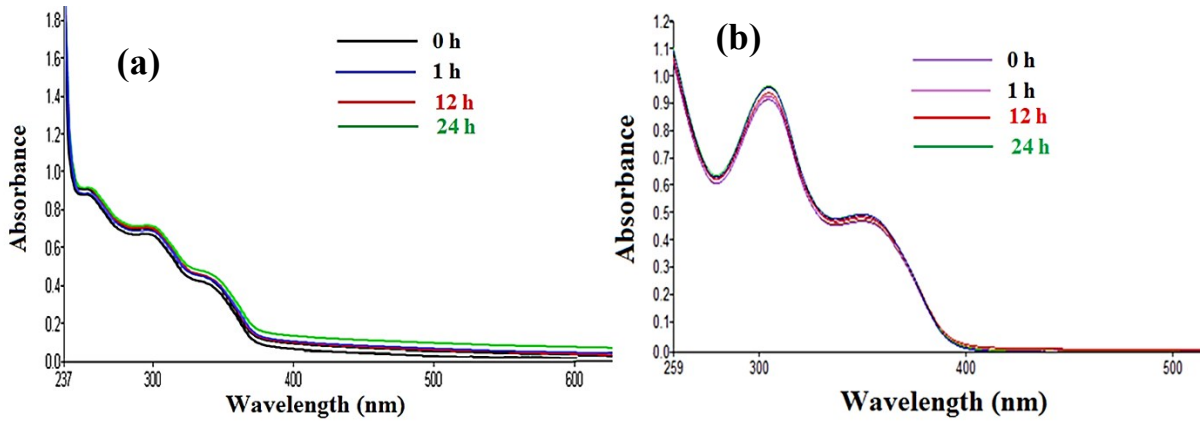


Fig. S3 UV–vis absorption spectra of (a) complex **1** and (b) 3–formylchromone ligand, in Tris buffer at pH 7.4 and 310 K (physiological conditions) and at different time intervals (0h, 6h, 12h, & 24h).

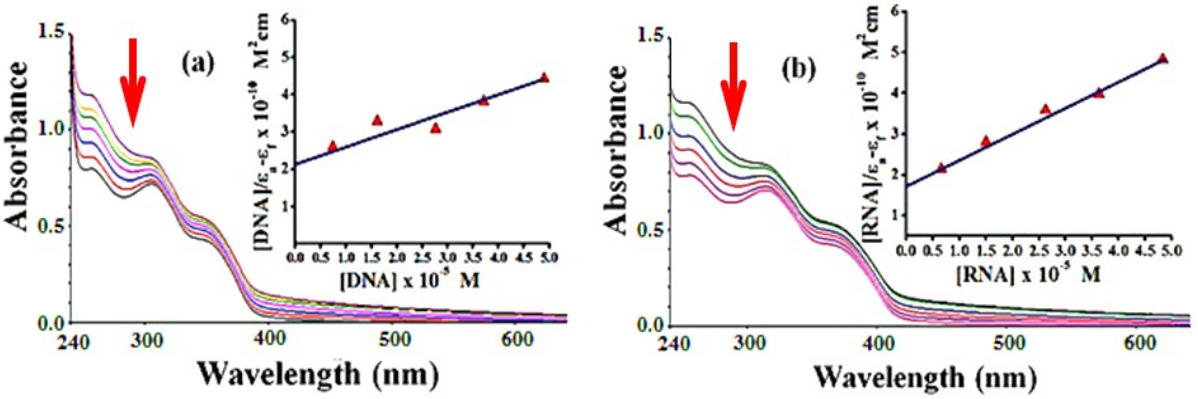


Fig. S4 Absorption spectra of Cu(II) complex in the absence and in presence of increasing amounts of (a) ct–DNA and (b) yeast tRNA in Tris–HCl buffer at pH 7.2. Inset: Plots of [DNA or RNA]/εa–εf (M^2 cm) vs. [DNA or RNA] for the titration with complex 1, ▲, experimental data points, full lines, linear fitting of the data. [DNA], [RNA] = 0.0–5.0 x 10^{-5} M, [Complex 1] = 1.67 x 10^{-4} M. The arrows indicate the change in absorbance with increasing [DNA/RNA].

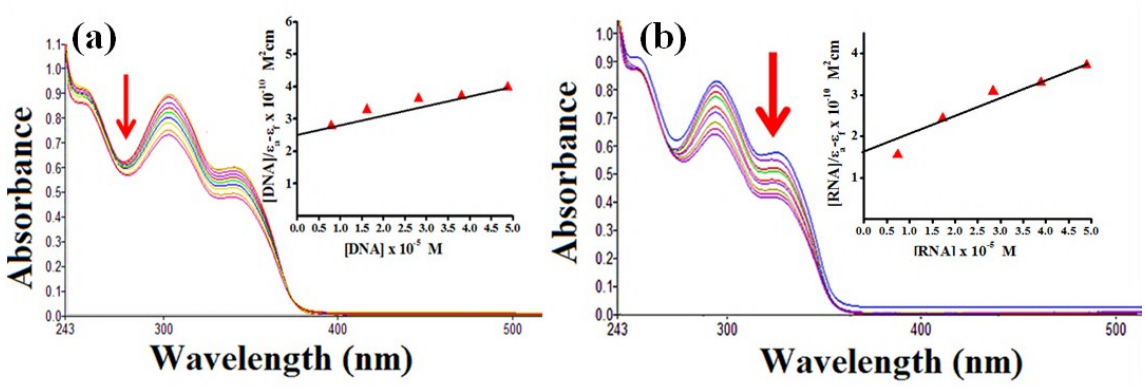


Fig. S5 Absorption spectral traces of 3-formylchromone in 5mM Tris HCl/50 mM NaCl buffer at pH 7.2 upon addition of (a) ct–DNA and (b) yeast tRNA. Inset: Plots of [DNA or RNA]/ε_a–ε_f (M^2 cm) *vs.* [DNA or RNA] for the titration with **1**, experimental data points, full lines, linear fitting of the data. [DNA], [RNA] = 0.0–5.0 x 10^{-5} M, [Compound] = 1.66 x 10^{-4} M. The arrows indicate the change in absorbance with increasing [DNA/RNA].

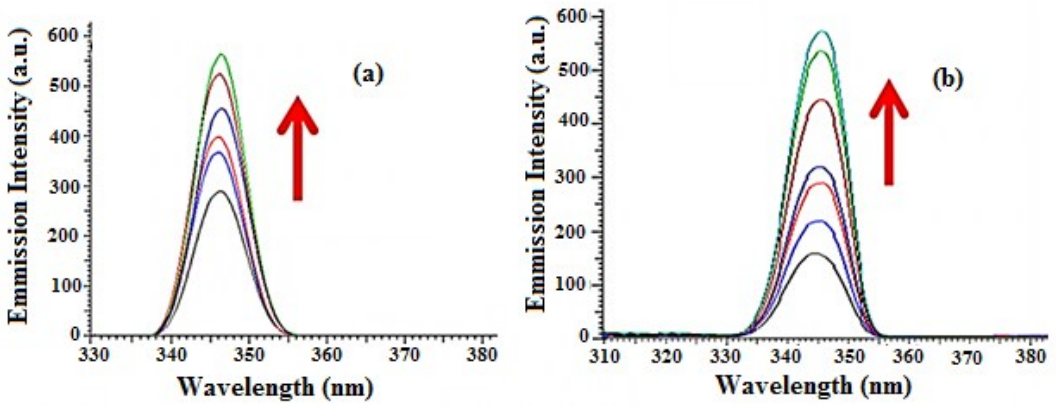


Fig. S6 Emission spectra of complex **1** in Tris–HCl buffer at pH 7.2 upon addition (a) ct–DNA and (b) yeast tRNA. [DNA], [RNA] = 0.00–4.00 x 10^{-5} M, [Complex **1**] = 1.67 x 10^{-4} M. Arrows show change in intensity with increasing concentration of DNA/RNA.

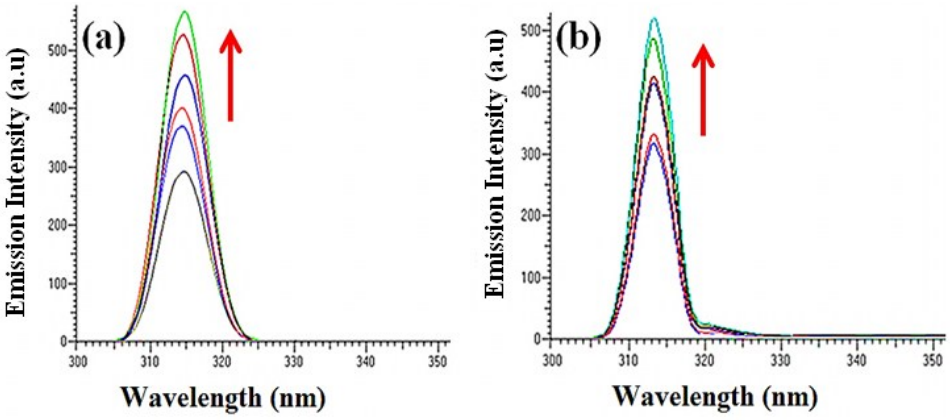


Fig. S7 Emission spectra of 3–formylchromone in Tris–HCl buffer at pH 7.2 upon addition (a) ct–DNA and (b) yeast tRNA. [DNA], [RNA] = 0.00–4.00 x 10^{-5} M, [Compound] = 1.01 x 10^{-5} M. Arrows show change in intensity with increasing concentration of DNA/RNA.

A three dimensional (3D) fluorescence spectroscopy was used to further investigate the interaction mode of complex **1** with the nucleic acids in the absence and presence of ct–DNA/RNA. As depicted in Fig. S7, two prominent peaks, peak A and peak B at λ_{em} = 333 and 369 nm respectively were observed upon excitation at 270 nm. However, upon addition of DNA/RNA (1.11 x 10^{-4} M) to complex **1**, a significant increase of the fluorescence intensity was observed due to strong interaction of **1** with the nucleic acids. The larger increase in **1**–tRNA system substantiates its larger binding propensity and more penetration into the hydrophobic environment of RNA than **1**–DNA system**.**

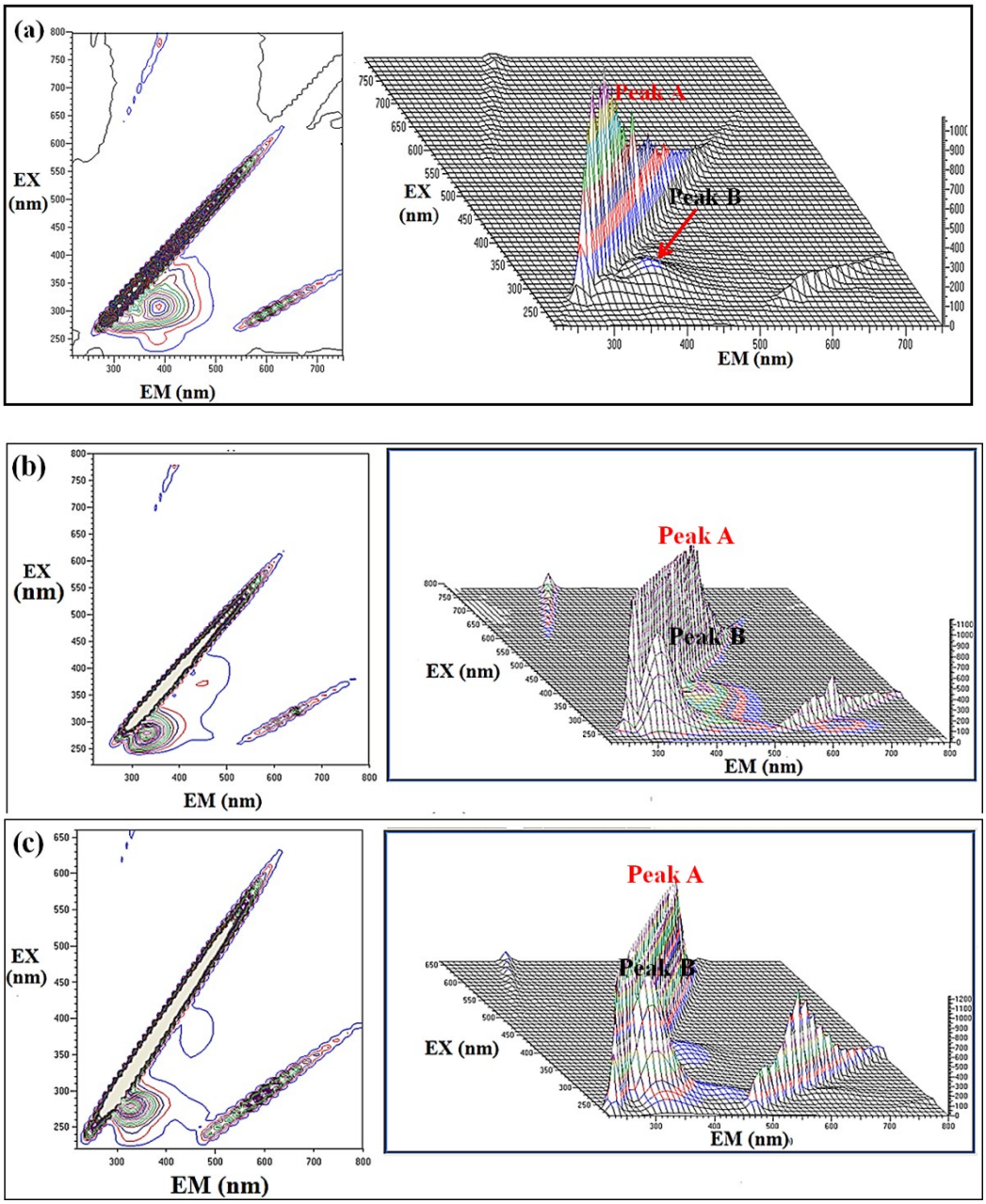


Fig. S8 3D fluorescence spectra and the corresponding contour diagram of (a) complex **1** alone and (b) complex **1**–DNA system (c) complex **1**–RNA system. The concentration of the complex **1** was 1.67 x 10^{-4} M and that of DNA/RNA was fixed at 1.11 x 10^{-4} M in Tris-HCl buffer at pH =7.3.

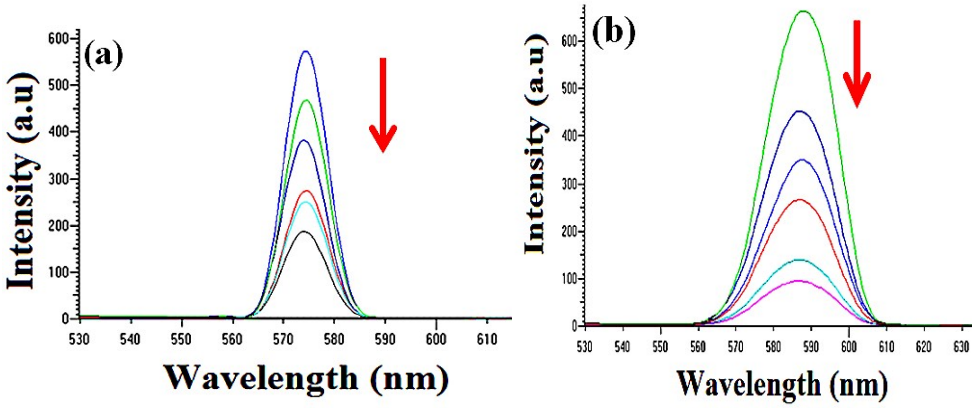


Fig. S9 Emission spectra of (a) EB–ct-DNA (b) EB–yeast tRNA in the absence and presence of complex **1** in Tris–HCl buffer at pH 7.2. [Complex **1**] = [EB] = [DNA] = 1.11 x 10^{-4} M. Arrow shows change in intensity with increasing concentration of complex **1**.

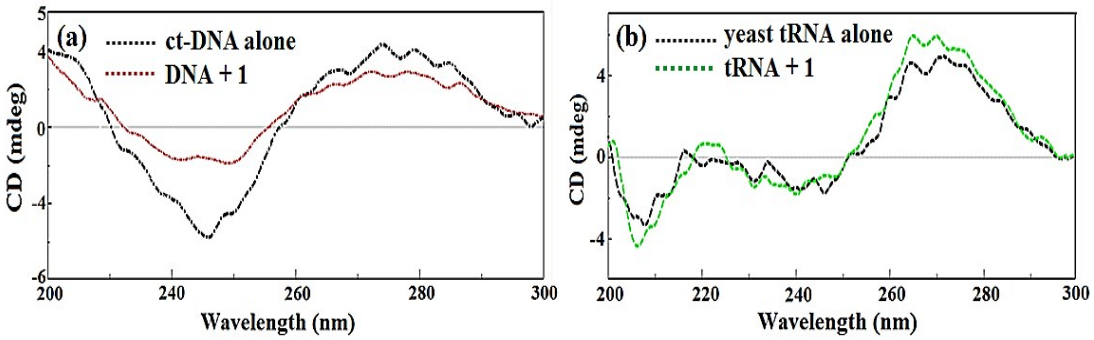


Fig. S10 Circular dichroic spectra in absence and presence of complex **1** (a) ct–DNA and (b) yeast tRNA.

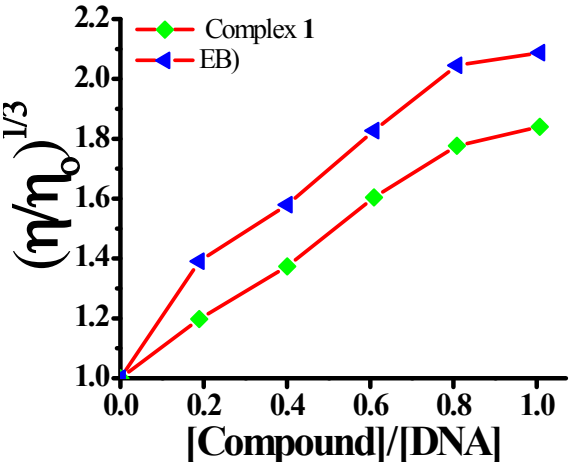


Fig. S11 Effect of increasing amount of **1** (green) and EB (blue) on the relative viscosities $(\eta/\eta_o)^{1/3}$of ct–DNA in Tris–HCl buffer at pH 7.2. The concentration of DNA was 0.10 mM, and the molar ratios of complex **1** or EB to DNA were 0.2, 0.4, 0.6, 0.8 and 1.0, respectively.