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 = ethylenediaminetetraacetic acid
DTNB = 5,5’-dithionitrobenzoic acid
TBARS = thiobarbituric acid–reactive substances
TBA = thiobarbituric acid

Figures

(a)
**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]/ε_a–ε_f (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 $\lambda_{em}$ = 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] = $1.11 \times 10^{-4}$ 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 $(\eta/\eta_0)^{1/3}$ 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.