Synthesis, characterization, plasmid cleavage and cytotoxicity of cancer cells by a copper(II) complex of anthracenyl–terpyridine

Amit Kumar,[a] Jugun Prakash Chinta,[b] Amrendra Kumar Ajay,[c] Manoj Kumar Bhat,[c] Chebrolu P. Rao,[a,b*]

[a]Department of Biosciences and Bioengineering & [b]Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai – 400 076, India. [c]National Centre for Cell Science, Ganeshkhind, Pune – 411 007, India

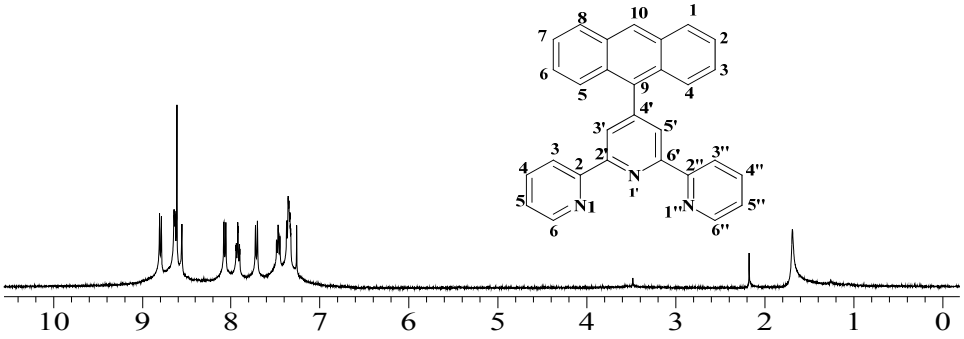
email: cprao@iitb.ac.in

Electronic Supplementary Information

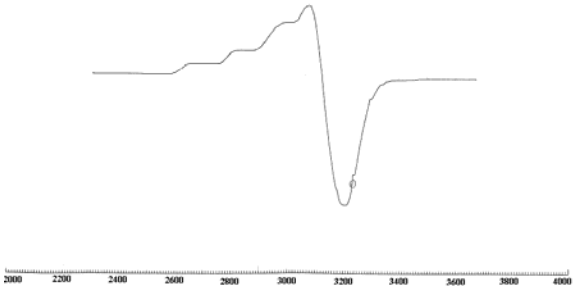
Contents **Page No.**

S01: Characterization for L, 1 and 2

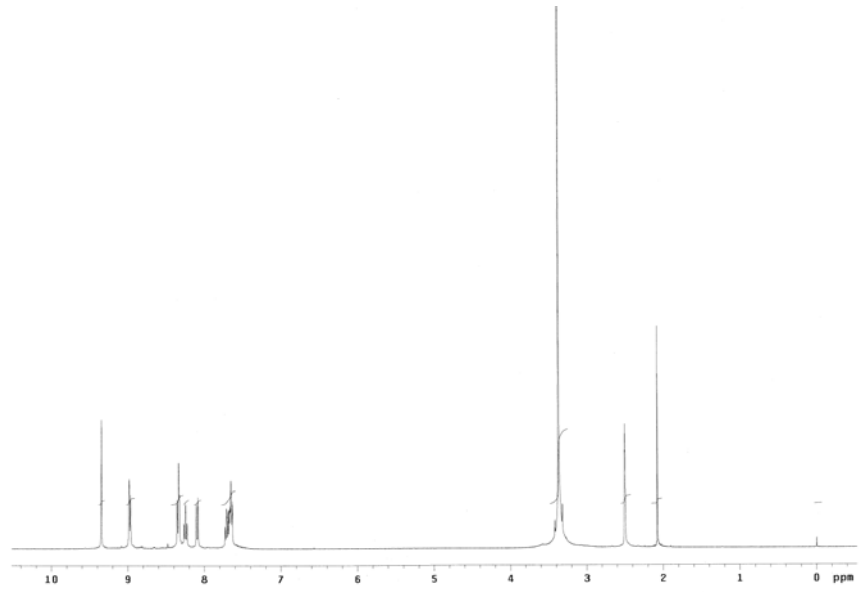
(a) ^{1}H NMR spectrum of L recorded in the $CDCl_3$



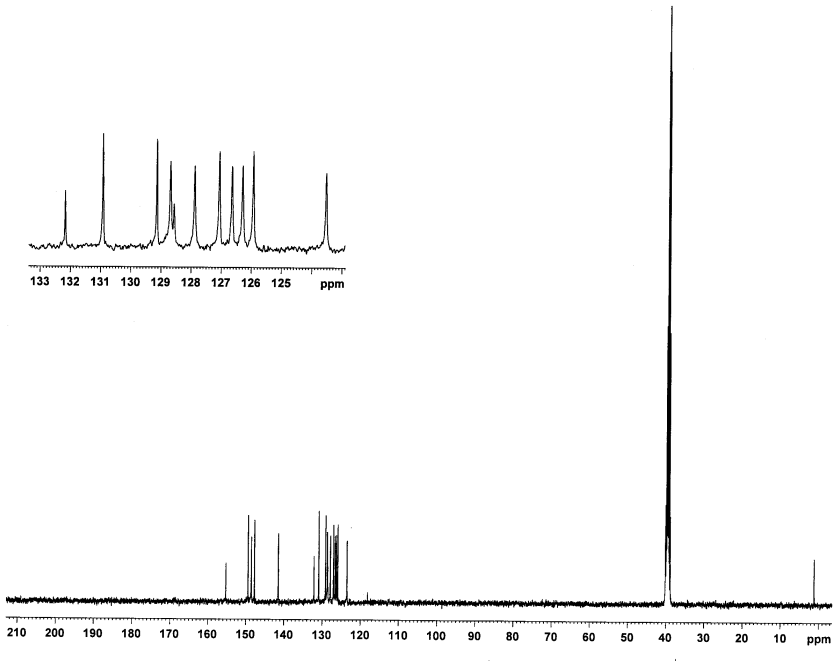
(b) EPR spectrum of 1 in acetonitrile solvent.



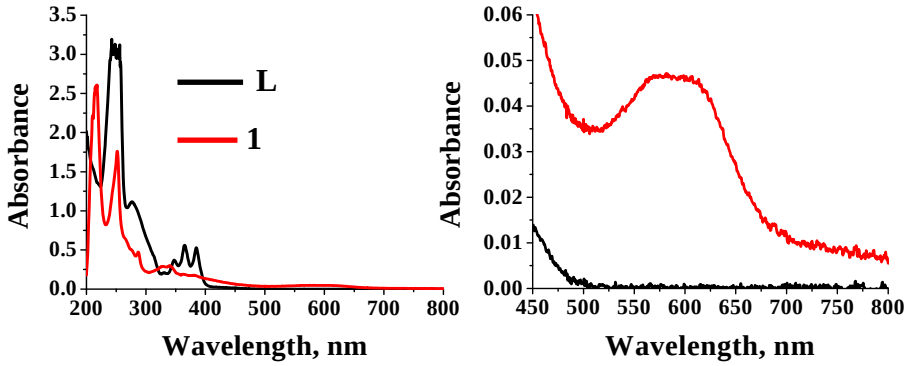
(c) ^{1}H NMR spectrum of 2 recorded in the DMSO-d6



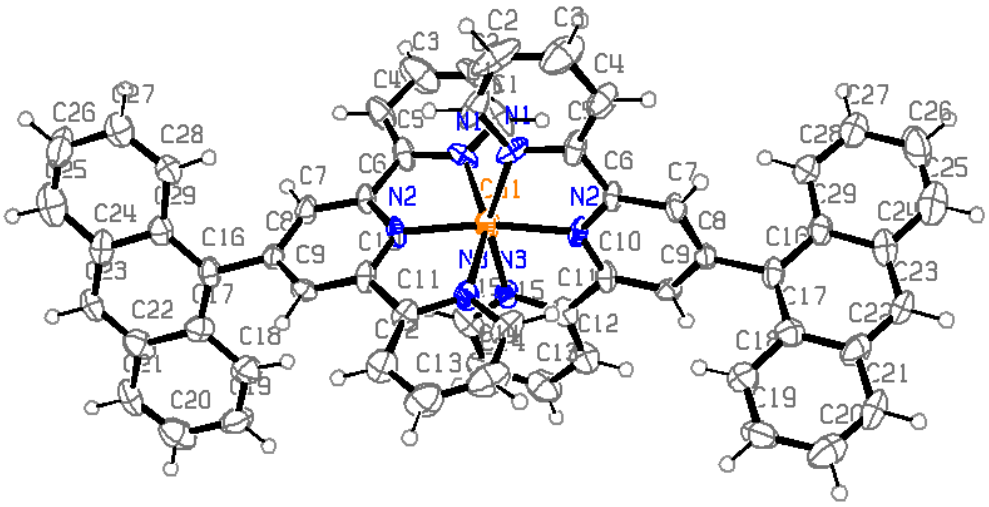
(d) ^{13}C NMR spectrum of 2 recorded in the DMSO-d6



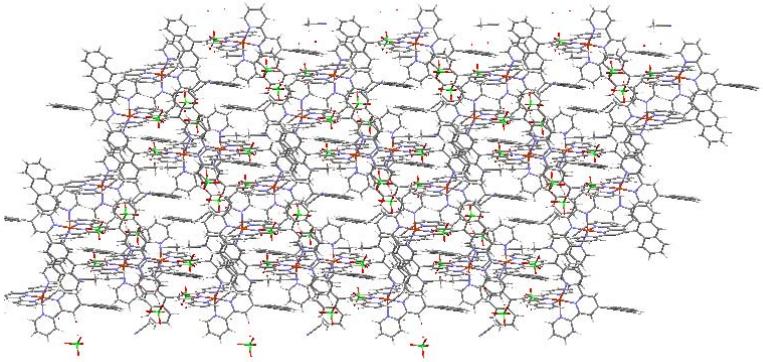
S02: UV-Visible spectra for L & 1:



S03: ORTEP diagram of 1.

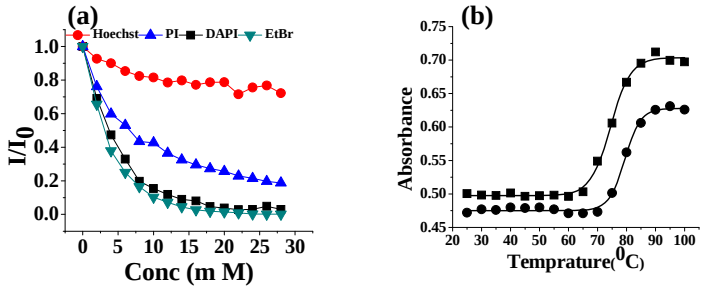


S04: Crystal packing diagram of 1



Interaction	Distance (Å)
An-py(π…π)	4.182
An-Py(C-H…π)	2.926
Solvent-An (C-H…π)	2.776

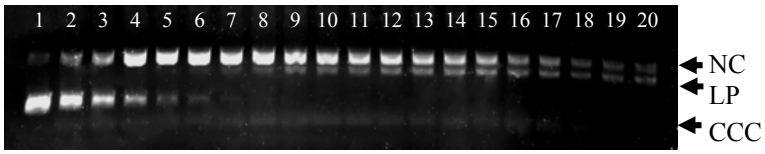
S05: Binding with CT-DNA:



Titration of DNA with **1**: (a) Relative fluorescence intensity plots obtained during the titration of displacement of DNA binding dyes by 1. (b) DNA melting curves of CT–DNA, in the absence (■) and in the presence (●) of **1**

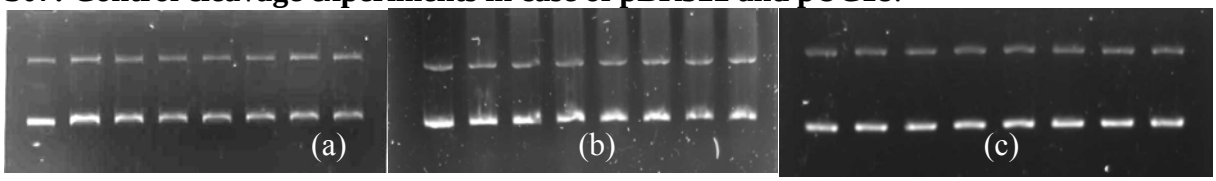
Plasmid cleavage:

S06: Cleavage of pUC18 by 1 under visible light:



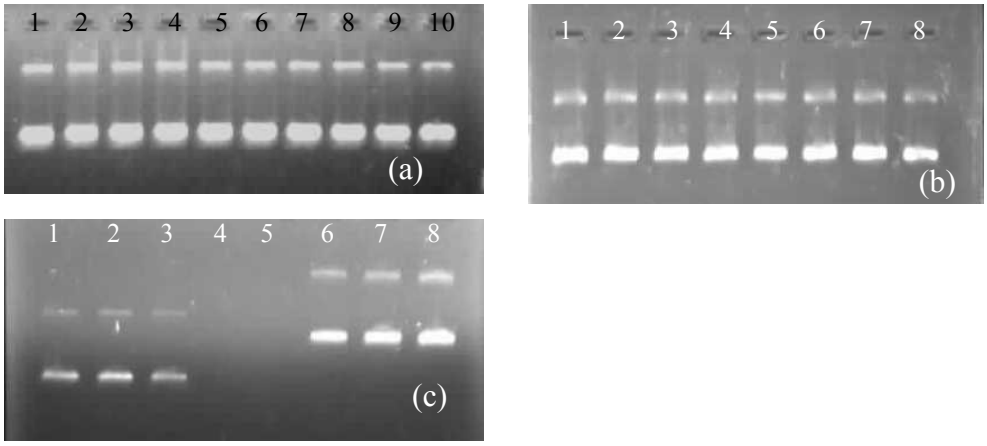
Agarose gel electrophoresis of pUC18 treated with **1** under visible light. Lane 1 is control (pUC18 incubated for 450 min in the absence of **1**, but under similar conditions), Lanes 2 – 20 are at 0, 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 480 min of incubation respectively.

S07: Control cleavage experiments in case of pBR322 and pUC18:



Plasmid cleavage in the presence of **L** without the copper ion. Reaction was carried out as a heterogeneous system as **L** is partially soluble in buffer. (a) pBR322, (b) pUC18. (c) Reaction of pBR322 with $Cu(ClO_4)_2$

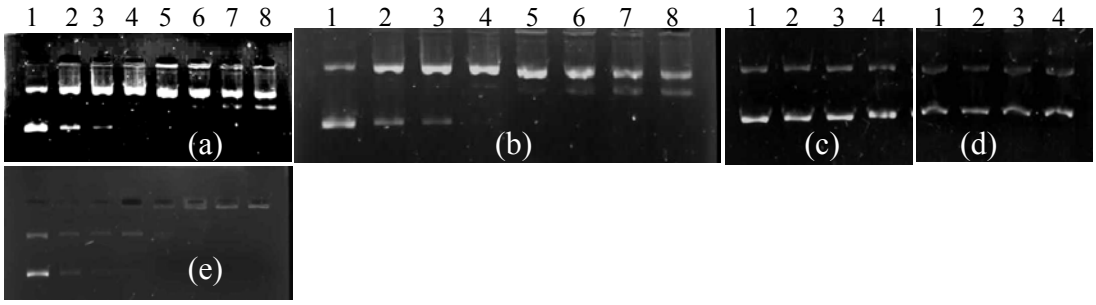
S08 Plasmid cleavage by Zn^{2+} complex of anthracenyl ter pyridine



Plasmid cleavage by Zn^{2+} complex of anthracenyl ter pyridine complex: (a) pBR322 cleavage. Lane no. 1 – 10 indicated the time of incubation from 0 – 8 h. (b) pUC18 cleavage. Lane no. 1 – 8 indicated the time of incubation from 0 – 8 h. (c) plasmid cleavage in the presence of zinc per-chlorate incubated for 8 h. lane 1-3 are for pUC18

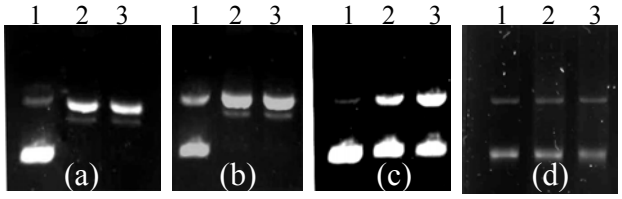
and lane 6 – 8 atr for pBR322. Lane 1 is control all cases and also 6 is control in case of figure (c).

S09: Nature of cleavage of pBR322 by 1 under visible light:



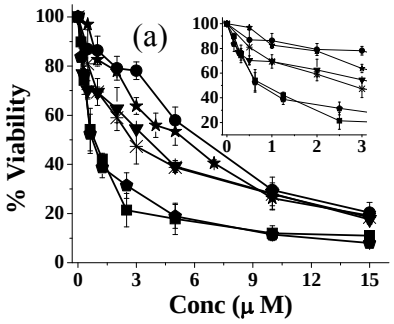
Plasmid cleavage (pBR322) in the presence of hydroxyl radical scavengers (viz., D-mannitol, DMSO) and singlet oxygen scavengers (viz., sodium azide, L-histidine) under visible light: (a) in the presence of D-mannitol, (b) DMSO, (c) sodium azide, (d) L-histidine. Lane 1 is control, lane 2 – 8 incubation from 0 to 8 h in (a) and (b), lane 2 to 4 0 – 8 h of incubation, (e) in D_2O as solvent.

S10: Nature of cleavage of pUC18 by 1 under visible light:

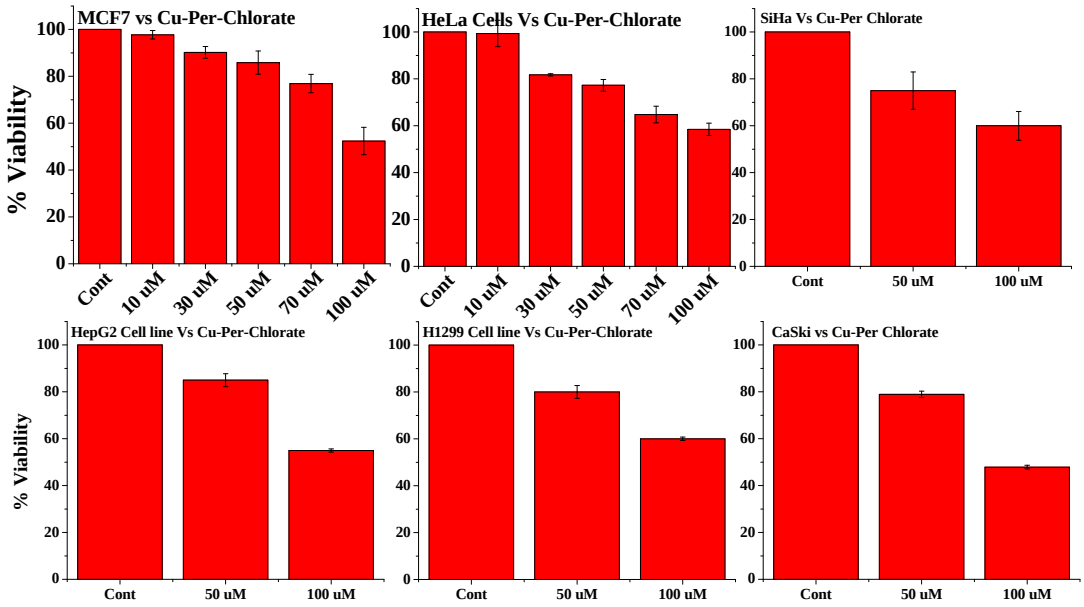


Plasmid cleavage (pUC18) in the presence of hydroxyl radical scavengers (viz., D-mannitol, DMSO) and singlet oxygen scavengers (viz., sodium azide, L-histidine). (a) In the presence of D-mannitol, (b) DMSO, (c) sodium azide, (d) L-histidine. Lane 1 is control; lane 2 and 3 (in duplicate) incubation for 8 h.

S11: Cell proliferation assay:



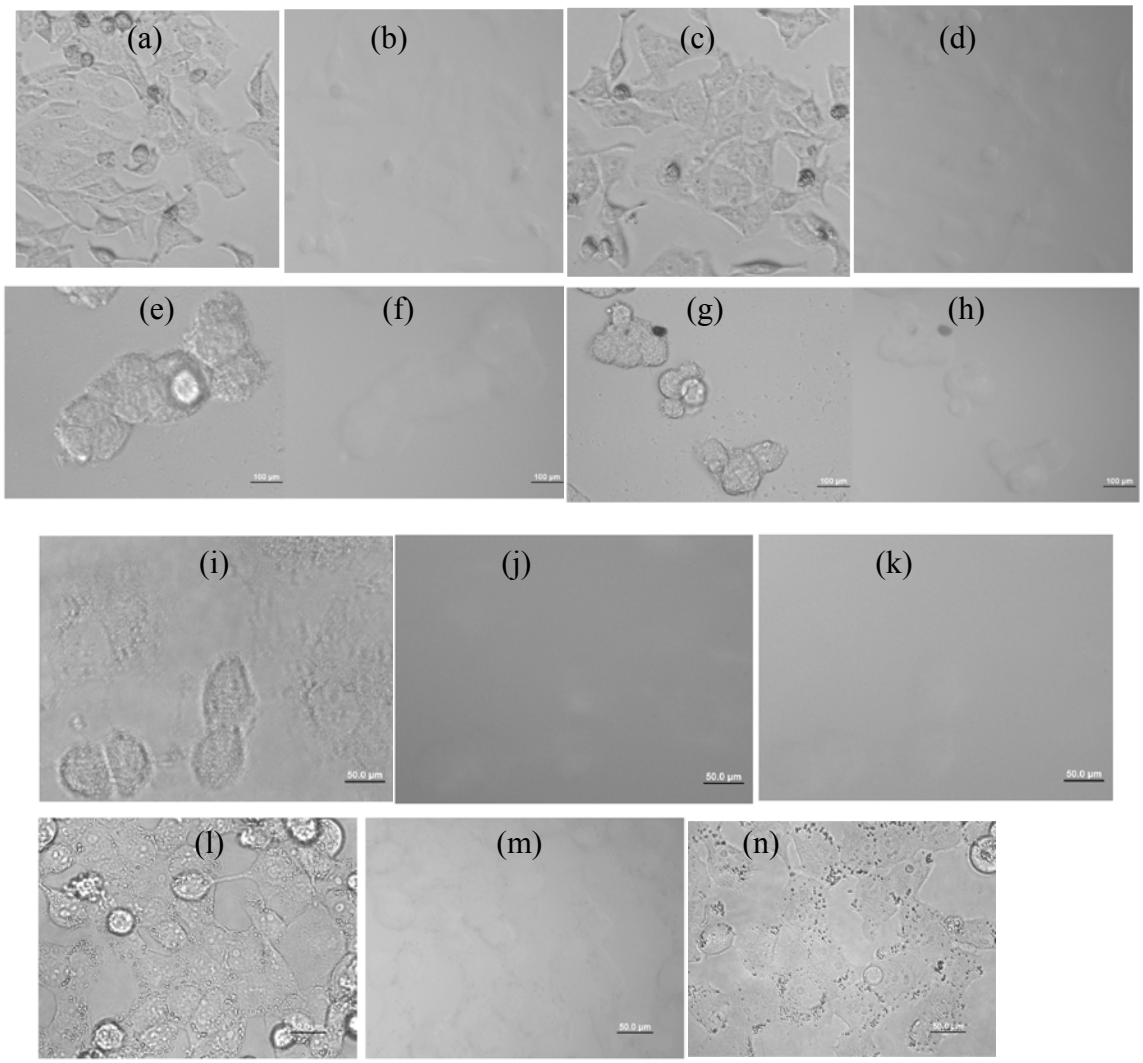
Dose response curve: Viability of cells (cytotoxicity) as a function of the concentration of **1**: (a) Dose response curves {Inset: expansion of X-axis from 0 to 3 μM}. The symbols are, ●= H1299, ★= MCF–7, ▼ = HepG2, ✳ = SiHa, ⬟ = CaSki, ■= HeLa, cell lines.



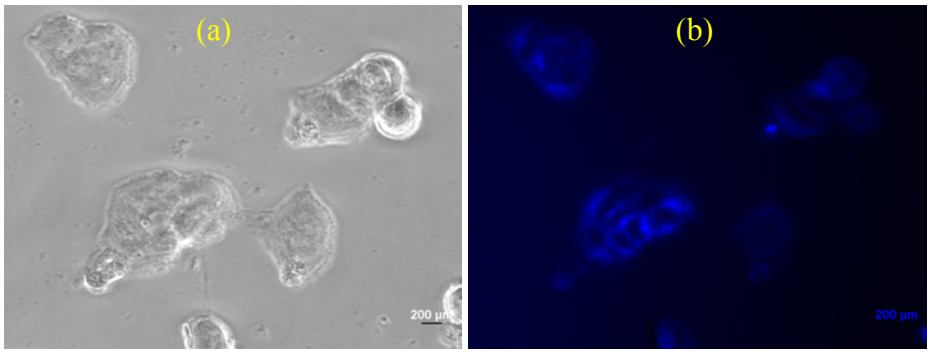
Percentage of cell viability in the presence of copper per chlorate.

Percentage of inhibition by copper per chlorate for different cell lines under identical conditions.

Cells	% inhibition at 100 mM of copper per chlorate
HeLa	42
SiHa	40
CaSki	52
MCF-7	48
HepG2	45
H1299	40

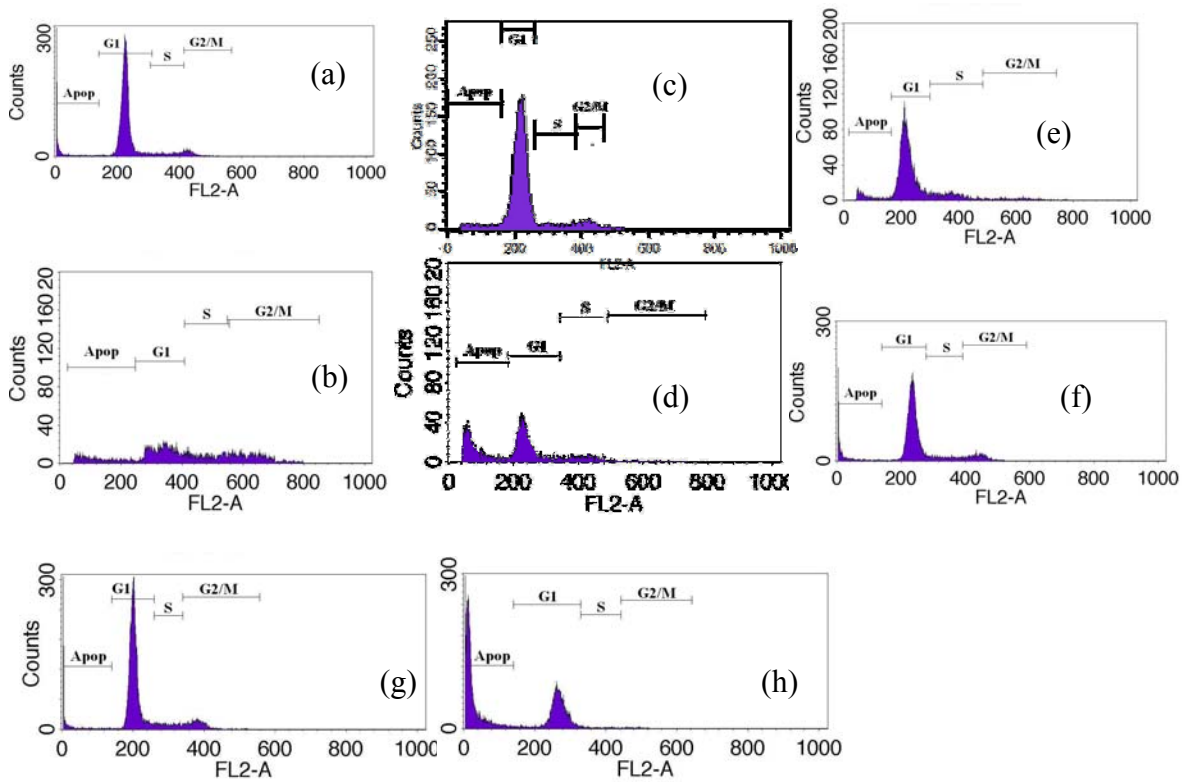


Control ells: Copper per chlorate treated / untreated cell, (a) copper per chlorate treated HaLa, (b) copper per chlorate treated HeLa under fluorescence microscope, (c) HeLa control, (d) HeLa under fluorescence microscope, (e) MCF-7 control, (f) MCF-7 under fluorescence microscope, (g) copper per chlorate treated MCF-7, (h) copper per chlorate threaded MCF-7 under fluorescence microscope, (i) copper perchlorate treated SiHa, (j) copper per chlorated treated SiHa under fluorescence microscope, (k) SiHa cell under fluorescence microscope, (l) H1299 copper per chlorate treated cells, (m) copper per chlorate treated cells under fluorescence microscope, (n) H1299 control cell without the copper perchlorate or copper complex showing the granular structure which is because of its cellular morphology. Either the copper per chlorate or untreated cell did no show any fluorescent granular structure.



Control transfected MCF-7 cells with simple DNA without e6 that do not exhibit granular structure. (a) phase contract image, (b) fluorescence microscopic image of same region.

S12: FACS analysis:

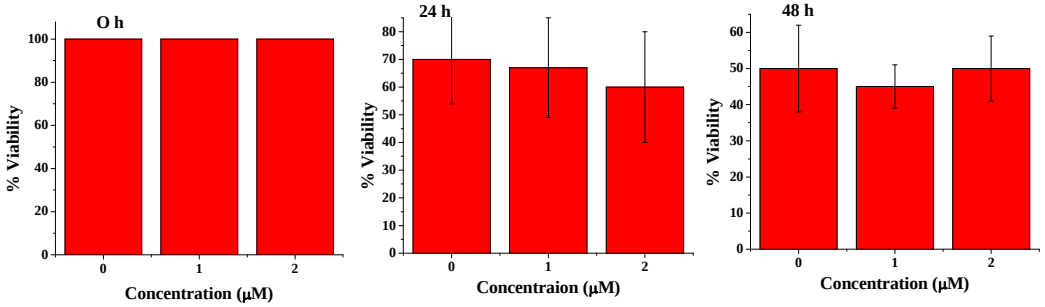


Distribution of cell in different cell cycle after the treatment with copper complex, as analyzed by the FACS. (a) MCF-7 control, (b) MCF-7 treated, (c) SiHa control, (d) SiHa treated, (e) CaSki control, (f) CaSki treated, (g) HeLa control, (h) HeLa treated.

FACS analysis of the different cell lines after the treatment with the copper complex

Phase	HeLa		SiHa		CaSki		MCF–7	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Apop	8	60	4	35	7	60	10	15
G1	80	38	88	50	86	39	80	38
S	7	2	4	12	9	1	6	22
G2/M	5	0.43	4	3	4	0.47	4	25

S13. Cell viability studies with healthy cell lines



Percentage of cell viability in the presence of **1** at various time intervals.