Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

A vanillin-based copper(II) metal complex with a DNA-mediated apoptotic activity

Wendy M. T. Q. de Medeiros [a], Mayara J. C. de Medeiros[a], Edinilton M. Carvalho[b], Jailma A. de Lima[c], Verônica da S. Oliveira[a], Ana C. F. de B. Pontes[a], Francisco O. N. da Silva[a], Javier A. Ellena[d], Hugo A. de O. Rocha[c], Eduardo H. S. de Sousa[b] and Daniel de L. Pontes*[a]

Figures S1 – S10

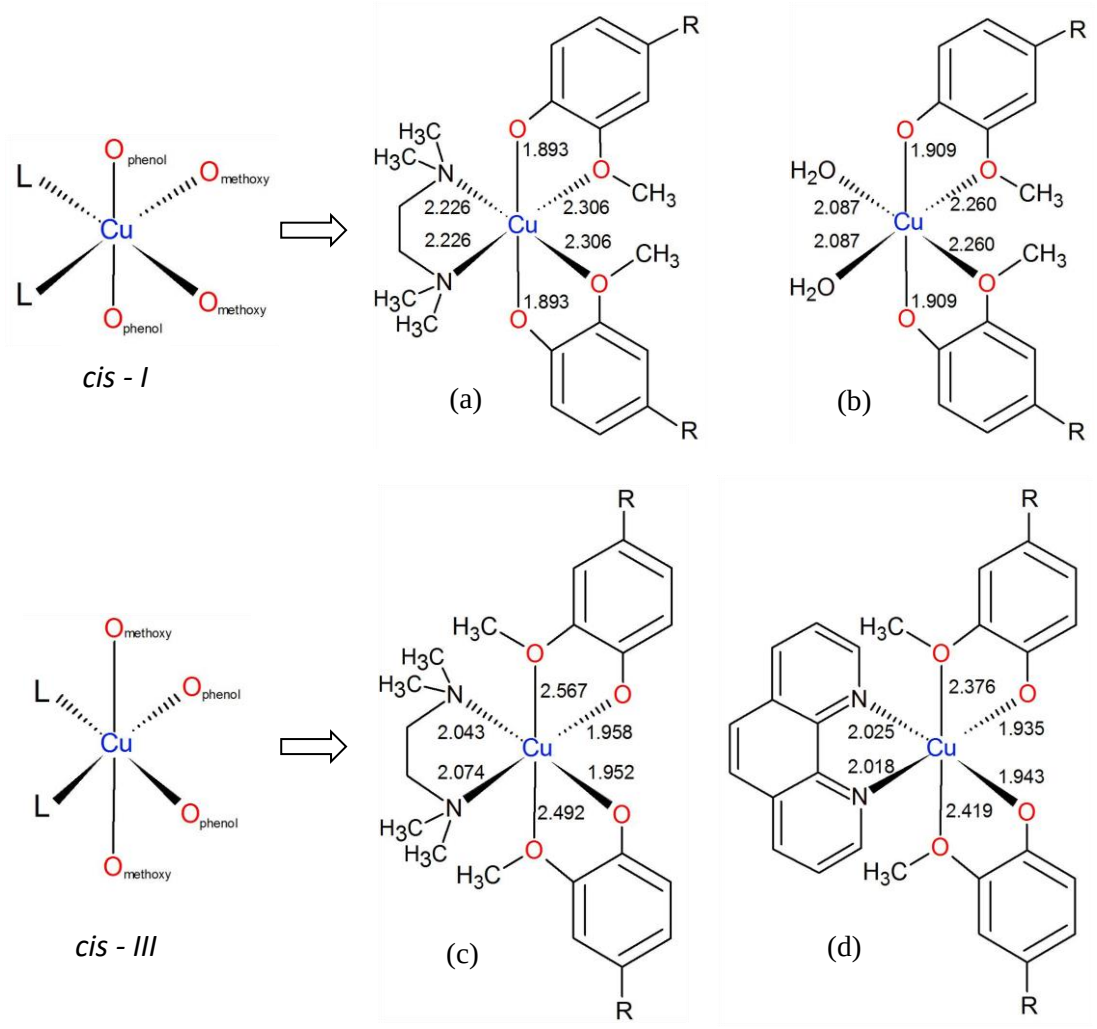

Figure S1. Bonding lengths of copper(II) complexes with vanillinate ions (R = aldehyde). (a) L,L = N,N,N',N'-tetramethylethylenediamine; (b) L = H_2O; (c) L,L = N,N,N',N'-tetramethylethylenediamine; (d) [Cu(phen)$(van)_2$] (this work).

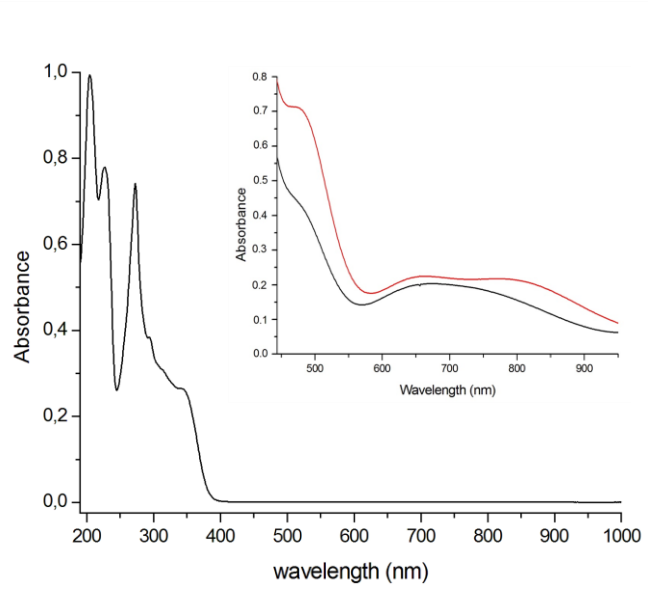

Figure S2. Electronic spectra of an aqueous solution of [Cu(phen)(van)$_2$], 2.6 x 10^{-5} mol L^{-1}. Inset shows the expanded region of the "d–d" transitions either in water (black trace, 3.6 x 10^{-3} mol L^{-1}) or in methanol (red trace, 1.7 x 10^{-3} mol L^{-1}).

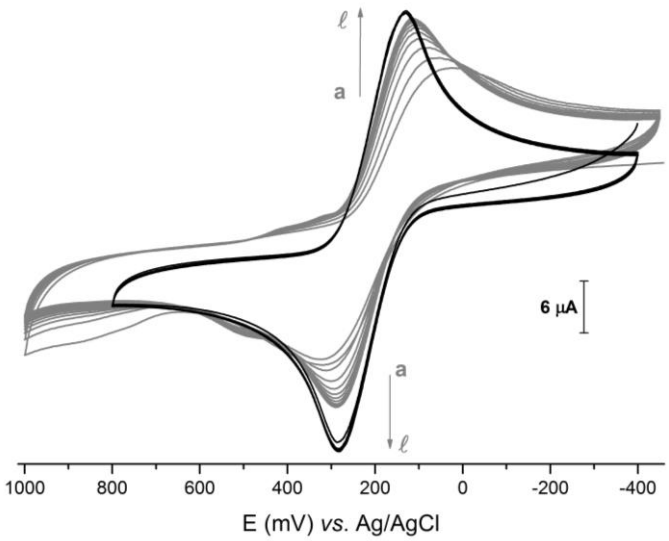

Figure S3. Cyclic voltammogram in KCl 0.1 mol L^{-1}, pH = 3.5, of $K_3[Fe(CN)_6]$ (2 mmol L^{-1}) obtained before (black curve) and just after (gray curves) conducting five cycles of the [Cu(phen)(van)$_2$] (1 mmol L^{-1}, KCl 0.1 mol L^{-1}, pH = 7.0) and without previously polishing the working electrode.

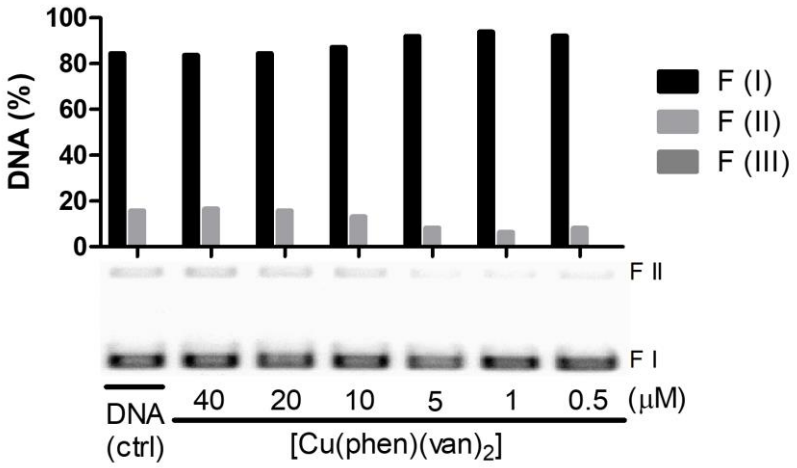

Figure S4. DNA cleavage assay employing plasmid DNA pBR322 (8.3 ng μL^{-1} per well) with metal complex [Cu(phen)$(van)_2$].

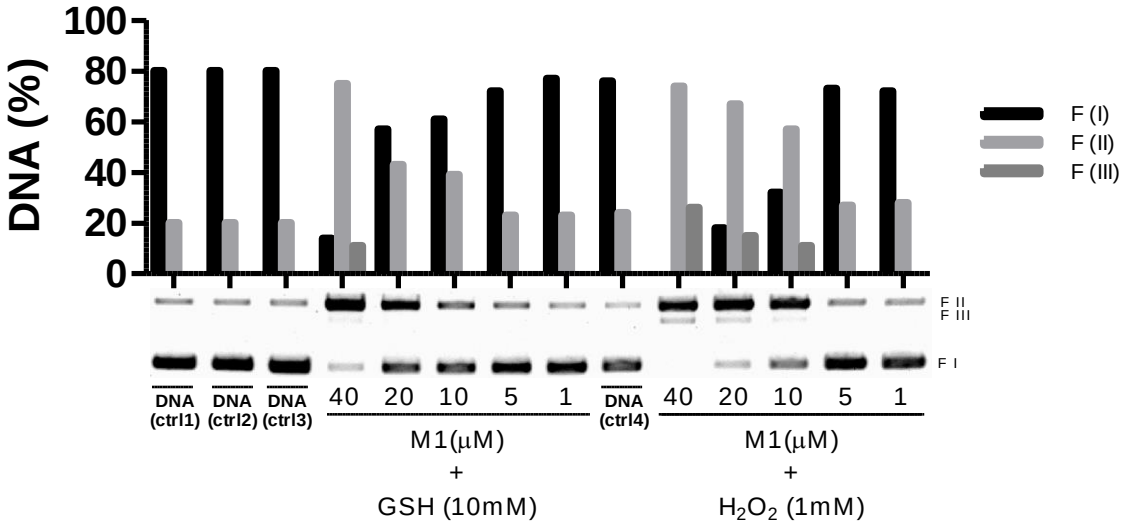

Figure S5. DNA cleavage study of copper precursor. It was employed plasmid DNA (8.3 ng μL^{-1} per well) and metal complex M1 at different concentrations (40, 20, 10, 5, and 1 μM). The reaction occurred in the presence of GSH 10 mM and H_2O_2 1 mM, in the dark for 90 minutes at 25 ^oC. DNA control (ctrl1) contains only DNA; DNA control 2 (ctrl2) contains DNA with M1 complex; DNA control 3 (ctrl3) contains DNA with GSH; DNA control 4 (ctrl4) contains DNA with H_2O_2.

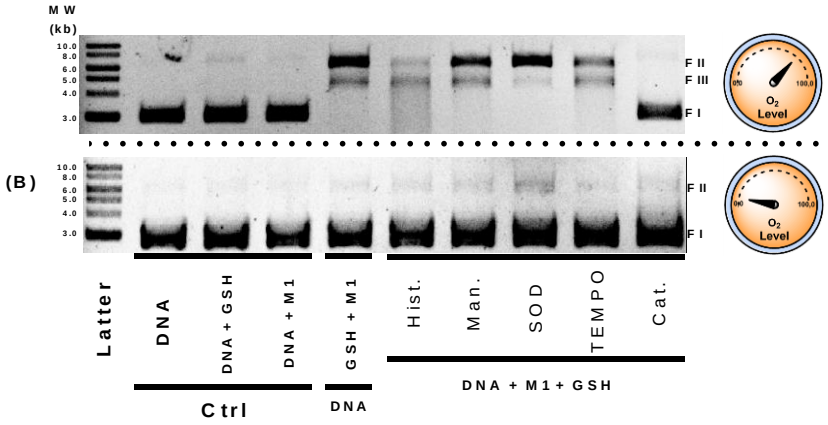

Figure S6. Mechanistic study of oxidizing species involved in the DNA cleavage. It was used plasmid DNA (8.3 ng/μL per well), GSH (1.5 mmol L^{-1}), [Cu(phen)Cl_2] (M1, 40 μmol L^{-1}) along with the inhibitors of free radicals: histidine (hist., 13 mmol L^{-1}), mannitol (man., 13 mmol L^{-1}), SOD (4 U μL^{-1}), TEMPO (3.9 mmol L^{-1}) e catalase (cat., 3.9 μmol L^{-1}), in reaction for 60 minutes at 25 °C. Reactions carried out under aerobic (TOP gel) or anaerobic (BOTTOM gel) conditions.

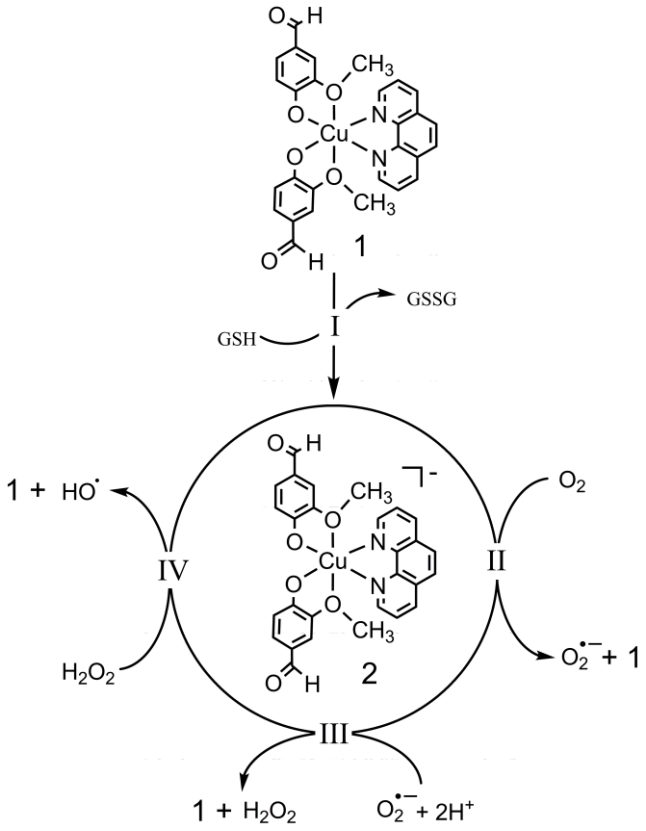

Figure S7. Proposed catalytic cycle for reduction of the [Cu(phen)$(van)_2$] (1) complex by GSH with production of superoxide: (I) reduction of Cu(II) by GSH; (II) production of $O_2^{\bullet-}$ via re-oxidation of Cu(I); (III) production of H_2O_2 via oxidation of Cu(I); and (IV) final production of $HO^{\bullet}$.

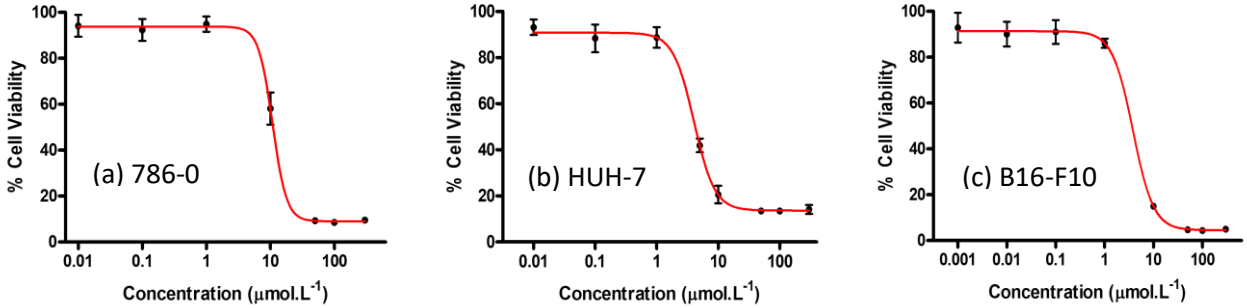

Figure S8. Cell viability of [Cu(phen)(van)$_2$] for (a) 786-0 (b) HUH-7, (c) B16-F10.

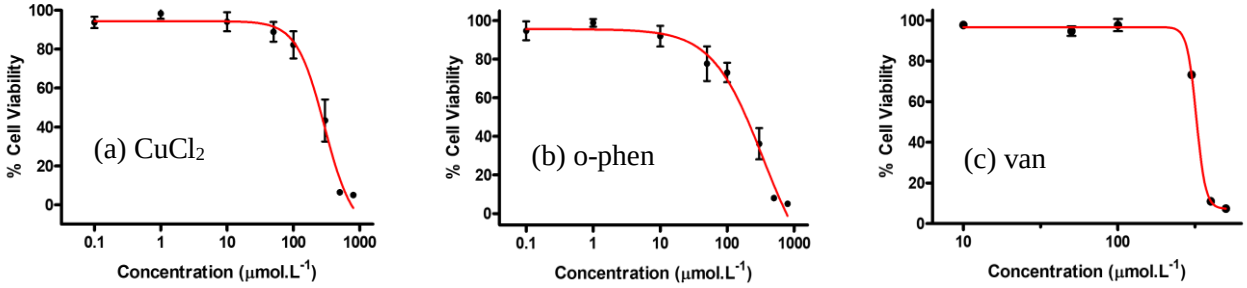

Figure S9. Cell viability of (a) $CuCl_2$ (b) o-phenanthroline and (c) vanillin against 786-0 cell line.

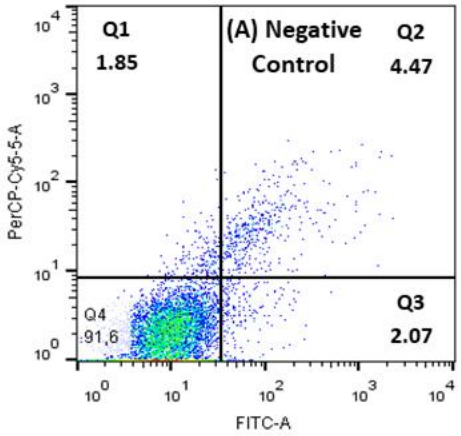

Figure S10. Flow cytometry study for untreated 786-0 cells (negative control).

Tables S1 – S3

Table S1. Crystal data and structure refinement parameters.

Empirical formula	$C_{28}H_{28}N_2O_9Cu$	
Formula weight	600.06	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 12.8200(16) Å	α= 104.639(6)°
	b = 14.9120(16) Å	β= 92.409(6)°
	c = 15.1150(12) Å	γ = 103.251(6)°
Volume	2705.9(5) $Å^3$	
Z	4	
Density (calculated)	1.473 mg/m^3	
Absorption coefficient	0.865 mm^{-1}	
F(000)	1244	
Crystal size	0.4 x 0.2 x 0.1 mm^3	
Theta range for data collection	2.787 to 27.532°	
Index ranges	$-16 \leq h \leq 16$, $-18 \leq k \leq 19$, $-19 \leq l \leq 19$	
Reflections collected	20967	
Independent reflections	11998 [R(int) = 0.1223]	
Completeness to theta = 25.242°	99.8 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.743 and 0.625	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	11998 / 0 / 725	
Goodness-of-fit on F^2	0.889	
Final R indices [I>2σ(I)]	R1 = 0.0566, wR2 = 0.1028	
R indices (all data)	R1 = 0.1324, wR2 = 0.1129	
Largest diff. peak and hole	0.674 and -0.417 $e.Å^{-3}$	

Table S2. Selected distances (Å) and angles (°) around the copper atom in [Cu(phen)(van)$_2$] complex.

Cu(1)-O(11):1.945(2)	O(11)-Cu(1)-O(12): 76.03(9)	O(21)-Cu(2)-O(22): 74.79(9)
Cu(1)-O(12): 2.377(3)	O(11)-Cu(1)-O(14): 94.9(1)	O(21)-Cu(2)-O(24): 97.6(1)
Cu(1)-O(14): 1.935(2)	O(11)-Cu(1)-O(15): 88.85(9)	O(21)-Cu(2)-O(25): 86.20(9)
Cu(1)-O(15): 2.419(3)	O(11)-Cu(1)-N(11): 92.6(1)	O(21)-Cu(2)-N(21): 91.1(1)
Cu(1)-N(11): 2.017(2)	O(11)-Cu(1)-N(12): 170.5(1)	O(21)-Cu(2)-N(22): 167.2(1)
Cu(1)-N(12): 2.023(3)	O(12)-Cu(1)-O(14): 90.42(9)	O(22)-Cu(2)-O(24): 91.00(9)
Cu(2)-O(21): 1.923(3)	O(12)-Cu(1)-O(15): 158.24(8)	O(22)-Cu(2)-O(25): 153.98(8)
Cu(2)-O(22): 2.425(2)	O(12)-Cu(1)-N(11): 94.08(9)	O(22)-Cu(2)-N(21): 98.69(9)
Cu(2)-O(24): 1.926(2)	O(12)-Cu(1)-N(12): 97.9(1)	O(22)-Cu(2)-N(22): 96.1(1)
Cu(2)-O(25): 2.454(2)	O(14)-Cu(1)-O(15): 75.18(9)	O(24)-Cu(2)-O(25): 73.80(9)
Cu(2)-N(21): 2.015(2)	O(14)-Cu(1)-N(11): 172.0(1)	O(24)-Cu(2)-N(21): 168.4(1)
Cu(2)-N(22): 2.022(4)	O(14)-Cu(1)-N(12): 92.4(1)	O(24)-Cu(2)-N(22): 91.4(1)
	O(15)-Cu(1)-N(11): 102.25(9)	O(25)-Cu(2)-N(21): 99.3(1)
	O(15)-Cu(1)-N(12): 98.9(1)	O(25)-Cu(2)-N(22): 105.1(1)
	N(11)-Cu(1)-N(12): 80.5(1)	N(21)-Cu(2)-N(22): 81.3(1)

Table S3. Electronic transition bands of [Cu(phen)(van)$_2$] in aqueous solutions

Wavelength (nm)	**Assignment**	**Molar Absortivity (L mol^{-1} cm^{-1})**
205	Intraligand, phen ($\pi \rightarrow \pi*$)	5.43 x 10^4
227	Intraligand, phen ($\pi \rightarrow \pi*$)	4.24 x 10^4
273	Intraligand, phen ($\pi \rightarrow \pi*$)	4.04 x 10^4
345	Intraligand, vanillinate ion ($\pi \rightarrow \pi*$)	1.45 x 10^4
469	d-d	1.25 x 10^2
650	d-d	5.53 x 10^1
775	d-d	4.78 x 10^1