Supporting Information

Copper (II) complex of methionine conjugated bis-pyrazole based ligand promotes dual pathway for DNA cleavage

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Table S1. Literature reports on DNA cleavage activity of methionine based metal complexes in presence of light and in dark.

complexes	Dark Cytotoxicity		Photo-cytotoxicity		ROS species involved	Ref
	Conc (μM)	%NC	Conc (μM)	%NC		
$[Cu_2(L^1)_2(H_2O)]$	80[a]		-	-	$^1O_2$	1
$[Cu_2(L^2)_2(H_2O)].H_2O$	88[a]		-	-		
$[(\eta^5\text{-}C_5Me_5)$ Ir $(H_2metOMe)$ $(dppz)](CF_3SO_3)_3$	70[b]		60[c]			2
[(η5-Cp*) Ir (AcmetOMe)(dppz)]$^{2+}$			60[c]	-		
[Cu(phen)(met)(OMe)](ClO_4)	200	14	200	89[d]	$^1O_2$	3
				40[e]		
				43[f,]		
[Cu(L-met)(phen)(MeOH)](ClO_4)	100	7	50[i]	40	$^{\bullet}OH^t, ^1O_2{}^u$	4
	100[h]	40				
[Cu(L-met)(bpy)(H_2O)] (ClO_4)	100[h]	10	50[i]	10		
[Cu(L-met)(dpq)(H_2O)](ClO_4)	100[h]	83	50[i]	77		
[Cu(L-met)(bppz)(H_2O)](ClO_4)	100[h]	87	50[i]	60		
[VO(salmet)(phen)]	67	15[h]/11[p]	33[j]/67[k]	15[j]/4[k]	$^{\bullet}OH^t/^1O_2$, $^{\bullet}OH^{u,n}$	5
[VO(salmet)(dpq)]		13[h]/12[o]/14[p]		82[j]/20[k]		
[VO(salmet)(dppz)]		9[g]/10[p]		91[j]/28[k]		
[Cu(Fc-met)(phen)](NO_3)	30	6	30	50[l]/60[m]	$^{\bullet}OH^u$	6
[Cu(Fc-met)(dppz)](NO_3)		5		91[l]/97[m]		
[Cu(Fc-met)(phen)](NO_3)	20	5	20	62[q]	$^{\bullet}OH^{t,u}$	7
	10	53[o] /68[p]	25	55[r]/58[s]		
[Cu(Fc-met)(dpq)](NO_3)	20	5	20	85[q]		
	10	78[o] /82[p]	25	78[r]/82[s]		
[Cu(Fc-met)(dppz)](NO_3)	20	6	20	95[q]		
	10	87[o] /94[p]	25	92[r]/97[s]		
[Cu(Fc-met)(nip)](NO_3)	20	5	20	82[q]		
	10	72[o] /79[p]	25	77[r]/79[s]		
[Cu(Ph-met)(phen)](NO_3)	20	12	20	33[q]		
	10	49[o] /32[p]	25	39[r]/38[s]		
[Cu(Ph-met)(dppz)](NO_3)	20	18	20	63[q]		
	10	85[o] /61[p]	25	75[r]/73[s]		

L^1 = (E)-2-(2 hydroxybenzylideneamino)-4-(methylthio)butanoic acid, L^2 = 2-(2-hydroxybenzylamino)-4-(methylthio)butanoic acid,bpy=2,2'–bipyridine, dpq=dipyrido[3,2-*d*:2',3'-*f*]quinoxaline,dppz=dipyrido[3,2-*a*:2',3'-*c*]phenazene, phen=1,10-phenanthroline, Fc-met=ferrocene conjugate reduced schiff base of methionine, salmet=N-salicylidene-L-methionate, Ph-met= reduced Schiff base from benzaldehyde and L-methionine, L-met=L-methionine [a] 25μM sodium ascorbate was used as reducing agent, [b] no external reducing agent used, [c] 500W Hg Lamp(3min), [d] 312nm (96W, 20 min), [e] 436 nm (125W,10 min), [f] 532 nm (125W, 30 min), [g] 312nm (5 min), [h] mercaptopropionic acid as reducing agent, [i] 365nm (12W, 5min), [j]365nm (12W, 2h),[k] >750nm(150mW, 2h irradiation), [l] 454nm(30mW,2h),[m] 633nm(12mW,2h), [n] only singlet oxygen involves and for >750nm both the ROS involved, [o] in presence of glutathione, [p] in presence of H_2O_2, [q] 454 nm (50mW, 2h), [r] 568 nm (50mW, 2h), [s] 647 nm (50mW, 2h), [t] in dark, [u] in presence of photo irradiation.

Table S2 Crystallographic data and structure refinement parameters for **1-3.**

	1	**2**.4CH_3CN.2H_2O	**3**.CH_2Cl_2
Empirical formula	$[C_{38}H_{56}Cu\ N_{12}O_6](ClO_4)_2$	$[C_{38}H_{60}CuN_{12}O_6](Cl)_2$ .4(CH_3CN).2H_2O	$[C_{37}H_{54}CuN_{10}O_6S_2](ClO_4)_2$.(CH_2Cl_2)
F_w(g mol^{-1})	1037.8	1115.65	1132.39
Temperature(K)	100(2)	100(2)	100(2)
Crystal system	Monoclinic	Triclinic	Triclinic
space group	*C*2/*c*	*P*$\bar{1}$	*P*1
a(Å)	39.869(4)	9.9692(10)	10.9216(11)
b(Å)	13.5647(12)	10.5011(11)	11.5633(11)
c(Å)	20.4661(18)	14.4625(16)	13.5707(13)
α(°)	90	69.076(2)	71.247(2)
β(°)	90.536(2)	70.144(2)	69.310(2)
γ(°)	90	82.168(2)	85.255(2)
V(Å^3)	11067.9(17)	1329.9(2)	1517.3(3)
Z	8	1	1
D_c (g cm^{-3})	1.246	1.393	1.239
μ(mm^{-1})	0.56	0.578	0.684
F(000)	4337.6	591	673
Limiting indices	-50<h<50, -17<k<17, -20<l<26	-13<h<13,-13<k<13, -19<l<19	-13<h<12, -14<k<14, -17<l<17
Reflections collected	91296	23922	18417
Unique reflections/R_{int}	12090/0.0388	6563/0.0262	9450/ 0.0219
Data / restraints / parameters	12090/0/639	6563/0/340	9450/3/625
Goodness-of-fit[a] on F^2	1.058	1.063	1.070
R_1[b], wR_2[c] [$I>2\sigma(I)$]	0.0423, 0.1156	0.0467, 0.1419	0.0524, 0.1468
R_1, wR_2 (all data)	0.0493, 0.1190	0.0497, 0.1447	0.0570, 0.1500
$\Delta\rho_{max/min}$ (e Å^{-3})	0.896/-0.595	0.584/-1.328	1.704/-0.559

[a]Goodness-of-fit=$[\Sigma w(F_o^2 - F_c^2)^2]/(N_{refln} - N_{params})]^{1/2}$, based on all data. [b] $R_1 = \Sigma\ (|F_o| - |F_c|)/|F_o|$. [c] $wR_2 = [R[\Sigma\ (F_o^2 - F_c^2)^2]/R[\Sigma(F_o^2)^2]]^{1/2}$.

Table S3 Bond Valence Sum calculation for **1-3** with two possible oxidation states

Complex	Cu^I	Cu^{II}
1	1.584621	1.657387
2	1.568981	1.649399
3	1.558736	1.628816

Table S4 Lipophilicity of metal complexes and the ligands in octanol/water system.

	$LogP_{o/w}$
L1	2.08(3)
L2	1.67(6)
1	-0.08(2)
2	-0.49(3)
3	-0.35(2)

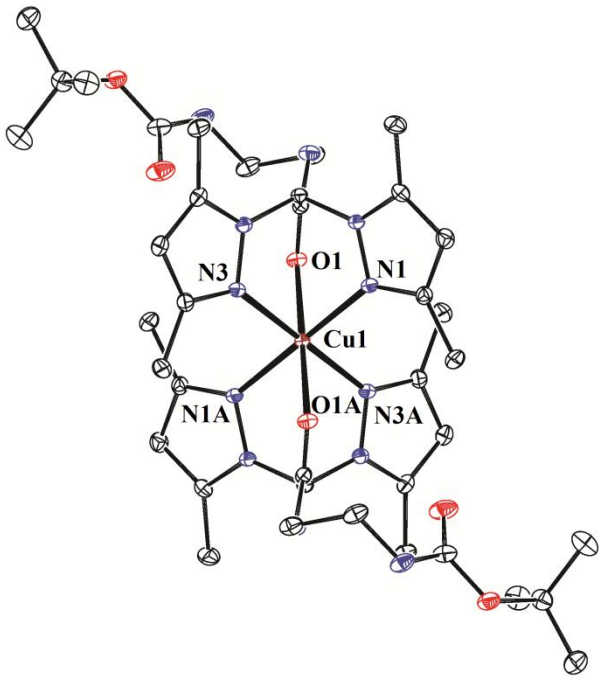

Fig. S1 ORTEP diagram of **2**.Thermal ellipsoids are drawn at 50% probability level. Hydrogen atoms, counter anions and solvents have been omitted for clarity. Symmetry transformations used to generate equivalent atoms A: : -x,-y+1,-z+1.

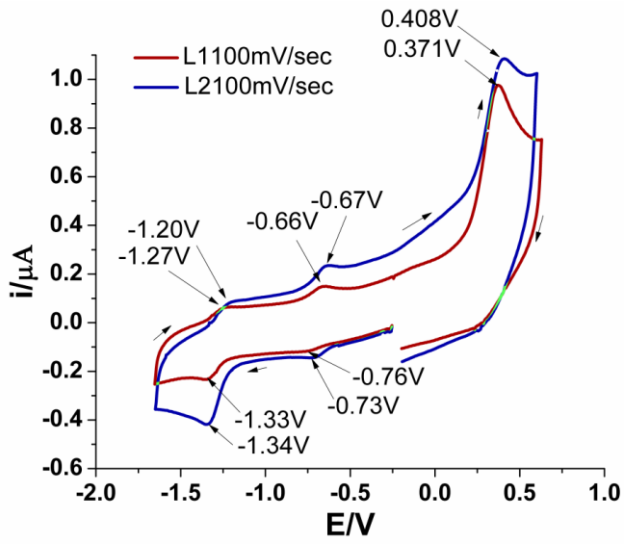

Fig. S2 Cyclic Voltammogram of a 1.0 mM solution of L1 & L2 in DMF and TBAP (0.1 M) as supporting electrolyte. Indicated peak potentials are in V *vs.* 0.01 M non aqueous Ag/Ag^{+} reference electrode (0.55 V vs. NHE, evaluated using Fc/Fc^{+} couple)

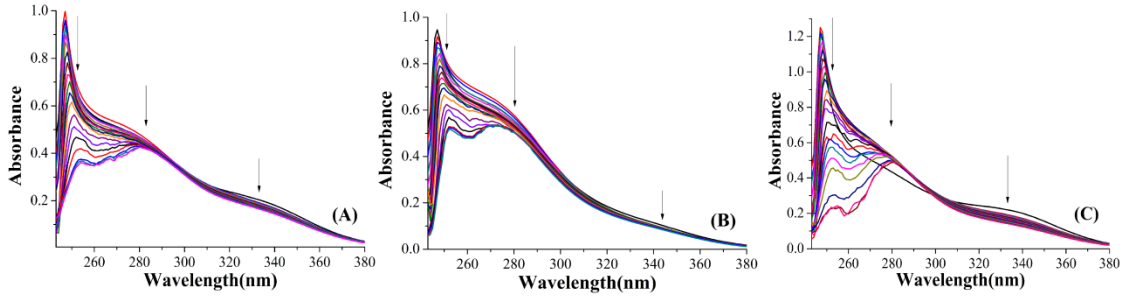

Fig. S3 Absorption spectral change after addition of increased amount of CT DNA (4×10^{-3} M) to complex (4×10^{-4}M) in Tris-NaCl/DMF (9:1) media (pH 7.4) at room 25°C: (A) **1** ,(B) **2** , (C) **3**. Inset: Plot of [DNA]/Δε vs [DNA] for calculation of apparent binding constant (K_b).

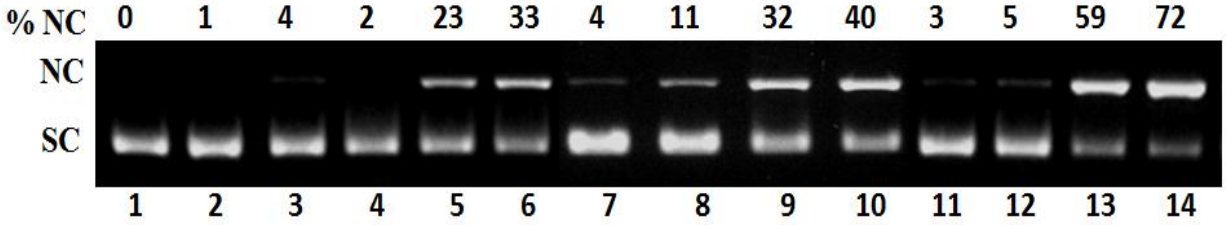

Fig. S4 DNA cleavage of pUC 19 DNA(300ng)in presence of hydrogen peroxide(H_2O_2) by **1-3** in 50mM Tris-HCl/NaCl buffer(pH=7.4) after 1.5h incubation at 37 °C .Lane 1,DNA control; lane 2, DNA+H_2O_2(700μM); lane 3, DNA+**1**(170μM); lane 4, DNA+**1**(100μM)+H_2O_2(700μM); lane 5, DNA+**1** (140μM)+H_2O_2(700μM); lane 6, DNA+**1**(170μM)+H_2O_2(700μM); lane 7, DNA+**2**(170μM); lane 8, DNA+**2**(100μM)+H_2O_2(700μM); lane 9, DNA+**2** (140μM)+H_2O_2(700μM); lane 10, DNA+**2**(170μM)+H_2O_2(700μM); lane 11, DNA+**3**(170μM); lane 12, DNA+**3**(100μM)+H_2O_2(700μM); lane 13, DNA+**3** (140μM)+H_2O_2(700μM); lane 14, DNA+**3**(170μM)+H_2O_2(700μM).

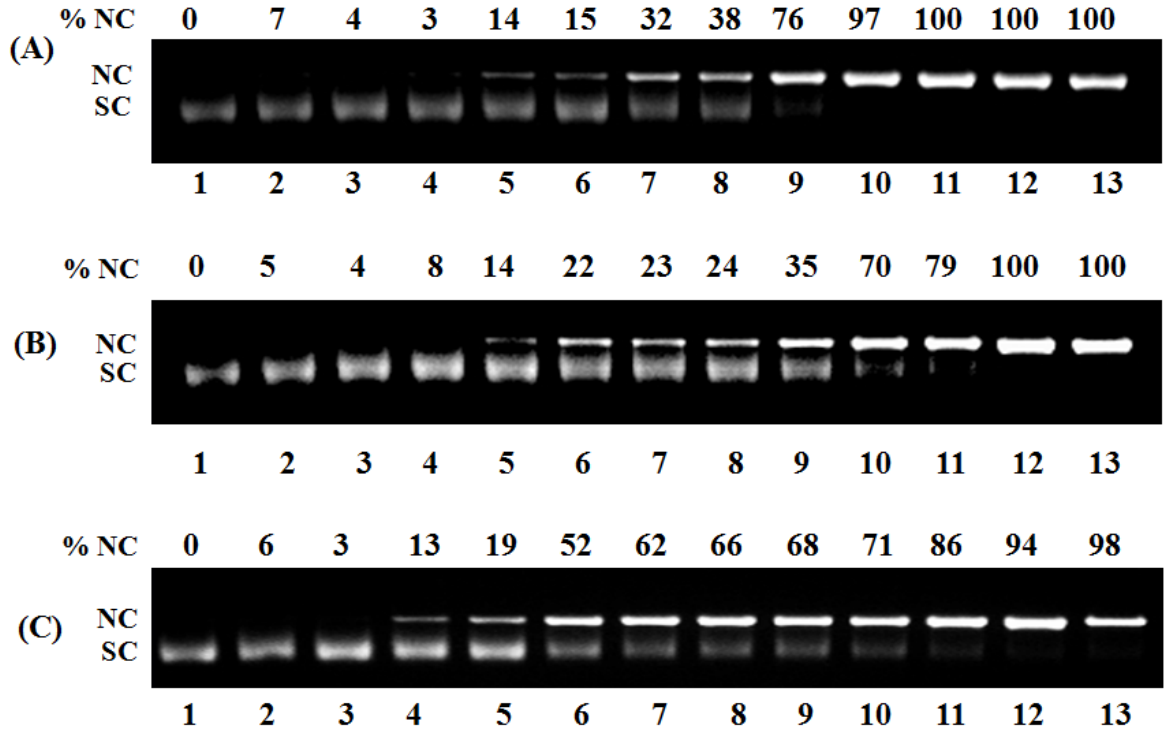

Fig. S5 pUC19 SC DNA cleavage activity of complexes **1-3** monitored by 1% agaraose gel electrophoresis, where [DNA] =300ng, [Complex] =100μM and [H_2A] =500μM in the same dilution and running buffer used for the usual gel electrophoresis. The gel images for (A) – (C) are for complexes **1-3** respectively. The representations are Lane 1, DNA control; lane 2, DNA + H_2A; lane 3, DNA+ complex; lane 4-13, DNA+complex+H_2A at 0, 5, 10 ,15, 20, 25, 30, 40, 50, 60 min respectively.

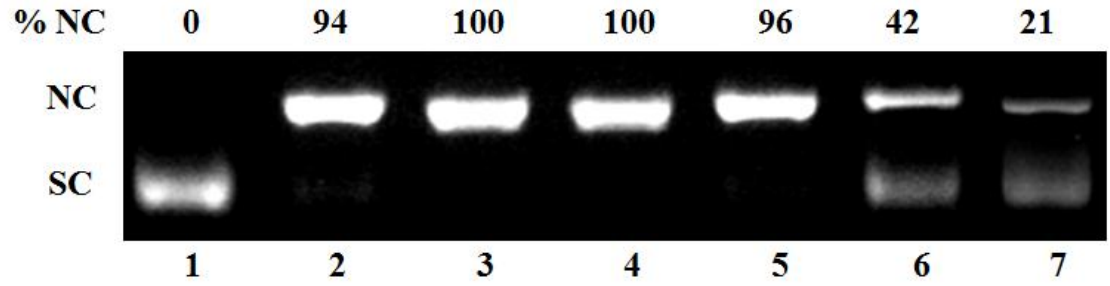

Fig. S6 Mechanistic study of oxidative DNA cleavage of pUC 19 DNA (300ng) in presence of 5 eq. ascorbic acid(H_2A) by complex **2** (100μM) in 50mM Tris-HCl/NaCl buffer(pH=7.4) after 1.5h incubation at 37 °C using DMSO(1eq.), NaN_3(1eq.), histidine(1 eq.), catalase(10U) and 4-carboxy TEMPO(1eq.). Lane 1, DNA control; lane 2, DNA+**2**; lane 3, DNA+**2**+DMSO; lane 4, DNA+**2**+NaN_3; lane 5, DNA+**2**+histidine; lane 6, DNA+**2**+catalase; lane 7, DNA+**2**+4-carboxy TEMPO.

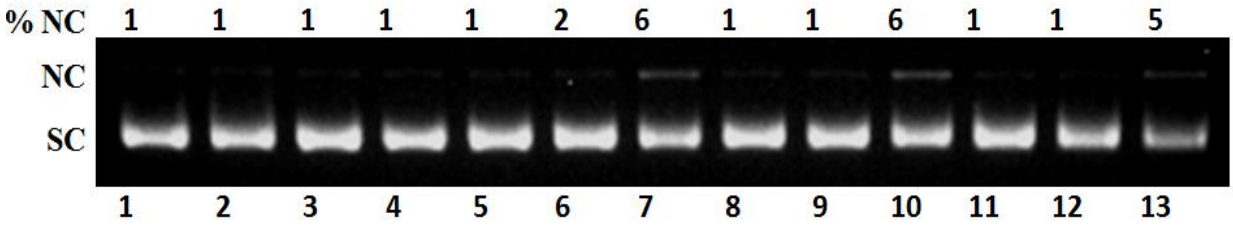

Fig. S7 DNA cleavage of pUC 19 DNA(300ng) after irradiation with 365nm UV light(4W,30min) followed by 1h dark incubation at 37 °C in presence of **1-3** in 50mM Tris-HCl/NaCl buffer(pH=7.4).Lane 1,DNA control; lane 2, DNA+**1**(100μM); lane 3, DNA+**2**(100μM); lane 4, DNA+**3**(100μM); lane 5, DNA+**1**(50μM); lane 6, DNA+**1**(100μM); lane 7, DNA+**1**(170μM); lane 8, DNA+**2**(50μM); lane 9, DNA+**2** (100μM); lane 10, DNA+**2**(170μM); lane 11, DNA+**3**(50μM); lane 12, DNA+**3**(100μM); lane 13, DNA+**3** (170μM).

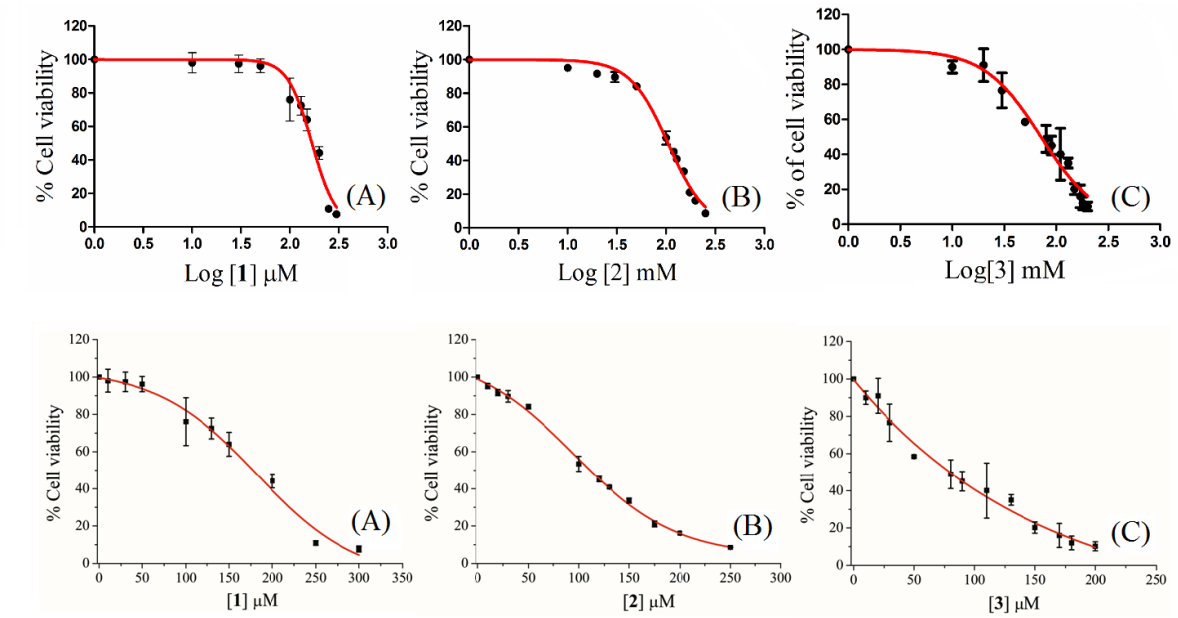

Fig. S8 Effects of treatment of the complexes **1-3** with MCF-7 tumor cell line for 48 h with rising concentrations: (A) **1**, (B) **2**, (C) **3**. Top panel of graphs showing % survival against log of concentration of complexes and bottom panel showing % survival against concentration of complexes).The IC_{50} obtained from the curves are calculated using GraphPad Prism. Data are means ± SD of three independent experiments.

References

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