Anticancer activity of a new copper (II) complex with a hydrazone ligand. Structural and spectroscopic characterization, computational simulations and cell mechanistic studies on 2D and 3D breast cancer cell models

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Supplementary Material

Table S1. Full bond lengths [Å] and angles [°] for $[Cu(HL)(H_2O)_2].NO_3$.

Bond lengths [Å]			
C(1)-C(6)	1.407(4)	C(11)-C(12)	1.381(4)
C(1)-C(2)	1.409(4)	C(12)-C(13)	1.385(5)
C(1)-C(8)	1.440(4)	C(13)-O(3)	1.348(4)
C(2)-O(1)	1.319(3)	C(13)-C(14)	1.385(5)
C(2)-C(3)	1.422(4)	C(14)-C(15)	1.375(4)
C(3)-O(4)	1.370(4)	C(16)-O(3)	1.432(5)
C(3)-C(4)	1.376(4)	N(1)-N(2)	1.384(3)
C(4)-C(5)	1.391(5)	N(1)-Cu	1.930(2)
C(5)-C(6)	1.364(5)	O(1)-Cu	1.889(2)
C(7)-O(4)	1.417(4)	O(2)-Cu	1.966(2)
C(8)-N(1)	1.286(4)	O(1W)-Cu	2.318(2)
C(9)-O(2)	1.256(3)	O(2W)-Cu	1.975(2)
C(9)-N(2)	1.343(4)	N(3)-O(33)	1.187(4)
C(9)-C(10)	1.471(3)	N(3)-O(32)	1.232(4)
C(10)-C(15)	1.392(4)	N(3)-O(31)	1.249(4)
C(10)-C(11)	1.395(4)		

Bond angles [°]			
C(6)-C(1)-C(2)	119.8(3)	C(12)-C(13)-C(14)	119.7(3)
C(6)-C(1)-C(8)	116.9(3)	C(15)-C(14)-C(13)	120.4(3)
C(2)-C(1)-C(8)	123.3(2)	C(14)-C(15)-C(10)	120.8(3)
O(1)-C(2)-C(1)	125.7(2)	C(8)-N(1)-N(2)	120.0(2)
O(1)-C(2)-C(3)	116.7(2)	C(8)-N(1)-Cu	128.1(2)
C(1)-C(2)-C(3)	117.6(2)	N(2)-N(1)-Cu	111.9(2)
O(4)-C(3)-C(4)	125.3(3)	C(9)-N(2)-N(1)	114.5(2)
O(4)-C(3)-C(2)	113.4(2)	C(2)-O(1)-Cu	126.1(2)
C(4)-C(3)-C(2)	121.3(3)	C(9)-O(2)-Cu	113.3(2)
C(3)-C(4)-C(5)	120.0(3)	C(13)-O(3)-C(16)	118.0(3)
C(6)-C(5)-C(4)	120.3(3)	C(3)-O(4)-C(7)	118.6(3)
C(5)-C(6)-C(1)	121.0(3)	O(1)-Cu-N(1)	93.51(9)
N(1)-C(8)-C(1)	123.1(2)	O(1)-Cu-O(2)	174.44(8)
O(2)-C(9)-N(2)	119.1(2)	N(1)-Cu-O(2)	81.10(9)
O(2)-C(9)-C(10)	120.4(2)	O(1)-Cu-O(2W)	91.61(9)
N(2)-C(9)-C(10)	120.4(2)	N(1)-Cu-O(2W)	158.4(1)
C(15)-C(10)-C(11)	118.3(2)	O(2)-Cu-O(2W)	92.93(9)
C(15)-C(10)-C(9)	118.1(2)	O(1)-Cu-O(1W)	90.75(9)
C(11)-C(10)-C(9)	123.5(3)	N(1)-Cu-O(1W)	107.75(9)
C(12)-C(11)-C(10)	121.0(3)	O(2)-Cu-O(1W)	92.20(9)
C(13)-C(12)-C(11)	119.8(3)	O(2W)-Cu-O(1W)	93.1(1)
O(3)-C(13)-C(12)	124.8(3)	O(33)-N(3)-O(32)	119.1(4)
O(3)-C(13)-C(14)	115.5(3)	O(33)-N(3)-O(31)	119.7(3)
		O(32)-N(3)-O(31)	121.1(3)

Table S2. Atomic coordinates (x 104) and equivalent isotropic displacement parameters (Å2 x 103) for $[Cu(HL)(H_2O)_2].NO_3$. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

atom	x	y	z	U(eq)
C(1)	4235(4)	3246(3)	7176(2)	41(1)
C(2)	5163(4)	1979(3)	7545(2)	40(1)
C(3)	5890(4)	1246(3)	6758(2)	44(1)
C(4)	5758(5)	1765(3)	5668(2)	52(1)
C(5)	4864(5)	3023(3)	5321(2)	58(1)
C(6)	4094(5)	3745(3)	6059(2)	52(1)
C(7)	7474(7)	-810(4)	6495(3)	75(1)

C(8)	3430(4)	4091(2)	7886(2)	41(1)
C(9)	3035(4)	4203(2)	10589(2)	41(1)
C(10)	2265(4)	5018(2)	11359(2)	42(1)
C(11)	1514(4)	6307(3)	11034(2)	47(1)
C(12)	846(4)	7029(3)	11791(3)	51(1)
C(13)	887(4)	6468(3)	12893(3)	50(1)
C(14)	1616(5)	5187(3)	13227(3)	57(1)
C(15)	2317(5)	4474(3)	12468(3)	52(1)
C(16)	-562(5)	8399(4)	13410(4)	70(1)
N(1)	3513(3)	3763(2)	8916(2)	39(1)
N(2)	2727(3)	4628(2)	9548(2)	42(1)
O(1)	5429(3)	1408(2)	8561(2)	49(1)
O(2)	3976(3)	3098(2)	10900(2)	50(1)
O(1W)	2279(3)	1005(2)	10595(2)	53(1)
O(3)	277(4)	7081(2)	13700(2)	65(1)
O(2W)	6780(4)	879(2)	10517(2)	62(1)
O(4)	6724(4)	20(2)	7196(2)	58(1)
Cu	4741(1)	2158(1)	9758(1)	45(1)
N(3)	7943(4)	2278(2)	12271(2)	54(1)
O(31)	9054(4)	2830(3)	11473(2)	80(1)
O(32)	7864(7)	1179(3)	12378(3)	107(1)
O(33)	6878(8)	2833(4)	12913(3)	127(2)

Table S3. Anisotropic displacement parameters ($Å^2$ x 10^3) for $[Cu(HL)(H_2O)_2].NO_3$. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$.

atom	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	43(1)	41(1)	38(1)	-11(1)	-4(1)	-11(1)
C(2)	46(1)	40(1)	37(1)	-11(1)	-5(1)	-13(1)
C(3)	52(1)	42(1)	41(1)	-14(1)	-6(1)	-11(1)
C(4)	60(2)	59(2)	38(1)	-21(1)	-4(1)	-10(1)
C(5)	70(2)	64(2)	34(1)	-9(1)	-9(1)	-7(2)

C(6)	60(2)	49(2)	41(1)	-6(1)	-10(1)	-4(1)
C(7)	113(3)	52(2)	63(2)	-32(2)	-9(2)	-6(2)
C(8)	46(1)	34(1)	41(1)	-8(1)	-8(1)	-5(1)
C(9)	49(1)	34(1)	44(1)	-14(1)	-8(1)	-10(1)
C(10)	47(1)	35(1)	48(1)	-19(1)	-7(1)	-7(1)
C(11)	52(1)	38(1)	51(2)	-14(1)	-9(1)	-7(1)
C(12)	52(1)	35(1)	67(2)	-22(1)	-8(1)	-2(1)
C(13)	46(1)	50(2)	62(2)	-31(1)	-7(1)	-9(1)
C(14)	72(2)	53(2)	49(2)	-19(1)	-16(1)	-4(1)
C(15)	67(2)	39(1)	52(2)	-16(1)	-15(1)	-4(1)
C(16)	61(2)	62(2)	99(3)	-53(2)	-9(2)	-2(2)
N(1)	46(1)	31(1)	42(1)	-13(1)	-6(1)	-6(1)
N(2)	51(1)	31(1)	44(1)	-15(1)	-8(1)	0(1)
O(1)	79(1)	33(1)	34(1)	-12(1)	-12(1)	-4(1)
O(2)	74(1)	33(1)	44(1)	-16(1)	-16(1)	1(1)
O(1W)	62(1)	42(1)	48(1)	-13(1)	-4(1)	-6(1)
O(3)	71(1)	63(1)	71(2)	-43(1)	-7(1)	-6(1)
O(2W)	89(2)	39(1)	63(1)	-20(1)	-35(1)	8(1)
O(4)	89(2)	39(1)	47(1)	-19(1)	-11(1)	-6(1)
Cu	68(1)	29(1)	38(1)	-13(1)	-13(1)	-3(1)
N(3)	68(2)	45(1)	43(1)	-13(1)	-15(1)	6(1)
O(31)	79(2)	69(2)	62(2)	-3(1)	-2(1)	10(1)
O(32)	167(4)	51(2)	106(3)	-15(2)	-53(3)	-9(2)
O(33)	174(4)	92(2)	90(3)	-48(2)	48(3)	-22(3)

Table S4. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for $[Cu(HL)(H_2O)_2].NO_3$.

atom	x	y	z	U(eq)
H(4)	6267	1273	5163	62
H(5)	4790	3374	4582	70
H(6)	3467	4580	5821	63
H(7A)	6428	-829	6151	113
H(7B)	7954	-1639	6918	113

H(7C)	8546	-524	5941	113
H(8)	2822	4913	7586	49
H(11)	1462	6686	10296	56
H(12)	370	7890	11561	61
H(14)	1630	4807	13968	69
H(15)	2832	3618	12700	63
H(16A)	375	8822	12851	105
H(16B)	-858	8728	14047	105
H(16C)	-1766	8531	13137	105
H(2)	2092	5377	9294	51
H(1A)	1250(40)	1510(30)	10820(30)	69(12)
H(1B)	2700(50)	500(30)	11168(17)	57(10)
H(2A)	7140(70)	890(40)	11090(20)	89(15)
H(2B)	7140(80)	131(19)	10450(40)	102(18)

Table S5. Hydrogen bond distances and angles in $[Cu(HL)(H_2O)_2].NO_3$.

D-H	d(D-H)	d(H..A)	∠(D-H..A)	d(D..A)	A	Symmetry operation
N2-H2	0.86	2.03	171.8	2.886(3)	O31	[-x+1, -y+1, -z+2]
O1W-H1A	0.85(1)	2.06(1)	171(4)	2.903(3)	O31	[x-1, y, z]
O1W-H1B	0.85(1)	2.21(2)	132(3)	2.847(3)	O1	[-x+1, -y, -z+2]
O1W-H1B	0.85(1)	2.14(2)	156(3)	2.936(3)	O4	[-x+1, -y, -z+2]
O2W-H2A	0.85(1)	1.99(1)	172(4)	2.839(5)	O32	

Table S6. Complete IR and Raman spectra of H_2L and $[Cu(HL)(H_2O)_2].NO_3$.

H_2L			**$[Cu(HL)(H_2O)_2]^+$**			
IR	**Raman**	**Assignment**	**IR**	**Raman**	**Calculated**	**Assignment**
3571 m		ν H_2O	3396 m,b			ν H_2O_{coord}
3213 m		ν NH	3178 w,b		3539	ν NH
3006 vw		ν OH	____			ν OH
3102 w		ν CHip MeBH	3114 vw		3232	ν CHip MeBH
3080 w	3083	ν CHip oVa	3087		3217	ν CHip oVa

	vw		vw			
2929 vw		ν_{as} CH_3 MeBH	3075 vw		3160/3090	ν_{as} CH_3 MeBH
2951 vw		ν_{as} CH_3 oVa	2985 vw		3142/5056	ν_{as} CH_3 oVa
2833 vw		ν C8H	2948 vw		3030	ν ν C8H
			2888		3025	ν_{s} CH_3 MeBH
2847 vw		ν_{s} CH_3 oVa	2855		3001	ν_{s} CH_3 oVa
			1767 vw			$\nu_1 + \nu_4$ NO_3^-
1650 s	1660 w	ν C=O				
1608 s (b)	1610 vs	ν C=N	1607 s	1609 vs	1688	ν C=N + ν C=O + ν ring oVa
			1590 w	1590 sh	1666	ν ring MeBH + Ar-C
1577 m	1581 s	ν ring + Ar-OCH_3 (MeBH + oVa)	1575 w	1564 m	1661	ν ring oVa
					1589	(ν ring + ν Ar-OCH_3) oVa
			1538 m	1543 sh	1648	ν C=O + ν ring MeBH
1514 m	1518 vw	ν ring + Ar-C (MeBH)	1512 m	1516 w	1606	ν ring MeBH + ν coord ring
1476 m	1485 m-w	ν C-NH + ν ring + ν Ar-OCH_3 (oVa)		1494 w	1550	C-NH +Ar-OCH_3 MeBH+ δ NH
1460 sh	1473 sh	ν ring + ν Ar-C (oVa) + ν Ar-OH + δ as CH_3 oVA	1468 w		1519 1517	δ as CH_3 oVa δ as CH_3 MeBH
1440 vw	1449 vw	ν ring + δ sim CH_3 oVa + ν C-NH + δ NH	1434 m	1435 w	1506	(ν ring + δ sim CH_3) oVa +δ NH
1419 vw		δ as CH_3 (MeBH)	1424 sh		1505	δ as CH_3 MeBH
1398 vw	1389 vw	δ as CH_3 oVa			1501	δ as CH_3 oVa
1371 m	1369 vvw	δ OH				
			1384 vs	1390 m	1473	ν ring (oVa) + ν Ar-O (oVa) coord ring + ν_3 NO_3^-
1337 sh	1344		1335 sh	1332 w	1369	(δ CH + ν ring) oVa + δ C8H
1327 m	1327 m	ν ring (oVa) + ν Ar-OH	1319 sh		1366	δ NH + δ C8H + ν ring (MeBH)
1315 m	1320 sh	δ CH + ν as N-C(O)-C+ ν OH	1304 w	1308 vw	1298	δ C8H + ν Ar-C MeBH

1296 m	1300 m	(ν C-OCH_3 + ν ring) MeBH	1272 mw		1310	(ν C-OCH_3 + ν ring) MeBH
	1268 sh					
1252 s	1259 w 1235 w	(ν C-OCH_3 + δ ring) oVA	1247 m		1284	(ν C-OCH_3 + δ ring) oVA
1182 m	1186 w	ν ring + ν Ar-OCH_3 (oVa)				
1124 sh	1130 vw	δ C8H + ν ring (oVa)	1217 s		1243	δ C8H + ν ring (oVa)
1150 w		ρ_r CH_3 + δ CH (oVa)			1221	ρ_r CH_3 oVa
					1212	ρ_r CH_3 MeBH
1168 w	1161 w	ν N-N + δ CH + ν_{as} NNC	1186 w	1192 sh	1158	ν N-N +- δ CH (rings)
			1177 w	1179 w	1140	ν N-N +- δ CH MeBH
1122 w	1220 vw	δ CH (MeBH)				
1106 sh 1098 w	1111 sh 1103 w	ν C-O(OCH_3) + δ ring breath (MeBH)	1125 w	1111 vw	1070	(ν C-O(OCH_3) + δ ring) MeBH
1080 w	1087 w	ν C-O(OCH_3) + δ ring (oVa)	1083 w		1118	(ν C-O(OCH_3) + δ ring)) oVa
1065 m	1066 vvw					
			1036 sh	1043 w	1001	(ν C-O(OCH_3) + δ ring) oVa
1035 m	1038 vw	δ ring (MeBH)	1023 m			δ ring MeBH
967 m 958 w		γ CH + γ_{ring} (MeBH)	977 w 972 sh	981 vvw	989	γ CH MeBH
893 m	899 w	γ CH (oVa)	906 w 885 vvw		956	γ C8H + ring (oVa)
893 m	899 w	γ CH (oVa)	856 sh		875	γ C8H + ring (oVa)
850 s		γ CH (MeBH)	846 m		863	γ CH MeBH
836 m	839 w	γ OH				
794 w 760 w	768 vw	γ CH + γ ring + γ CO (MeBH)				
736 s		γ CH + δ ring (oVa)	743 m		870	δ ring oVA
					814	δ ring MeBH
680 w	680 vw	δ N-N-C + γ C8H	648 w	644 vvw	916	δ coord. ring
610 m		γ NH	625 m	625	482	γ NH

				vvw		
			546 vw		570/541	ν Cu-O
472 vw	476 vw	γ NH	482 vw			γ NH
			436 vw, b		463	γ NH + ν N-Cu
418 vw		δ Ar-O-CH_3 + δ C(O)-NH-N	416 vvw			δ Ar-OCH_3 + δ C(O)-NH-N

Calculated frequencies are not scaled. vs: very strong; s: strong; m: medium; w: weak; vw: very weak; vvw: very,very weak; sh: shoulder. ν: stretching; δ: in-plane deformation; γ: out-of-plane deformation; ρr: rocking. coord.ring = N-N-C=O-Cu

Table S7. Electronic absorption spectra of $3 \times 10-5$ M methanolic solution of the ligand and Cu complex. Results from TD-DFT are also shown. Percentage contributions of calculated transitions are given in parentheses. Absorption maxima are given in nm. Oscillator strengths, shown in parenthesis, are in a.u.

H_2L	**$[Cu(HL)(H_2O)_2]^+$**			
Experimental	**Experimental**	**Calculated**	**Assignment**	
		251 (0.1246)	HOMO-1→LUMO+5 (24%) HOMO-1→LUMO+6 (16%) HOMO→LUMO+6 (13%)	Intra-ligand transition
220	230 sh ($1.95x10^5$)	256 (0.0804)	HOMO-6→LUMO+1 (12%) HOMO-4→LUMO+1 (47%)	Intra-ligand transition
260 sh	290 sh ($1.11x10^5$)	279 (0.1612)	HOMO-7→LUMO (45%) HOMO-6→LUMO (26%) HOMO-4→LUMO (11%)	Intra-ligand transition Charge transfer transition (L→M)
310 330 sh	322 ($1.20x10^5$)	306 (0.2182)	HOMO-7→LUMO (34%) HOMO-6→LUMO (19%) HOMO-4→LUMO (34%)	Intra-ligand transition Charge transfer transition (L→M)
	402 ($1.22x10^5$)	334 (0.1389)	HOMO-2→LUMO (16%) HOMO-1→LUMO+1 (56%)	Charge transfer transition (L→M)

		340 (0.4314)	HOMO→LUMO+1 (20%) HOMO-2→LUMO (77%)	Charge transfer transition (L→M)
	710* (125.9)	532 (0.0039)	HOMO→LUMO (27%)	d → d

(L→M): Ligand to Metal

*found using a more concentrated solution of the complex ($1x10^{-3}$M).

Figure S1. Drawings of Molecular orbitals most involved in electronic transitions.

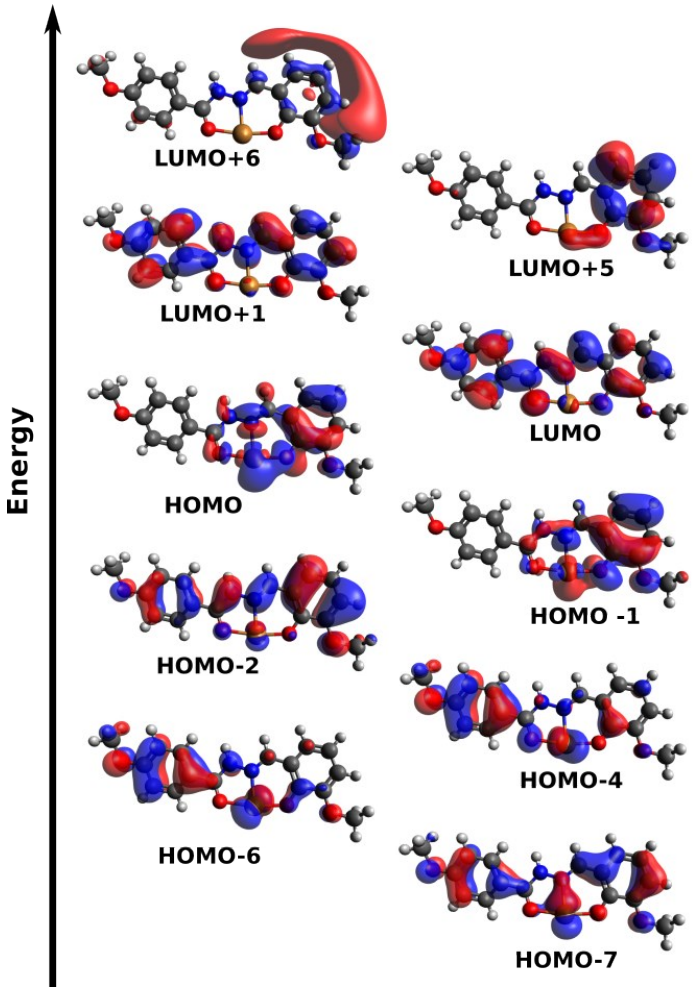


Figure S2. Root-mean-square deviation (RMSD) of dodecamer (blue line) and **CuHL** complex (red line) versus the dynamics simulation time.

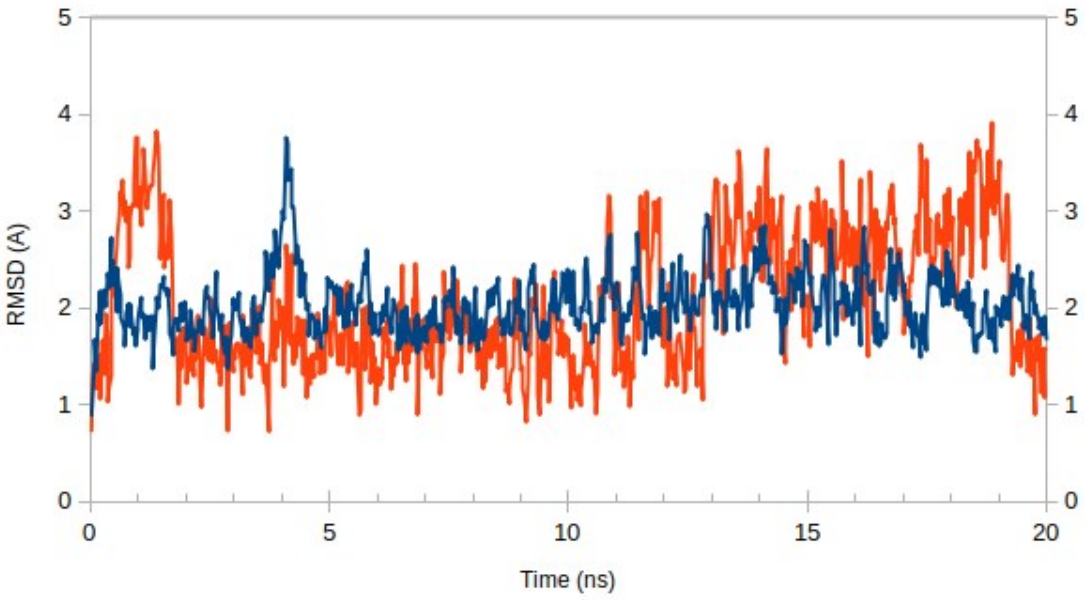


Figure S3. Energy decomposition of **CuHL** with nucleotides, based on molecular dynamics trajectory and using MM/GBSA model: total energy (green), interaction with Cu(II) (red) and interaction with the ligand (yellow). The original 1BNA PDB numbering was respected.

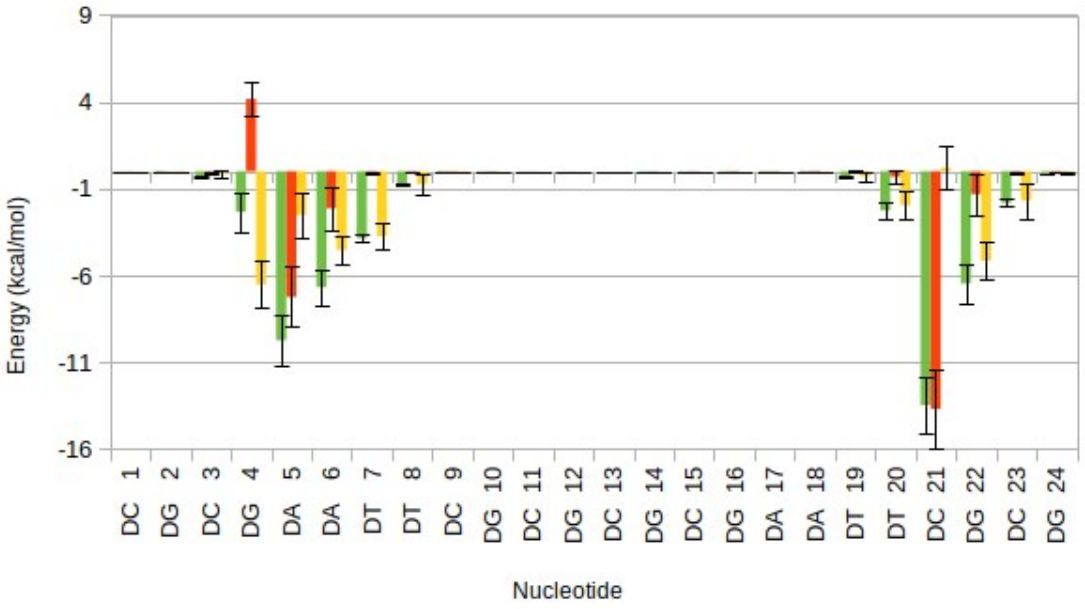


Figure S4 Apoptosis assay. The plots are representative of three independent experiments.

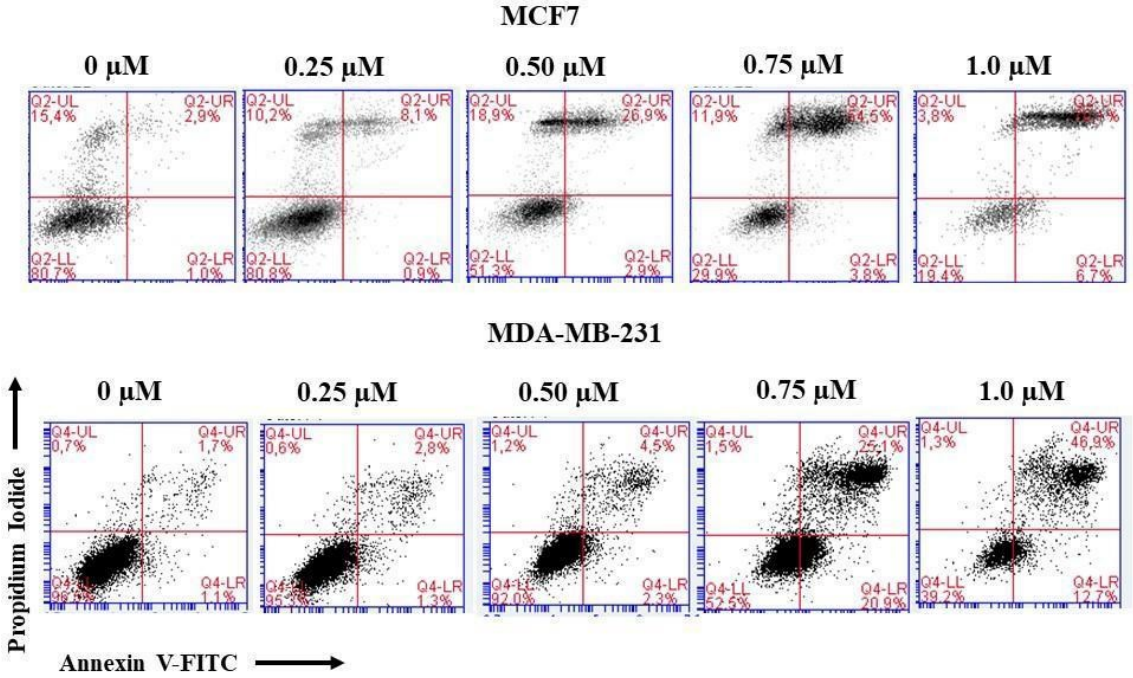
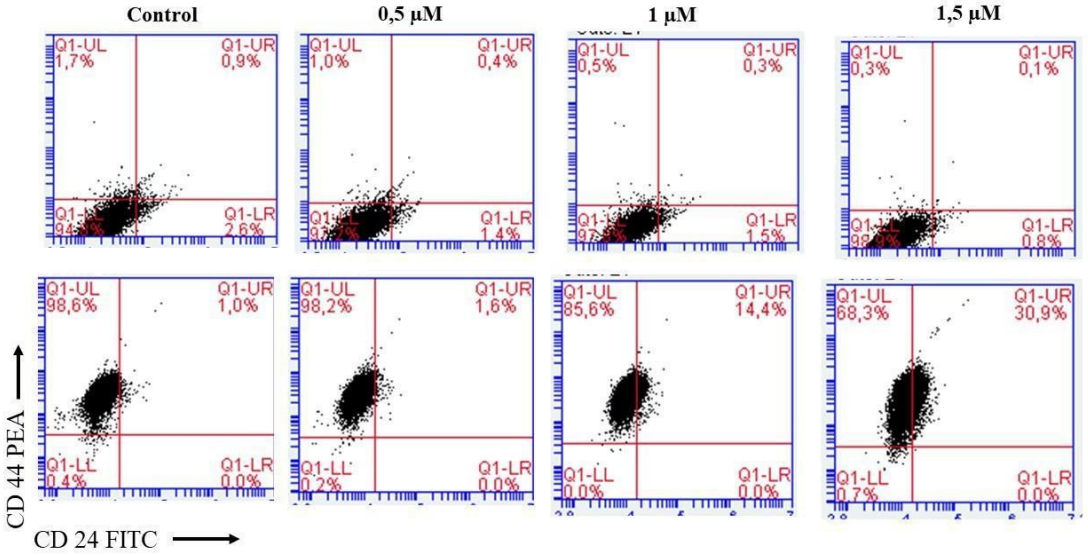


Figure S5 Cancer Stem Cell studies. The plots are representative of three independent experiments.


Control
0,5 µM
1 µM
1,5 µM
Q1-UL 1,7%
Q1-UR 0,9%
Q1-LR 2,6%
Q1-UL 1,0%
Q1-UR 0,4%
Q1-LR 1,4%
Q1-UL 0,5%
Q1-UR 0,3%
Q1-LR 1,5%
Q1-UL 0,3%
Q1-UR 0,1%
Q1-LR 0,8%
Q1-UL 98,6%
Q1-UR 1,0%
Q1-LL 0,4%
Q1-LR 0,0%
Q1-UL 98,2%
Q1-UR 1,6%
Q1-LL 0,2%
Q1-LR 0,0%
Q1-UL 85,6%
Q1-UR 14,4%
Q1-LL 0,0%
Q1-LR 0,0%
Q1-UL 68,3%
Q1-UR 30,9%
Q1-LL 0,7%
Q1-LR 0,0%
CD 44 PEA
CD 24 FITC