Supplementary Information

DNA interaction of non-chelating tinidazole-based coordination compounds. Structural, redox and cytotoxic properties.

Rodrigo Castro-Ramírez,[a] Naytzé Ortiz-Pastrana,[f] Ana B. Caballero,[b,g] Matthew T. Zimmerman,[c] Bradley S. Stadelman,[c] Andrea A. E. Gaertner,[c] Julia L. Brumaghim,[c] Luís Korrodi-Gregório,[d] Ricardo Pérez-Tomás,[d] Patrick Gamez,[b,e,g] and Norah Barba-Behrens,*[a]

Table S1. Crystallographic data and structure refinement of compounds **3**, **7**, **9** and **13**

Compounds	**(3)**	**(7)**	**(9)**	**(13)**
Chemical formula	$C_{16}H_{26}N_8O_{14}S_2Co$	$C_{16}H_{21}N_6O_8S_2CI_2Cu$	$C_{16}H_{26}N_8O_{14}S_2Cu$	$C_{16}H_{26}N_8O_{14}S_2Zn$
F. W. (g mol^{-1})	677.03	1253.96	681.03	683.94
Space group	$P2_1/n$	$P2_1/c$	$P2_1/c$	$P2_1/n$
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
a (Å)	20.903	7.181	7.2258(5)	20.7776(11)
b (Å)	6.522	26.896	10.8229(8)	6.5108(4)
c (Å)	21.396	13.182	16.4296(12)	21.3007(11)
α (°)	90.00	90.00	90.00	90.00
β (°)	113.45	97.64	90.376(5)	114.0800(10)
γ (°)	90.00	90.00	90.00	90.00
V (Å^3)	2675.9	2523.4	1284.83(16)	2630.8(3)
Z	4	4	4	4
D_{cal} (mg m^{-3})	1.682	1.642	1.763	1.727
Indices (min)	[-28,-8,-26]	[-9,-24,-12]	[-5,-14,-18]	[-26,-8,-27]
Indices (max)	[28,9,29]	[8,36,18]	[9,14,21]	[25,8,25]
Parameters	378	306	189	374
F(000)	1396	1272	702	1408
μ (mm^{-1})	0.880	1.296	1.098	1.176
θ range (°) data collection			2.25-27.53	2.95-27.23
Measured reflections	19422	12238	10970	26676
Independent reflections	6553	5955	2953	6004
Reflections observed [I >2σ(I)]	4879	4007	2184	4451
R_{int}	0.0357	0.0397	0.0786	0.0927
R[F^2 > 2σ(F^2)]	0.0493	0.0628	0.0469	0.0411
wR_2 (F^2)	0.1195	0.1266	0.0955	0.0736
Goodness of fit (S)	0.706	1.029	1.035	0.971
$\Delta\rho_{max}$ (e Å^{-3})	0.454	2.057	0.442	0.513
$\Delta\rho_{min}$ (e Å^{-3})	-0.406	-1.556	-0.542	-1.219

Table S2. Gel electrophoresis results for $[Cu(tnz)(\mu_2\text{-}AcO)_2]_2$ **10** DNA damage assays with 50μM H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	95.63 ± 1.74	4.37 ± 1.74	–	–
2: p + H_2O_2	0	95.67 ± 1.73	4.33 ± 1.73	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	0.29 ± 0.34	99.71 ± 0.34	0	–
4: p + Compound + H_2O_2	0.1	93.32 ± 0.55	6.68 ± 0.55	1.34 ± 1.18	0.188
5	1	50.56 ± 1.06	49.44 ± 1.06	44.10 ± 0.68	< 0.001
6	4	0.84 ± 0.61	99.16 ± 0.61	93.82 ± 1.17	< 0.001
7	6	0.82 ± 0.86	99.18 ± 0.86	93.84 ± 1.03	< 0.001
8	9	0.15 ± 0.24	99.85 ± 0.24	94.52 ± 1.78	< 0.001

[a]Data are reported as the average of three trials with calculated standard deviations shown.

Table S3. Gel electrophoresis results for $[Cu(tnz)_2Br_2]$ **8** DNA damage assays with 50 μM H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	95.62 ± 0.91	4.38 ± 0.91	–	–
2: p + H_2O_2	0	93.82 ± 2.37	6.18 ± 2.37	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	15.13 ± 3.01	84.87 ± 3.01	0	–
4: p + Compound + H_2O_2	0.1	93.37 ± 1.18	6.63 ± 1.18	-0.55 ± 1.45	0.579
5	1	79.81 ± 2.54	20.19 ± 2.54	13.01 ± 1.49	0.004
6	4	32.87 ± 9.44	67.13 ± 9.44	59.95 ± 10.18	0.009
7	6	1.84 ± 2.25	98.16 ± 2.25	90.98 ± 3.15	< 0.001
8	9	0.54 ± 0.71	99.46 ± 0.71	92.28 ± 2.54	< 0.001

[a]Data are reported as the average of three assays with calculated standard deviations shown.

Table S4. Gel electrophoresis results for $[Cu(tnz)_2(NO_3)_2]$ **9** DNA damage assays with 50 μM H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	95.11 ± 1.87	4.89 ± 1.87	–	–
2: p + H_2O_2	0	96.48 ± 2.29	3.52 ± 2.29	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	2.97 ± 3.59	97.03 ± 3.59	0	–
4: p + Compound + H_2O_2	0.1	94.13 ± 0.96	5.87 ± 0.96	1.76 ± 1.33	0.148
5	1	80.93 ± 6.22	19.07 ± 6.22	12.16 ± 4.31	0.039
6	4	15.03 ± 3.20	84.97 ± 3.20	81.14 ± 5.28	0.001
7	6	7.30 ± 2.51	92.70 ± 2.51	88.38 ± 4.32	< 0.001
8	9	7.06 ± 1.83	92.94 ± 1.83	90.07 ± 3.96	< 0.001

[a]Data are reported as the average of three assays with calculated standard deviations shown.

Table S5. Gel electrophoresis results for $CuSO_4$ DNA damage assays without 50 μM H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	99.94 ± 0.09	0.06 ± 0.09	–	–
2: p + H_2O_2	0	99.85 ± 0.27	0.15 ± 0.27	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	12.44 ± 3.47	87.56 ± 3.47	0	–
4: p + Cu^{II} + Ascorbic Acid	1	100 ± 0	0 ± 0	-1.15 ± 0.27	< 0.001
5	6	97.03 ± 1.18	2.97 ± 1.18	1.82 ± 1.09	0.102
6	9	96.57 ± 0.57	3.43 ± 0.57	2.28 ± 0.70	0.030
7	15	93.10 ± 1.45	6.90 ± 1.45	5.74 ± 1.63	0.026
8	25	46.72 ± 3.87	53.28 ± 3.87	52.13 ± 4.14	0.002
9	35	28.03 ± 0.62	71.97 ± 0.62	70.82 ± 0.86	< 0.001
10	50	15.39 ± 7.00	84.61 ± 7.00	83.45 ± 6.82	0.002
11	75	2.50 ± 2.71	97.50 ± 2.71	96.34 ± 2.50	< 0.001
12	100	0.00 ± 0	100.00 ± 0	98.85 ± 0.27	< 0.001
13	500	0.14 ± 0.24	99.86 ± 0.24	98.71 ± 0.51	< 0.001
14	1000	0.49 ± 0.69	99.51 ± 0.69	98.36 ± 0.64	< 0.001

[a]Data are reported as the average of three assays with calculated standard deviations shown.

Table S6. Gel electrophoresis results for $[Cu(tnz)(\mu_2\text{-}AcO)_2]_2$ **10** DNA damage assays without H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	100 ± 0	0 ± 0	–	–
2: p + H_2O_2	0	100 ± 0	0 ± 0	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	7.60 ± 4.11	92.40 ± 4.11	0	–
4: p + Compound + Ascorbic Acid	1	100 ± 0	0 ± 0	-1.00 ± 0	< 0.001
5	6	97.71 ± 2.42	2.29 ± 2.42	1.29 ± 2.42	0.453
6	9	87.12 ± 4.59	12.88 ± 4.59	11.88 ± 4.59	0.046
7	15	70.34 ± 5.64	29.66 ± 5.64	28.66 ± 5.64	0.013
8	25	46.58 ± 3.06	53.42 ± 3.06	52.42 ± 3.06	0.001
9	35	45.52 ± 7.51	54.48 ± 7.51	53.48 ± 7.51	0.007
10	50	3.32 ± 5.75	96.68 ± 5.75	95.68 ± 5.75	0.001
11	75	0.80 ± 1.01	99.20 ± 1.01	98.20 ± 1.01	< 0.001
12	100	2.18 ± 2.78	97.82 ± 2.78	96.82 ± 2.78	< 0.001
13	500	0.70 ± 1.21	99.30 ± 1.21	98.30 ± 1.21	< 0.001
14	1000	0.80 ± 1.10	99.20 ± 1.10	98.20 ± 0.56	< 0.001

[a]Data are reported as the average of three trials with calculated standard deviations shown.

Table S7. Gel electrophoresis results for $[Cu(tnz)_2Br_2]$ **8** DNA damage assays without H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	100.00 ± 0	0.00 ± 0	–	–
2: p + H_2O_2	0	100.00 ± 0	0.00 ± 0	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	14.50 ± 4.54	85.50 ± 4.54	0	–
4: p + Compound + Ascorbic Acid	0.1	100.00 ± 0	0.00 ± 0	-1.00 ± 0	< 0.001
5	1	99.99 ± 0.01	0.01 ± 0.01	-0.99 ± 0.1	0.003
6	6	96.87 ± 5.43	3.13 ± 5.43	2.13 ± 5.43	0.567
7	9	99.52 ± 0.57	0.48 ± 0.57	-0.52 ± 0.57	0.255
8	15	99.77 ± 0.29	0.23 ± 0.29	-0.77 ± 0.29	0.44
9	25	59.88 ± 2.70	40.12 ± 2.70	39.12 ± 2.70	0.002
10	50	2.73 ± 2.24	97.27 ± 2.24	96.27 ± 2.24	< 0.001
11	75	1.34 ± 1.01	98.66 ± 1.01	97.66 ± 1.10	< 0.001
12	100	3.07 ± 3.57	96.93 ± 3.57	95.93 ± 3.57	< 0.001
13	500	2.17 ± 2.54	97.83 ± 2.54	96.83 ± 2.54	< 0.001
14	1000	0.05 ± 0.09	99.95 ± 0.09	98.95 ± 0.09	< 0.001

[a]Data are reported as the average of three assays with calculated standard deviations shown.

Table S8. Gel electrophoresis results for $[Cu(tnz)_2(NO_3)_2]$ **9** DNA damage assays without H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	100 ± 0	0 ± 0	–	–
2: p + H_2O_2	0	100 ± 0	0 ± 0	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	8.83 ± 2.7	91.17 ± 2.7	0	–
4: p + Compound + Ascorbic Acid	0.1	100.00 ± 0	0.00 ± 0	-1.00 ± 0	< 0.001
5	1	100.00 ± 0	0.00 ± 0	-1.00 ± 0	< 0.001
6	6	100.00 ± 0	0.00 ± 0	-1.00 ± 0	< 0.001
7	9	100.00 ± 0	0.00 ± 0	-1.00 ± 0	< 0.001
8	15	100.00 ± 0	0.00 ± 0	-1.00 ± 0	< 0.001
9	25	24.38 ± 7.35	75.62 ± 7.35	74.62 ± 7.35	0.003
10	50	0.44 ± 0.14	99.56 ± 0.14	98.56 ± 0.14	< 0.001
11	75	2.65 ± 1.81	97.35 ± 1.81	96.35 ± 1.81	< 0.001
12	100	0.24 ± 0.25	99.76 ± 0.25	98.76 ± 0.25	< 0.001
13	500	0.69 ± 0.42	99.31 ± 0.42	98.31 ± 0.42	< 0.001
14	1000	8.74 ± 1.84	91.26 ± 1.84	90.26 ± 1.82	< 0.001

[a]Data are reported as the average of three assays with calculated standard deviations shown.

Table S9. Gel electrophoresis results for $CuSO_4$ DNA damage assays without H_2O_2.[a]

Gel lane	[Compound], μM	% Supercoiled	% Nicked	% Damage	*p* Value
1: plasmid DNA (p)	0	99.94 ± 0.09	0.06 ± 0.09	–	–
2: p + H_2O_2	0	99.85 ± 0.27	0.15 ± 0.27	–	–
3: p + Cu^{II} + Ascorbic Acid + H_2O_2	0	12.44 ± 3.47	87.56 ± 3.47	0	–
4: p + Cu^{II} + Ascorbic Acid	0.1	100.00 ± 0	0.00 ± 0	-1.0 ± 0	< 0.001
5	1	97.03 ± 1.18	2.97 ± 1.18	1.97 ± 1.18	0.007
6	6	96.57 ± 0.57	3.43 ± 0.57	2.43 ± 0.57	< 0.001
7	9	93.10 ± 1.45	6.90 ± 1.45	5.90 ± 1.45	< 0.001
8	15	46.72 ± 3.87	53.28 ± 3.87	52.28 ± 3.87	< 0.001
9	25	28.03 ± 0.62	71.97 ± 0.62	70.97 ± 0.62	0.003
10	35	15.39 ± 6.99	84.61 ± 6.99	83.61 ± 6.99	
11	50	2.50 ± 2.71	97.50 ± 2.71	96.50 ± 2.71	< 0.001
12	75	0.00 ± 0	100.00 ± 0	99.00 ± 0	< 0.001
13	100	0.14 ± 0.24	99.86 ± 0.24	98.86 ± 0.24	< 0.001
14	500	0.49 ± 0.68	99.51 ± 0.68	98.51 ± 0.68	< 0.001
15	1000	99.96 ± 0.06	0.04 ± 0.06	-0.96 ± 0.06	< 0.001

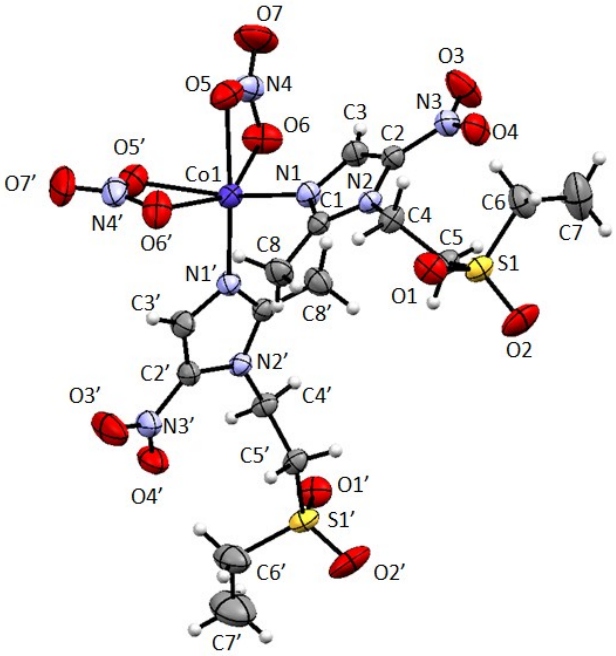

Fig. S1. ORTEP representation for $[Co(tnz)_2(NO_3)_2]$ **3**, ellipsoids at 50% probability.

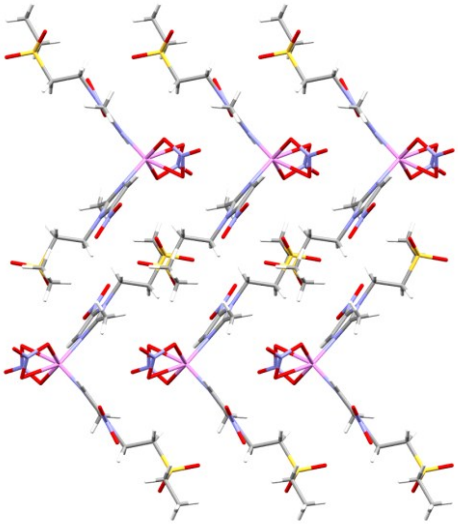

Fig. S2. Supramolecular arrangement for $[Zn(tnz)_2(NO_3)_2]$ **13.**

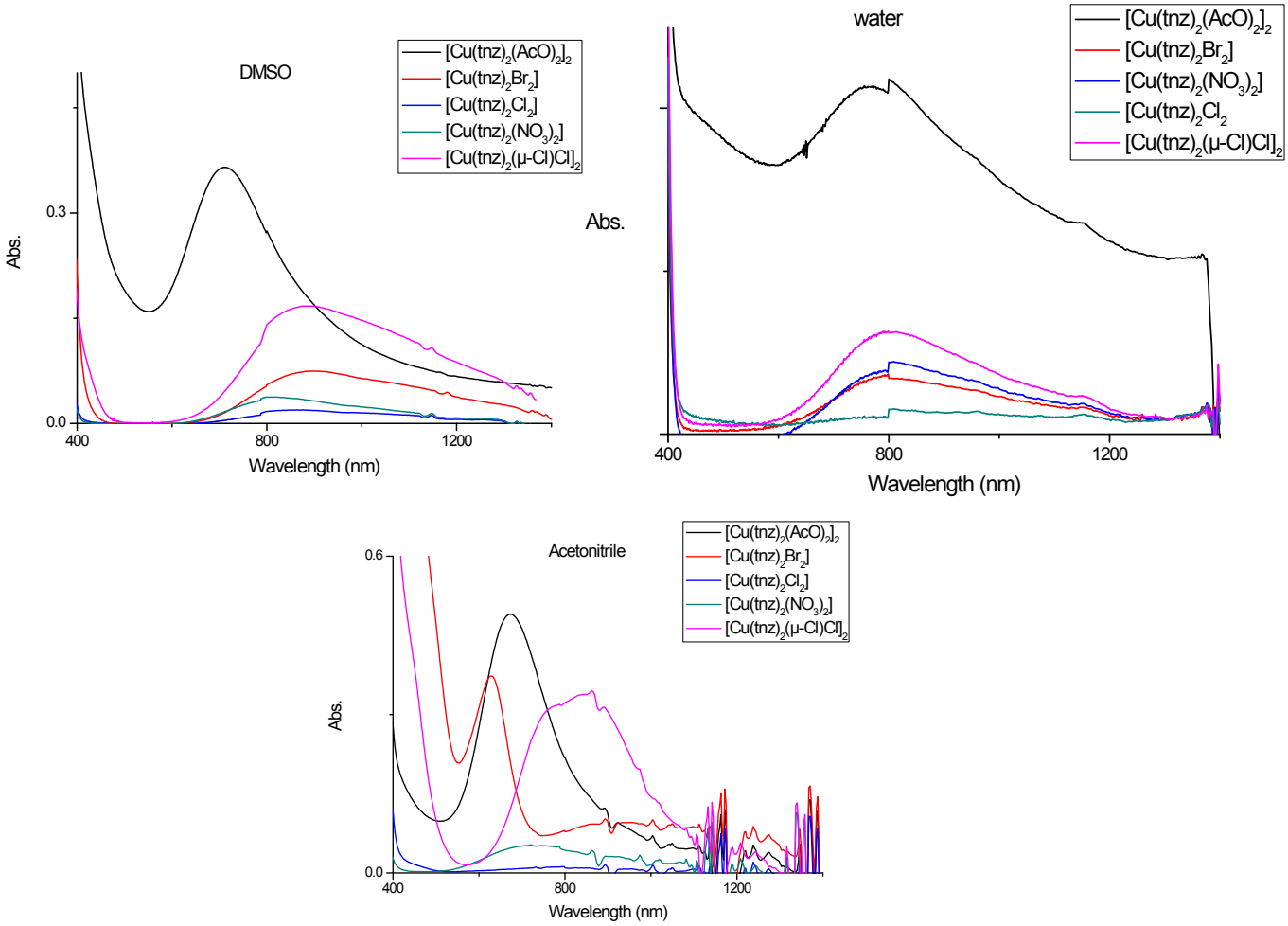

Fig. S3. Electronic spectra for Cu^{II} tnz complexes in DMSO, H_2O and acetonitrile (10^{-3} M).

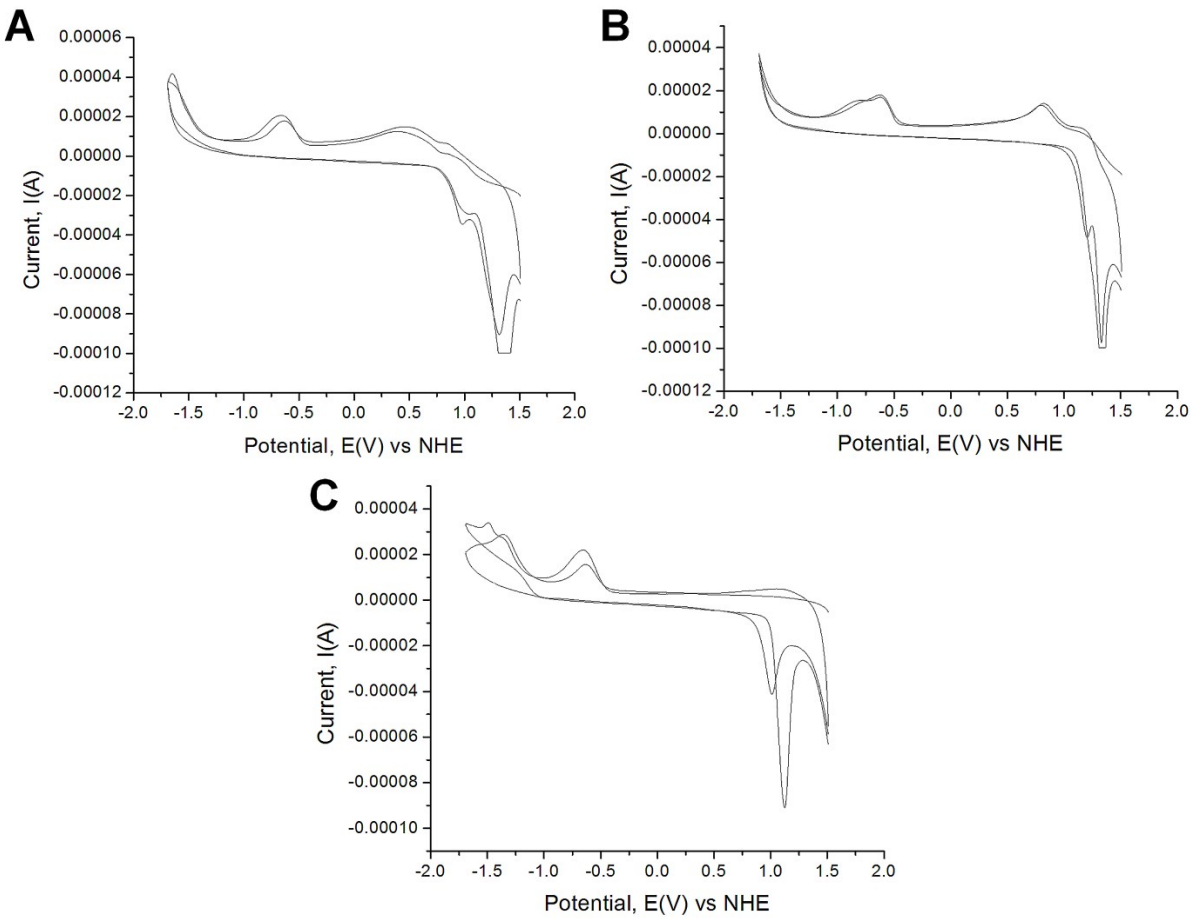

Fig. S4. Cyclic voltammograms vs. NHE for A) $[Co(tnz)_2Br_2]$ **2**, B) $[Co(tnz)_2Cl_2]$ **1**, and C) $[Co(tnz)_2(NO_3)_2]$ **3**. Samples are 1 mM in acetonitrile with 100 mM $TBAPF_6$ as the supporting electrolyte.

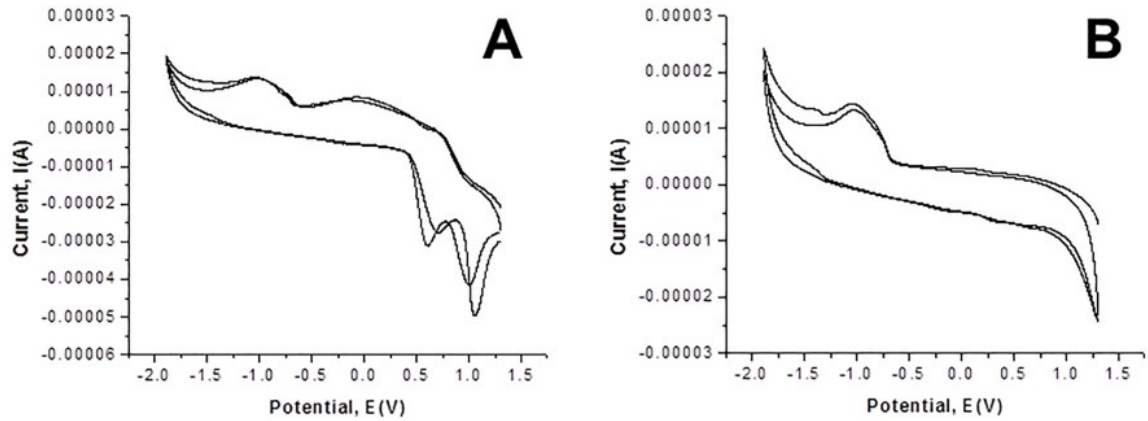

Fig. S5. Cyclic voltammograms vs. NHE for A) $[Ni(tnz)_2Br_2]$ **4** and B) $[Ni(tnz)_2(NO_3)_2]$ **5**. Samples are 1 mM in acetonitrile with 100 mM $TBAPF_6$ as the supporting electrolyte.

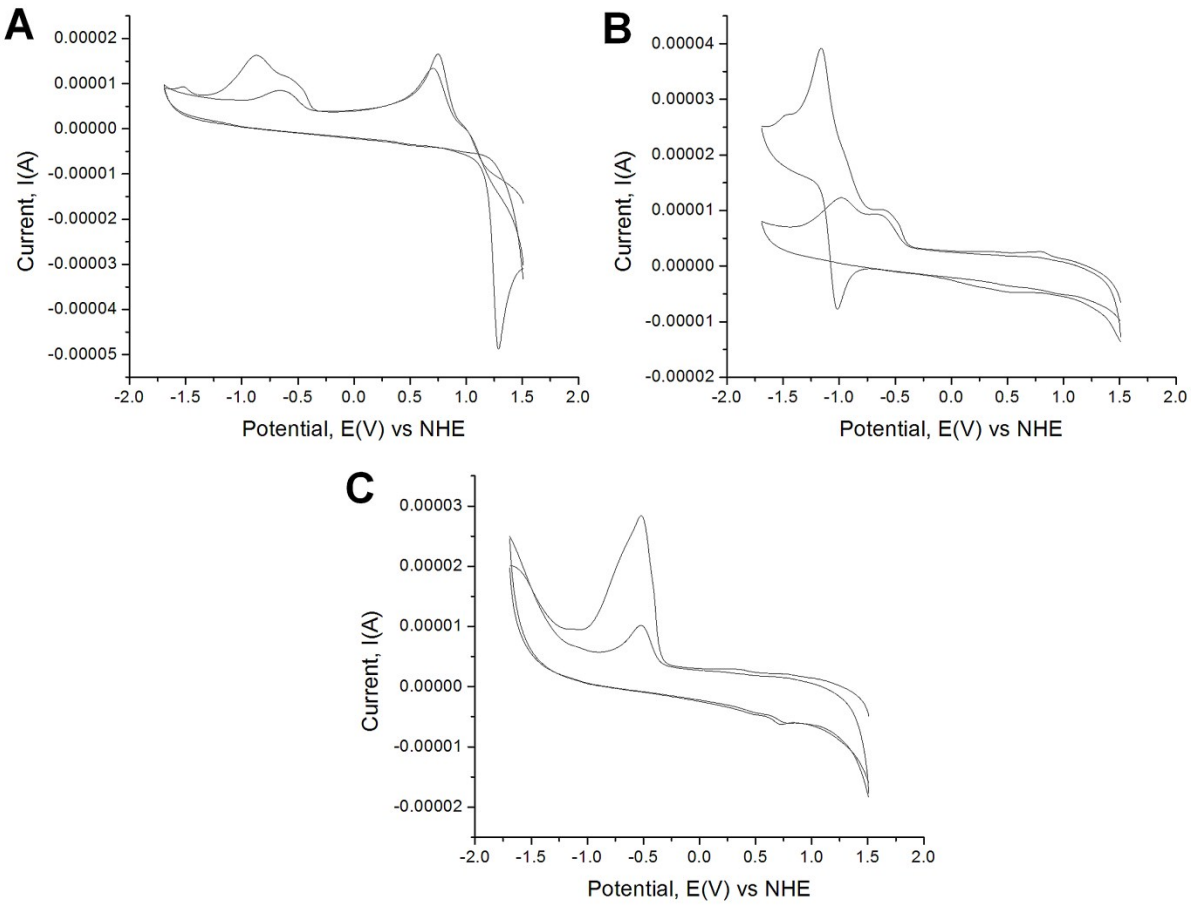

Fig. S6. Cyclic voltammograms vs. NHE for A) $[Zn(tnz)_2Br_2]$ **12**, B) $[Zn(tnz)_2Cl_2]$ **11**, and C) $[Zn(tnz)_2(NO_3)_2]$ **13.** Samples are 1 mM in acetonitrile with 100 mM $TBAPF_6$ as the supporting electrolyte.

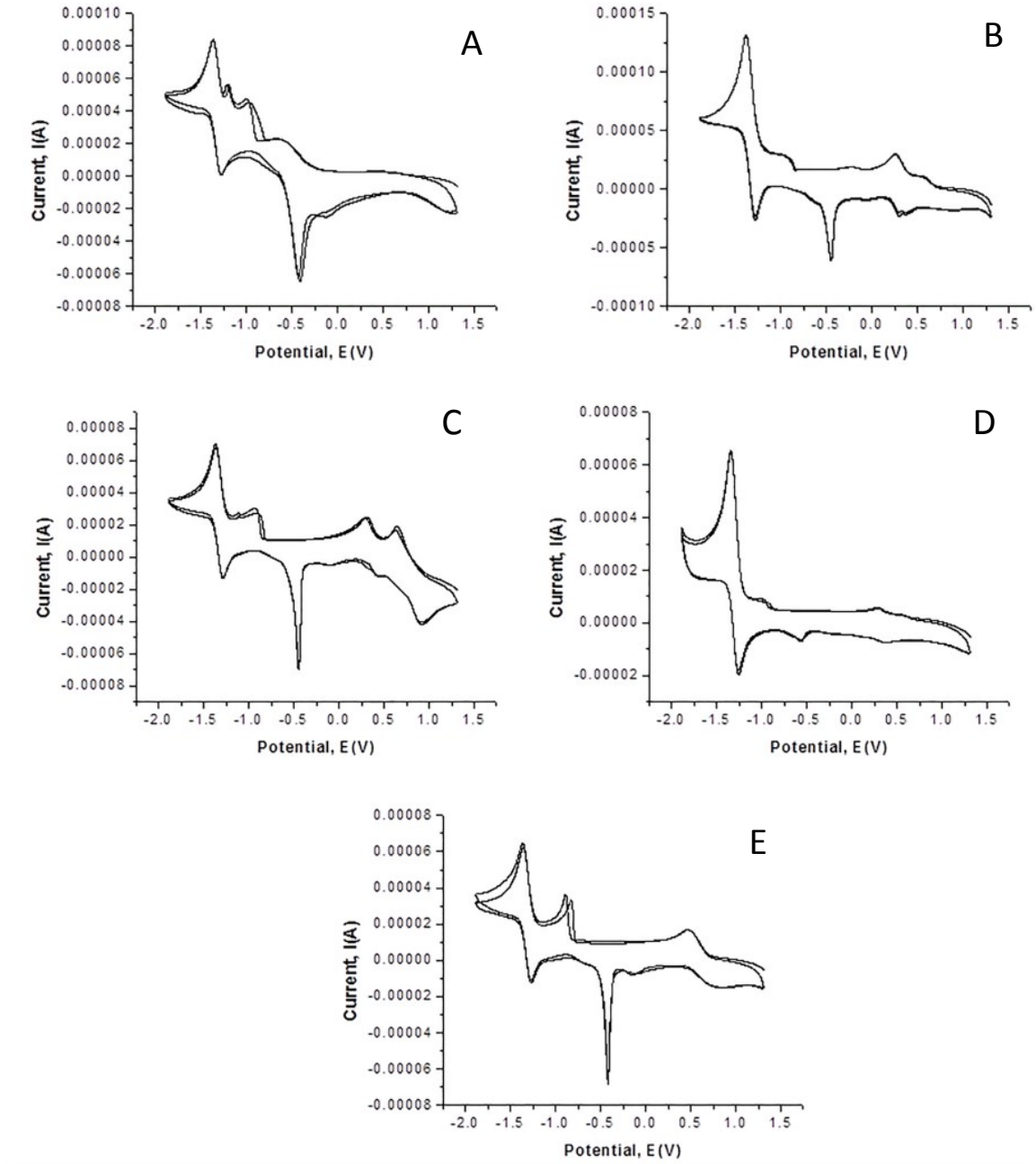

Fig. S7. Cyclic voltammograms vs. NHE for A) $[Cu(tnz)(\mu\text{-}OAc)_2]_2$, B) $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$, C) $[Cu(tnz)_2Br_2]$, D) $[Cu(tnz)_2Cl_2]$, and E) $[Cu(tnz)_2(NO_3)_2]$. 1 mM in acetonitrile with 100 mM $TBAPF_6$ as supporting electrolyte.

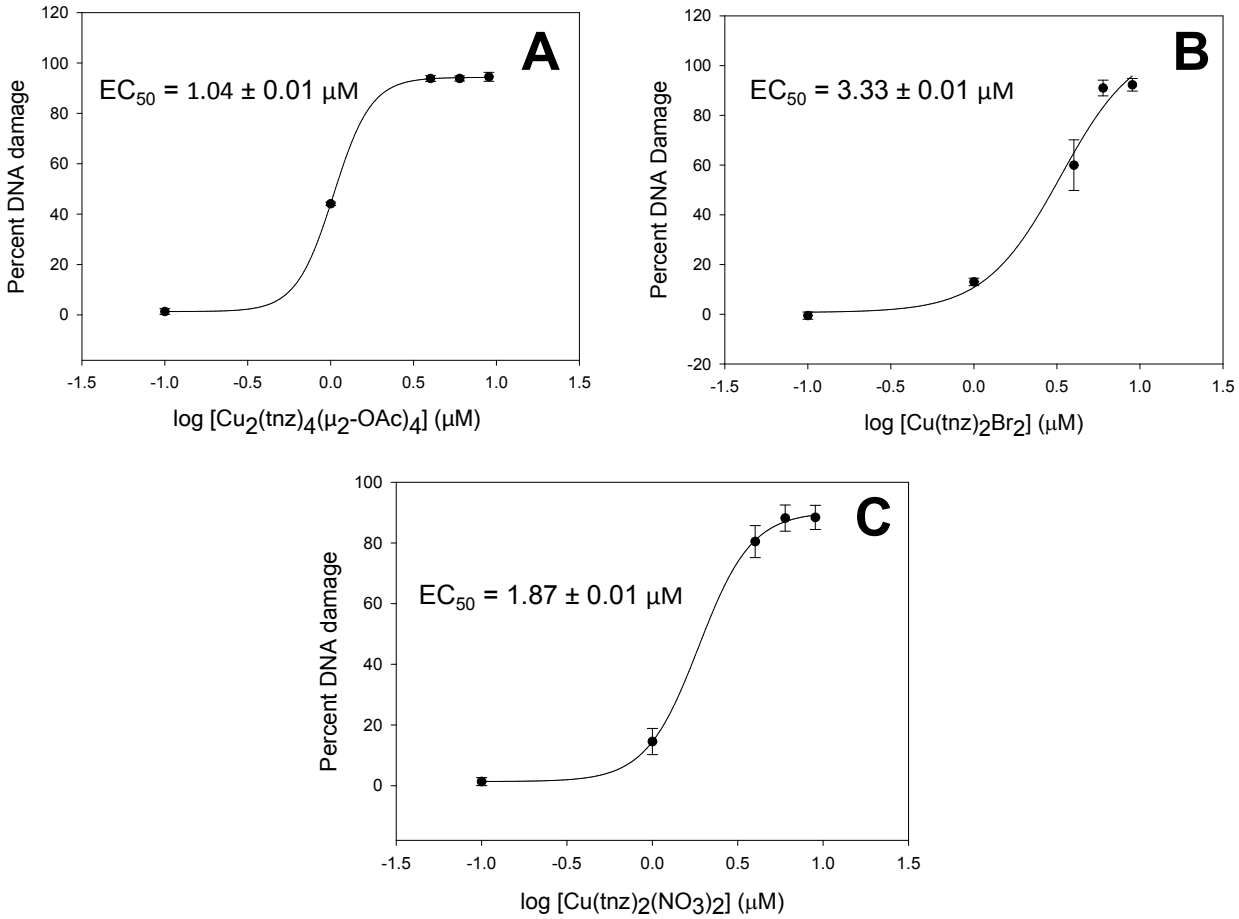

Fig. S8. Dose-response curves for DNA damage by addition of 50 μM H_2O_2: A) [Cu(tnz)(μ^2-AcO_2)$_2$] **10**, B)[Cu(tnz)$_2$Br$_2$] **8**, and C) [Cu(tnz)$_2$(NO_3)$_2$] **9**.

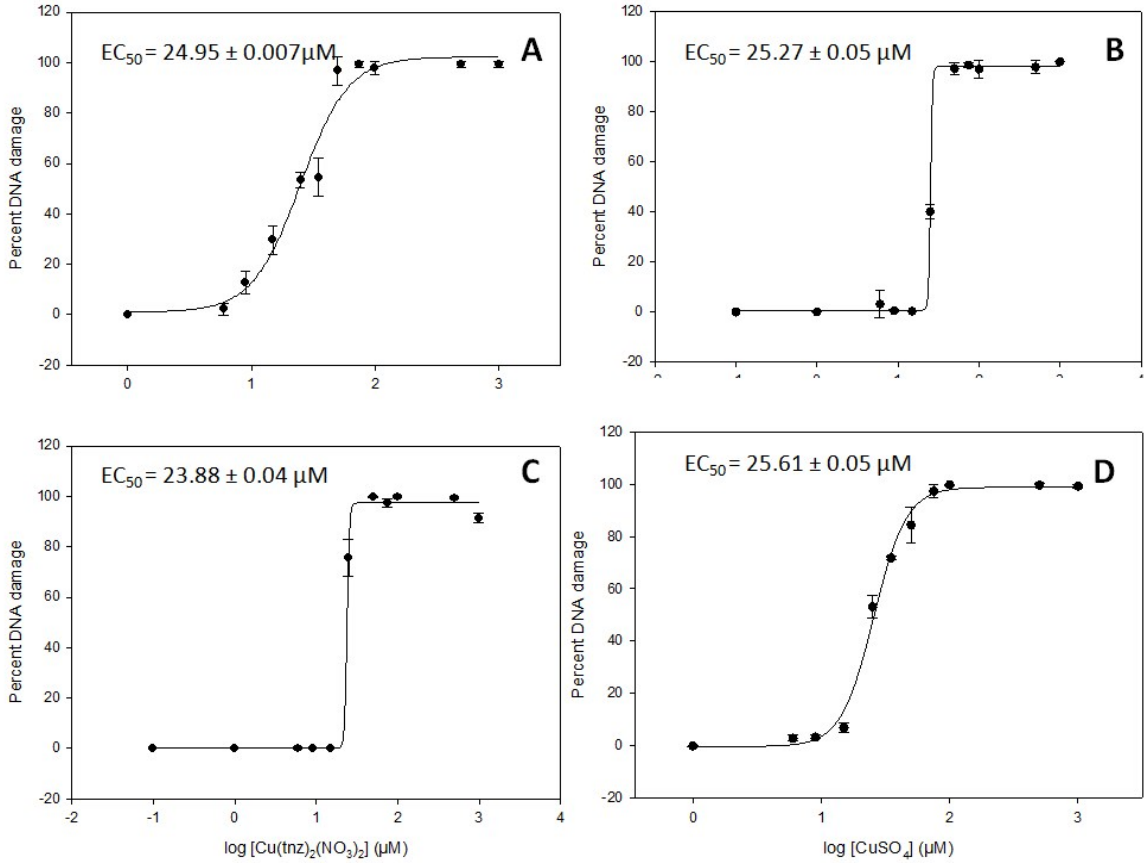

Fig. S9. Dose-response curves for DNA damage with ascorbic acid and without the addition of H_2O_2: A) [Cu(tnz)(μ^2-AcO_2)$_2$] **10**, B) [Cu(tnz)$_2$Br$_2$] **8**, C) [Cu(tnz)$_2$(NO_3)$_2$] **9** and D) $CuSO_4$

Table S10. Cell-viability percentage for copper(II) tnz complexes against different cell lines[a].

	A549		SKOV-3		A375		MCF-7		SW620	
	10 mM	50 mM	10 mM	50 mM	10 mM	50 mM	10 mM	50 mM	10 mM	50 mM
tinidazole	94.3	94.6	95.6	94.9	70.5	86.4	94.6	96.5	99	101.6
[Cu(tnz)$_2$(µ-Cl)Cl]$_2$	60.6	10.9	71.5	4.9	53.9	7.8	32.4	5.5	106.3	1.8
[Cu(tnz)(µ-AcO)$_2$]$_2$	57.5	18.9	79.5	20.8	92.1	33	50.8	10	109.5	10.7
[Cu(tnz)$_2$Br$_2$]	68.3	20	78.6	14.7	63.6	35.2	48.5	17.3	99.6	16.4
[Cu(tnz)$_2$Cl$_2$]	86.9	65.5	87.2	65.3	102.4	64	63.7	34.4	102.6	106
[Cu(tnz)$_2$(NO_3)$_2$]	75.8	25.6	80.2	26.2	105	34.2	48	36.6	96.8	33.4

[a]Cell viability was determined twice for each compound.

Table S11. Inhibitory concentrations for copper(II) tnz complexes against different cell lines at 24h and selectivity index (MCF-7 vs MCF-10A)

		MCF-7		SW620		MCF-10A		Selectivity index (IC_{50} MCF-7/IC_{50} MCF-10A)
Compound Name	**IC**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	
Tinidazol	IC_{75}	>100	n.a.	>100	n.a.	>100	n.a.	n.d
	IC_{50}	>100	n.a.	>100	n.a.	>100	n.a.	
	IC_{25}	>100	n.a.	>100	n.a.	>100	n.a.	
$[Cu(tnz)_2Br_2]$	IC_{75}	50.39	10.87	47.87	1.51	71.90	1.13	0.88
	IC_{50}	28.87	4.54	37.43	2.79	32.86	14.72	
	IC_{25}	3.06	9.28	27.04	9.40	5.76	12.06	
$[Cu(tnz)_2(u\text{-}Cl)Cl]_2$	IC_{75}	35.87	9.89	26.65	8.32	60.35	10.40	0.75
	IC_{50}	9.20	6.13	17.60	2.77	12.22	1.54	
	IC_{25}	2.18	22.19	9.99	16.47	3.73	42.48	
$[Cu(tnz)_2Cl_2]$	IC_{75}	71.77	10.92	>100	n.a.	50.62	4.60	2.66
	IC_{50}	31.79	8.93	>100	n.a.	11.96	4.68	
	IC_{25}	2.95	9.92	>100	n.a.	3.21	13.54	
$[Cu(tnz)_2(NO_3)_2]$	IC_{75}	57.26	2.32	66.52	3.02	>100	n.a.	1.52
	IC_{50}	30.51	6.61	22.64	8.38	20.06	10.11	
	IC_{25}	5.10	8.47	4.47	14.23	2.79	12.28	
$[Cu(tnz)(\mu\text{-}AcO)_2]_2$	IC_{75}	41.76	1.20	35.76	1.97	62.02	3.02	1.50
	IC_{50}	25.70	0.96	19.71	5.96	17.08	6.41	
	IC_{25}	5.48	2.34	5.11	0.84	3.28	14.56	

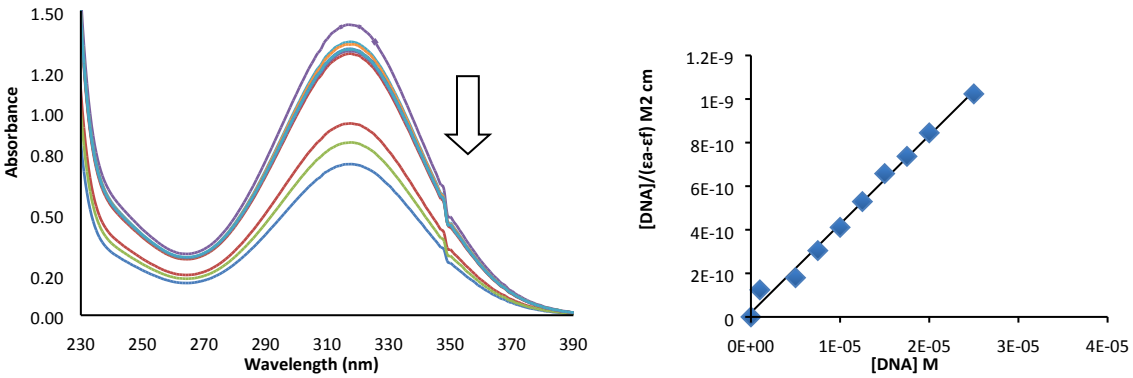

Fig. S10. Absorption spectra for $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$ **7** (25 μM in sodium cacodylate 1 mM – 20 mM of $NaCl_2$ buffer, pH = 7.25; ct-DNA: 0-25 μM). Linear [DNA]/εa-εf vs [DNA] plot for the determination of the intrinsic binding constant K_b. The arrow shows an increase in ct-[DNA].

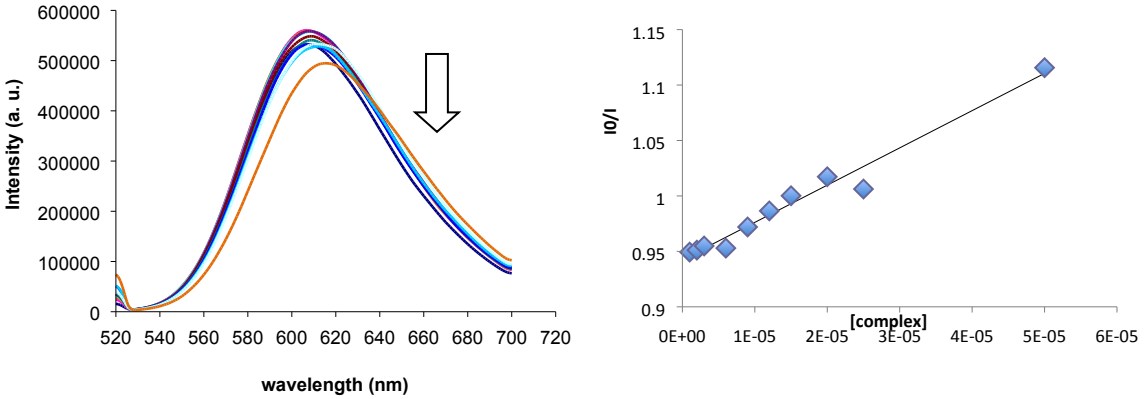

Fig. S11. Emission spectra for EB-DNA upon increasing concentration of $[Cu(tnz)_2(\mu\text{-}Cl)Cl]_2$ **7** (1-50 μM). λ_{ex} = 514 nm y λ_{em} = 607 nm. Lineal I_0/I vs [complex] plot for the determination of the Stern-Volmer K_{sv}. The arrow shows an increase of [complex].