Cytotoxic activity of copper(II), nickel(II) and platinum(II) thiosemicarbazone derivatives: interaction with DNA and the H2A histone peptide

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Synthetic procedures

Synthesis and characterization of S-citronellal-thiosemicarbazone (Htcitr). To a stirred solution of thiosemicarbazide (177 mg, 1.94 mmol) in 30 ml of ethanol 350 µL of S-citronellal (1.94 mmol) were added. The solution was heated to reflux for six hours. The mixture was then evaporated under reduced pressure and dried under vacuum. The product is a light brown oil obtained with a yield of 95% (419 mg, 1.84 mmol). 1H-NMR (400MHz, DMSO-D6) δ 0.87 (d, J = 8 Hz, 3H); δ 1.16 (m, 1H); δ 1.29 (m, 1H); δ 1.57 (s, 3H); δ 1.65 (s, 3H); δ 1.69 (m, 1H); δ 1.96 (m, 2H); δ 2.04 (m, 1H); δ 2.17 (m, 1H); δ 5.08 (s, 1H); δ 7.41 (t, J = 8 Hz, 1H); δ 7.44 (s, 1H); δ 7.98 (s, 1H); δ 11.02 (s, 1H). ATR-FTIR (cm^{-1}) 3410 (s) and 3265 (s, br) ν (NH_2); 3164 (s) ν (NH); 3024 (m) ν (CH_3); 2965 (s) and 2917 (s) ν (CH_2); 1596 (s) ν (CN) + ν (C C); 925 (w) and 824 (m) ν (CS). ESI-MS (m/z) 226.39 $(M-H)^+$.

Synthesis and characterization of bis(S-citronellal-thiosemicarbazonate)nickel(II) ([Ni(tcitr)$_2$]). [Ni(tcitr)$_2$] was prepared following a modified procedure reported elsewhere[1]. Briefly, a solution of $Ni(CH_3COO)_2{\cdot}4H_2O$ (26 mg, 0.154 mmol) (ethanol, 5 mL) was added dropwise to a solution of S-citronellalthiosemicarbazone (Htcitr) (70 mg, 0.308 mmol) in 10 mL of ethanol, at room temperature with stirring, in a 1:2 molar ratio Ni:ligand. The complex was isolated as dark green powder. 1H-NMR (400MHz, DMSO-D6) δ 0.83 (s, 3H); δ 1.13 (m, 2H); δ 1.23 (m, 2H); δ 1.45 (m, 1H); δ 1.57 (s, 3H); δ 1.64 (s, 3H); δ 1.92 (m, 2H); δ 2.31 (m, 1H); δ 5.06 (s, 1H); δ 6.65 (s, 1H); δ 6.73 (s, 1H); δ 7.19 (s, 1H). ATR-FTIR (cm^{-1}) 3464 (m), 3328 (s) and 3150 (s) ν (NH_2); 2960 (m) ν (CH_3); 2911 (m) ν (CH_2); 1614 (m) ν (C C); 1523 (vs) ν (CN); 871 (w) ν (CS). ESI-MS (m/z) 511.21 $(M-CH_3)^+$.

Synthesis and characterization of bis(S-citronellal-thiosemicarbazonate)platinum(II) ([Pt(tcitr)$_2$]). A solution of $PtCl_2$ (41 mg, 0.154 mmol) (acetonitrile, 25 mL, reflux) was added dropwise to a solution of S-citronellalthiosemicarbazone (Htcitr) (70 mg, 0.308 mmol) in 10 mL of ethanol, at room temperature with stirring, in a 1:2 molar ratio of Pt:ligand. The complex was isolated as a yellow powder. 1H-NMR (400MHz, DMSO-D6) δ 0.89 (m, 3H); δ 1.24 (m, 3H); δ 1.57 (s, 3H); δ 1.65 (s, 3H); δ 1.73 (m, 1H); δ 1.95 (bs, 2H); δ 2.42 (m, 1H); δ 5.08 (s, 1H); δ 7.11 (bs, 1H); δ 7.90 (s, 1H); δ 8.17 (s, 1H). ATR-FTIR (cm^{-1}) 3249 (m) and 3138 (m) ν (NH_2); 2955 (s) ν (CH_3); 2920 (s) ν (CH_2); 1618 (s) ν (C C); 1565 (s) ν (CN); 824 (w) ν (CS). ESI-MS (m/z) 648.47 $(M-CH_3)^+$. The complex was also recrystallized from DMSO, obtaining crystals apt for X-ray diffraction analysis.

Synthesis and characterization of bis(S-citronellal-thiosemicarbazonate)copper(II) ([Cu(tcitr)$_2$]). [Cu(tcitr)$_2$] was prepared following a modified procedure reported elsewhere [1]. Briefly, a solution of $CuCl_2{\cdot}2H_2O$ (26 mg, 0.154 mmol) (ethanol,5 mL) was added dropwise to a solution of S-citronellalthiosemicarbazone (Htictr) (70 mg, 0.308 mmol) in 10 mL of ethanol, at room temperature under magnetic stirring, in a 1:2 molar ratio of Cu:ligand. The complex was isolated as black powder. ATR-FTIR (cm^{-1})) 3243 (s) and 3103 (s) ν (NH_2); 2964 (s) ν (CH_3); 2917 (s) ν (CH_2); 1615 (s) ν (C C); 1511 (vs) ν (CN); 716 (w) ν (CS) ESI-MS (m/z) 513.35 $(M-NH_2)^+$.

Crystallography

The crystallographic data of compound [Pt(tcitr)$_2$] were collected with a SMART APEX2 diffractometer with Bruker AXS CCD detector using Mo-Kα radiation and a graphite crystal monochromator [λ(Mo-Kα) 0.71073 Å]. The SAINT [2] software was used for integrating reflection intensities and scaling, and SADABS [3] for absorption correction. The structure was solved by direct methods using SHELXS [4] and refined by full-matrix least-squares on all F^2 using SHELXL [5] implemented in the OLEX2_1.1 package [6] . All the non-hydrogen atoms in the molecules were refined anisotropically. The hydrogen atoms were partly found and partly placed in the ideal positions using riding models. The structure was solved by direct methods and difference Fourier synthesis using the SHELX suite of programs as implemented within the OLEX software. Thermal ellipsoid plots were generated using OLEX. The crystal system is triclinic, space group P_1; cell parameters: a = 6.189(1), b = 12.221(2), c =12.196(2) Å, α = 86.035(2), β = 75.346(2), γ = 79.549(2)°, V = 877.4(3) $Å^3$. The asymmetric unit is formed by a molecule of complex and two molecules of DMSO of formula $C_{26}H_{52}N_6O_2Pt_1S_4$, Mr = 804.06, Z = 1, Dc = 1.522 g cm^{-3}, m = 4.267 mm^{-1}, F (000) = 408. A semi-empirical absorption correction, based on multiple scanned equivalent re-flections, has been carried out and gave 0.4008 < T < 0.7457. A total of 12082 reflections were collected up to a θ range of 28.5° (±8h,±16 k, ±16 l), 8671 unique reflections (R_{int} = 0.02). All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in ideal positions and refined using riding models.

CCDC1894193 contains the supplementary crystallographic data. (http://www.ccdc.cam.ac.uk/data_request/cif).

Table S1 ^{1}H-NMR assignment (ppm) of TESSHK in the absence or in the presence of [Ni(tcitr)$_2$] and [Pt(tcitr)$_2$].

Fragment	Free	In the presence of [Ni(tcitr)$_2$]	In the presence of [Pt(tcitr)$_2$]	Fragment	Free	In the presence of [Ni(tcitr)$_2$]	In the presence of [Pt(tcitr)$_2$]
Thr α	4.14	4.18	4.12	His^3 α	4.55	4.41	4.56
β	3.98	3.99	3.95	β	3.01	3.44	2.95
γ	1.07	1.07	1.06	NH_{back}	8.10	/	8.14
$NH_{back.}$	7.93	/	7.93	C_2	7.58	/	7.58
OH	4.99	?	?	C_5	7.29	/	7.34
Glu α	4.30	4.37	4.26	His^4 α	4.51	4.32	4.53
β	1.73	1.73	1.72	β	3.09	3.52	2.91
γ	2.26	2.26	2.22	$NH_{back.}$	8.24	/	8.27
$NH_{back.}$	7.97	/	7.99	C_2	7.33	/	7.38
COOH	14.07	/	14.12	C_5	7.23	/	?
Ser α	4.18	4.11	4.18	Lys α	4.23	4.26	4.21
β	3.55	3.53	?	β	1.68	1.64	1.68
$NH_{back.}$	7.86	/	7.87	γ	1.31	1.38	1.31
OH	5.16	?	?	δ	1.56	1.57	1.50
				ε	2.75	2.80	2.75
				$NH_{back.}$	8.16	/	8.17
				NH_2	7.68	7.68	7.72

Table S2. Metal complex concentrations that inhibit growth at 50% (GI_{50}, 50% Growth Inhibition) for cell line U937 after 24 and 72h of treatment.

GI_{50} (μM)		
	24h	**72h**
[Cu(tcitr)$_2$]	33.0	30.0
[Pt(tcitr)$_2$]	7.0	13.0
[Ni(tcitr)$_2$]	10.0	8.0

Table S3. Mutagenicity data in *S. typhimurium* TA98 and TA100 strains treated with [Cu(tcitr)$_2$], with and without S9 activation. Results are expressed as revertants/plate (mean ± standard deviation) and mutagenicity ratio (MR). Positive controls: 2NF [20.0 μg per plate]:170±15 for TA98-S9. 2AA [10.0 μg per plate]: 86±3 for TA98+S9. SA [15.0 μg per plate]: 606±46 for TA100-S9. 2AA [10.0 μg per plate]: 413±44 for TA100+S9.

[Cu(tcitr)$_2$]	**TA98 - S9**		**TA98 + S9**		**TA100 - S9**		**TA100 + S9**	
μg/plate	Mean ± sd	MR	Mean ± sd	MR	Mean ± sd	MR	Mean ± sd	MR
DMSO	17±6	1.0	10±1	1.0	106±6	1.0	138±23	1.0
2.5	38±11	2.2	17±4	1.6	127±16	1.2	127±0	0.9
5.0	31±11	1.8	21±0	2.0	109±2	1.0	117±16	0.8
10.0	32±17	1.9	22±4	2.1	107±20	1.0	119±15	0.9
50.0	30±15	1.7	22±8	2.1	107±7	1.0	116±11	0.8
100.0	31±11	1.8	14±1	1.3	99±2	0.9	100±6	0.7

Table S4. Mutagenicity data in *S. typhimurium* TA98 and TA100 strains treated with [Pt(tcitr)$_2$], with and without S9 activation. Results are expressed as revertants/plate (mean ± standard deviation) and mutagenicity ratio (MR). Positive controls: 2NF [20.0 μg per plate]:170±15 for TA98-S9. 2AA [10.0 μg per plate]: 86±3 for TA98+S9. SA [15.0 μg per plate]: 606±46 for TA100-S9. 2AA [10.0 μg per plate]: 413±44 for TA100+S9.

[Pt(tcitr)$_2$]	**TA98 - S9**		**TA98 + S9**		**TA100 - S9**		**TA100 + S9**	
μg/plate	Mean ± sd	MR	Mean ± sd	MR	Mean ± sd	MR	Mean ± sd	MR
DMSO	17±6	1.0	10±1	1.0	106±6	1.0	137±22	1.0
2.5	26±7	1.5	20±0	1.9	109±5	1.0	132±4	1.0
5.0	23±16	1.3	20±3	2.0	93±13	0.9	119±4	0.9
10.0	22±7	1.3	29±7	2.8	92±5	0.9	126±15	0.9
50.0	28±5	1.6	30±8	2.9	94±16	0.9	120±9	0.9
100.0	16±11	0.9	36±6	3.4	112±3	1.1	110±21	0.8

Fig. S1. The crystal packing in the plane (7 0 -14).

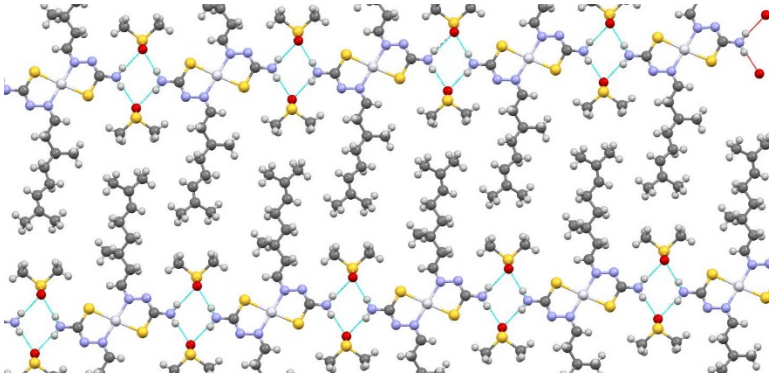
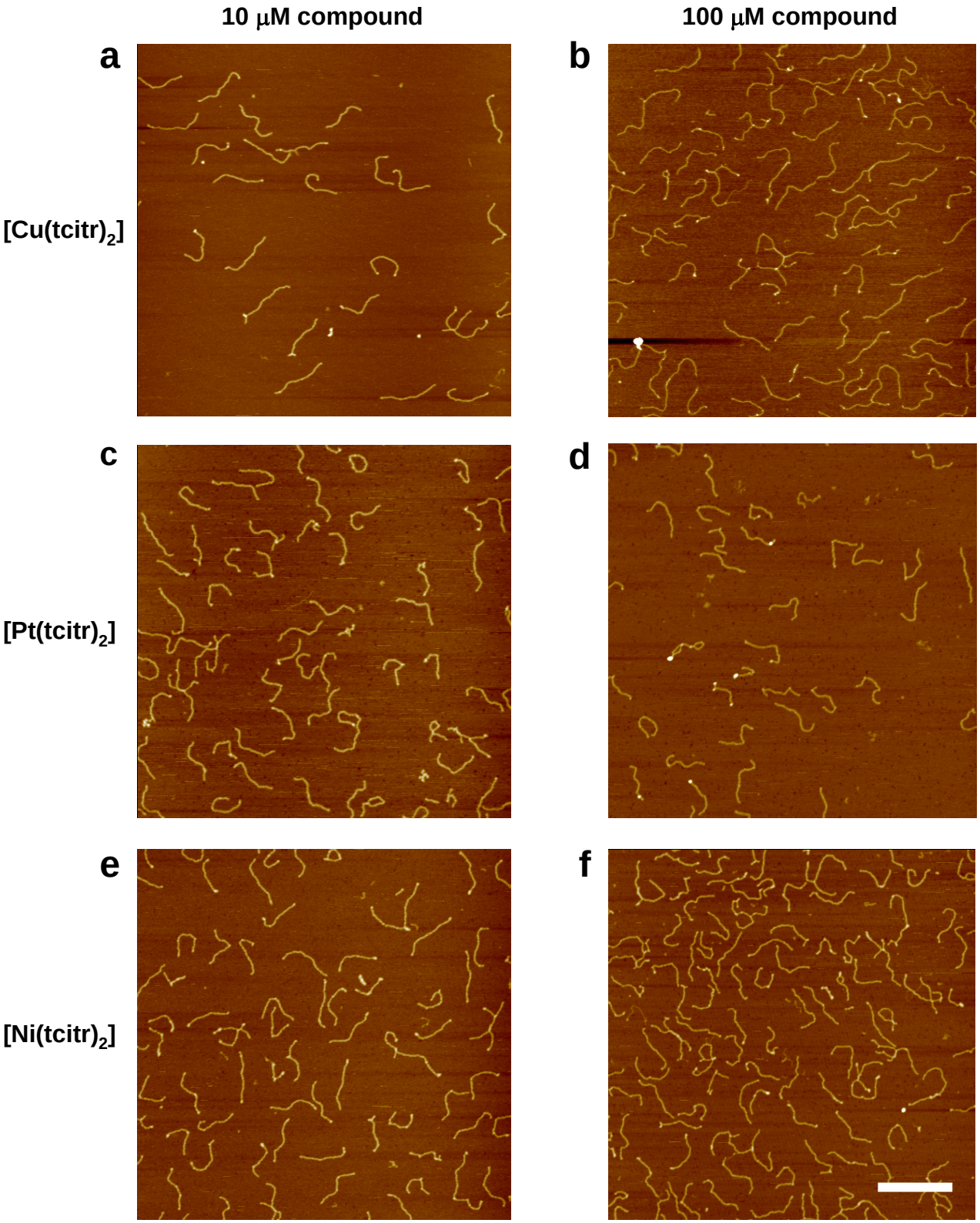


Fig. S2. AFM images of 965 bp DNA fragments in the presence of 10 mM (left column) and 100 mM (right column) [Cu(tcitr)$_2$] (**a, b**), [Pt(tcitr)$_2$] (**c, d**) and [Ni(tcitr)$_2$] (**e, f**). Scale bar 400 nm.



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