Electronic Supporting Information

An engineered POSS drug delivery system for copper(II) anticancer metallodrugs in selective application toward melanoma cells

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Index

Scheme of the syntheses of metal complexes:

1st step: ligand formation by condensation reaction of isatin with an amine;
2nd step: metalation of the ligand (depending of the adjusted pH, different tautomeric species can be obtained preferentially and isolated)

Characterization of the Copper(II) Complexes

Data from: *J. Inorg. Biochem*. 2008, **102**, 1090–1103. doi:10.1016/j.jinorgbio.2007.12.033.

[Cu(isaepy)H_2O]ClO_4 1

Using the previous reported procedure [G. Cerchiaro, G.A. Micke, M.F.M. Tavares, A.M.D.C. Ferreira, J. Mol. Catal. A: Chem. 221 (2004) 29–39.], a yield of 66% of complex **1** was obtained. Anal. Found: C, 42.46%; H, 3.64%; N, 8.77%. Calcd. for $C_{15}H_{14}N_3O_2$-Cu(ClO_4): C, 41.78%; H, 3.25%; N, 9.70%. **MS (ESI+)**: m/z = 314.1 [MW = 431.29; in CH_3OH/H_2O, fragment $C_{15}H_{12}N_3OCu$]; 316.1 [isotopic pattern ($Cu^{63/65}$) monocation]; 564.1 [keto-form, fragment $C_{30}H_{24}N_6O_2Cu$ (compound **1**, MW = 566.11)]; 566.2 [isotopic pattern ($Cu^{63/65}$) monocation]. Although the elemental analysis value (for C and N) is not totally satisfactory, the MS(ESI^+) data are consistent with the proposed formula of this compound ***3***.

[Cu(isaepy)$_2$](ClO_4)$_2$ 3

Using the same reported procedure, a yield of 72% for complex **3** was obtained. Anal., Found: C, 47.13%; H, 3.49%; N, 10.70%. Calcd. for $C_{30}H_{26}N_6O_2Cu(ClO_4)_2$: C, 47.09%; H, 3.39%; N, 10.98%. **MS (ESI^+)**: m/z = 563.1. [MW = 566.11; in CH_3CN/H_2O, fragment monocation $C_{30}H_{24}N_6O_2Cu$]; 565.1 [isotopic pattern ($Cu^{63/65}$) monocation]; 312.0 [enol-form, fragment$C_{15}H_{12}N_3OCu$ (compound **1**); 314.1 [isotopic pattern ($Cu^{63/65}$) monocation].

At pH 7.4, both tautomeric form (keto and enol) of the ligand *isaepy* co-exist in solution, giving two possible and isolated complexes **1** and **3**.

Data from: G. Cerchiaro, G.A. Micke, M.F.M. Tavares, A.M.D.C. Ferreira, J. Mol. Catal. A: Chem. 2004, **221**, 29–39.

[Cu(isapn)]$(ClO_4)_2$ 2

The isatin-diimine copper(II) complex (1), Cu(isapn)$(ClO4)_2$, was analogously synthesized by condensation of 1,3-diaminopropane (pn) with isatin (isa) dissolved in EtOH solution, followed by coordination to copper(II) ions, added as perchorate the salt. During the reaction, an apparent pH of 5.5 was maintained, so the tautomer keto-keto was preferentially formed. The isolated crystals were recrystallized from ethanol diethyl ether solution. Yield: 85%. (Found: C, 38.5; H, 2.9; N, 9.5; Cu, 10.5. Calcd . for $C_{19}H_{16}N_4O_2$ $Cu(ClO_4)_2$.: C, 38.4; H, 2.75; N, 9.4; Cu, 10.7%.) Λ_M 81.5 S cm^2 $mol^{-}1$ in H_2O, and 57.6 S cm^2 $mol^{-}1$ in MeOH (both at 25°C). FT-IR (cm)1, KBr): 3447 (m, NH or OH) and 3334 (m, NH); 3102–2774 (w, $C_{sp2}H$ and C=C); 1734 (s, CO amide); 1665 (s, O-H); 1616 (s, N-H); 1591 (s, C=N); 1459 (m, C-O); 1102 and 625 (s, ClO_4 no coordinated). MS (ESI^+): m/z found: 395.02 (calcd.: 395.07, for $C_{19}H_{16}N_4O_2Cu$).

Characterization of the Materials

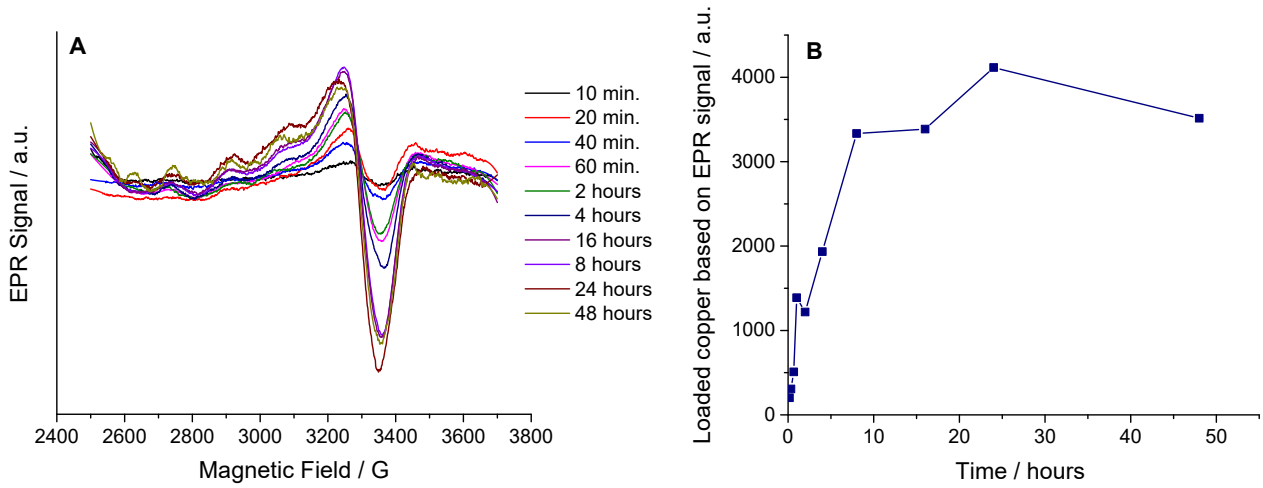

Figure S1. (A) $[Cu(isapn)]^+$ loading kinetics on POSS-atzac matrix assisted by X-band EPR spectroscopy at different period of time. Experimental conditions: Temperature = 298 K, Frequency = 9.4 GHz, , Power Attenuation = 20 dB, Modulation Amplitude = 10 G, Center Field = 3200 G, Power = 2 mW and Modulation Frequency = 100 kHz. **(B)** Loaded copper based on EPR signal as a function of time.

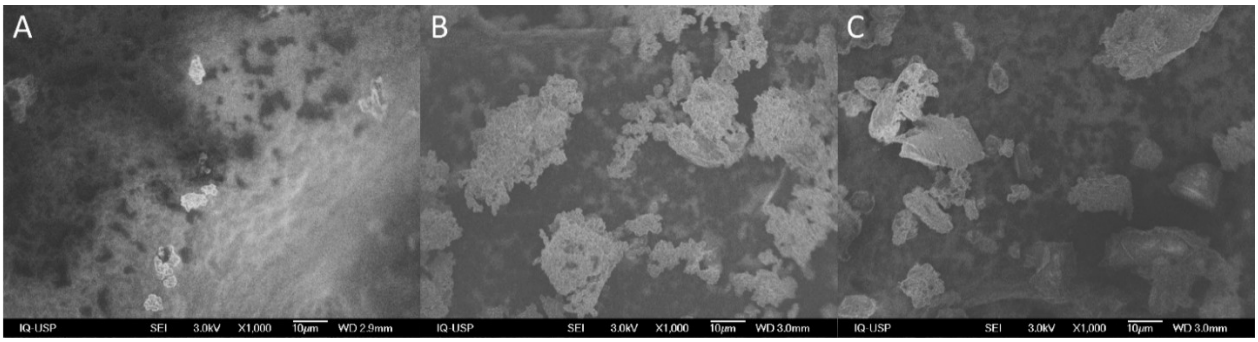

Figure S2. Scanning electron micrographs of materials **A**. POSS-atzac, **B**. [Cu(isapn)]@POSS-atzac and **C**. [Cu(isaepy)]@POSS-atzac, at 1000x magnification.

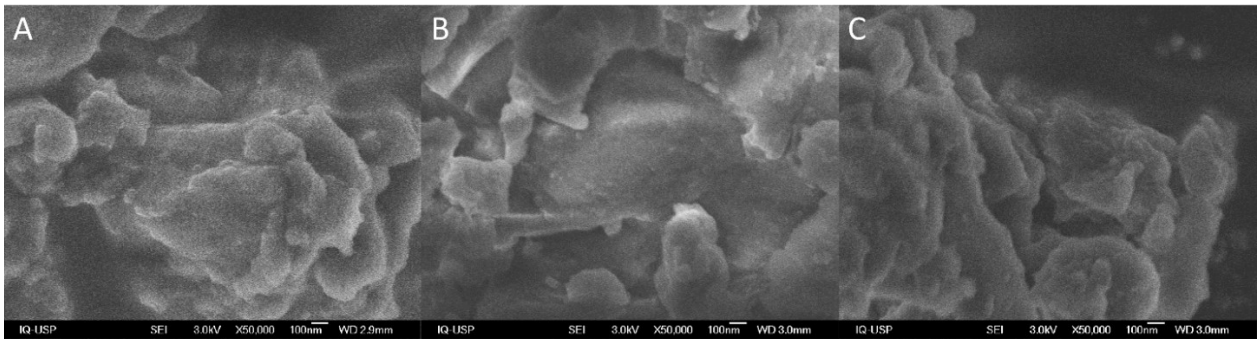

Figure S3. Scanning electron micrographs of materials **A**. POSS-atzac, **B**. [Cu(isapn)]@POSS-atzac and **C**. [Cu(isaepy)]@POSS-atzac at a 50,000x magnification.

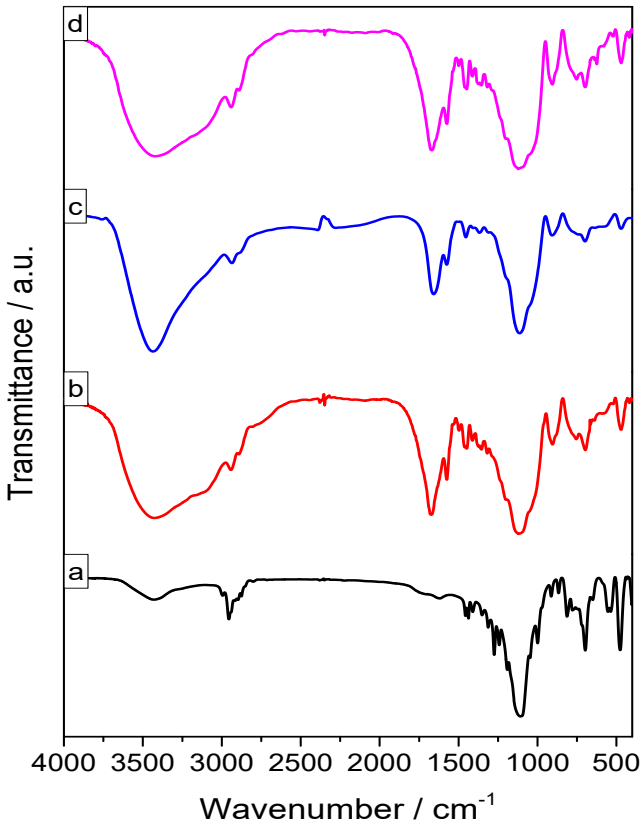

Figure S4. IR vibrational spectra acquired in KBr pellets for hybrid materials (a) POSS-Cl **3**, (b) POSS-atzac **4**, (c) [Cu(isapn)]@POSS-atzac **5**, and (d) [Cu(isaepy)]@POSS-atzac **6**.

Main IR bands:

POSS-Cl *[$Si_8O_{12}(C_3H_6Cl)_8$]*
FTIR (KBr pellet, cm^{-1}): ν(C-H) 2954, ν(C-H) 1430, ν_{as}(Si-O-Si) 1110 and ν(C-Cl) 699.

POSS-atzac

FTIR (KBr pellet, cm^{-1}): ν(C-H) 2942, ν(C=O) 1674, ν(C=N) 1574, ν(C-N) 1449, ν_{as}(Si-O-Si) 1114 and ν(C-Cl) 696.

[Cu(isapn)]@POSS-atzac

FTIR (KBr pellet, cm^{-1}): ν(C-H) 2935, ν(C=O) 1656, ν(C=N) 1568, ν(C-N) 1454, ν_{as}(Si-O-Si) 1111, and ν(C-Cl) 703.

[Cu(isaepy)]@POSS-atzac

FTIR (KBr pellet, cm^{-1}): ν(C-H) 2942, ν(C=O) 1670, ν(C=N) 1573, ν(C-N) 1460, ν_{as}(Si-O-Si) 1116 and ν(C-Cl) 699.

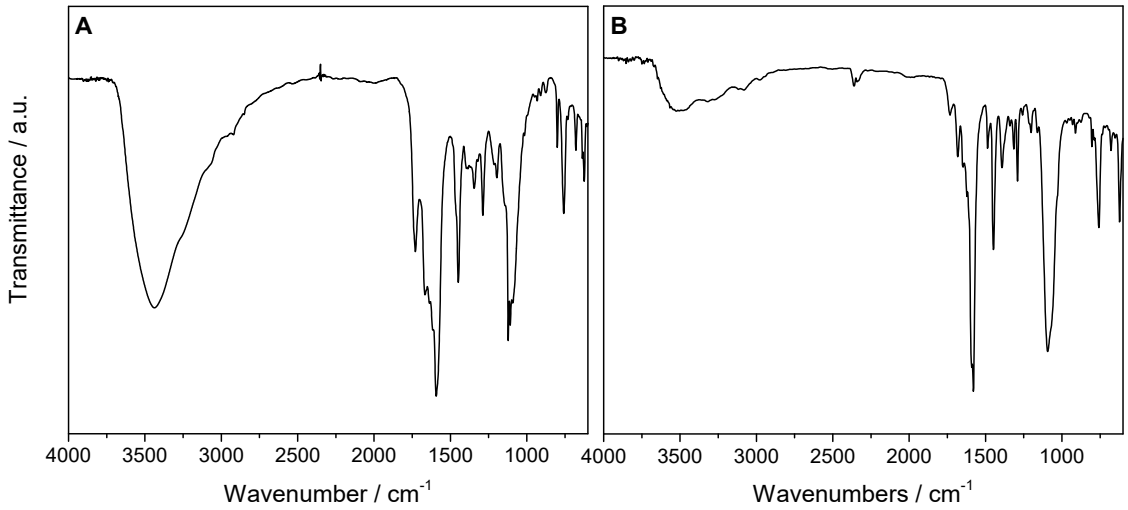

Figure S5. IR vibrational spectra acquired in KBr pellets for complexes [Cu(isapn)]ClO_4 (**A**) and [Cu(isaepy)(H_2O)]ClO_4 (**B**).

Main IR bands:

[Cu(isapn)]@POSS-atzac

FTIR (KBr pellet, cm^{-1}): ν(C-H) 2935, ν(C=O) 1656, ν(C=N) 1568, ν(C-N) 1454, ν_{as}(Si-O-Si) 1111, and ν(C-Cl) 703.

[Cu(isaepy)]@POSS-atzac

FTIR (KBr pellet, cm^{-1}): ν(C-H) 2942, ν(C=O) 1670, ν(C=N) 1573, ν(C-N) 1460, ν_{as}(Si-O-Si) 1116 and ν(C-Cl) 699.

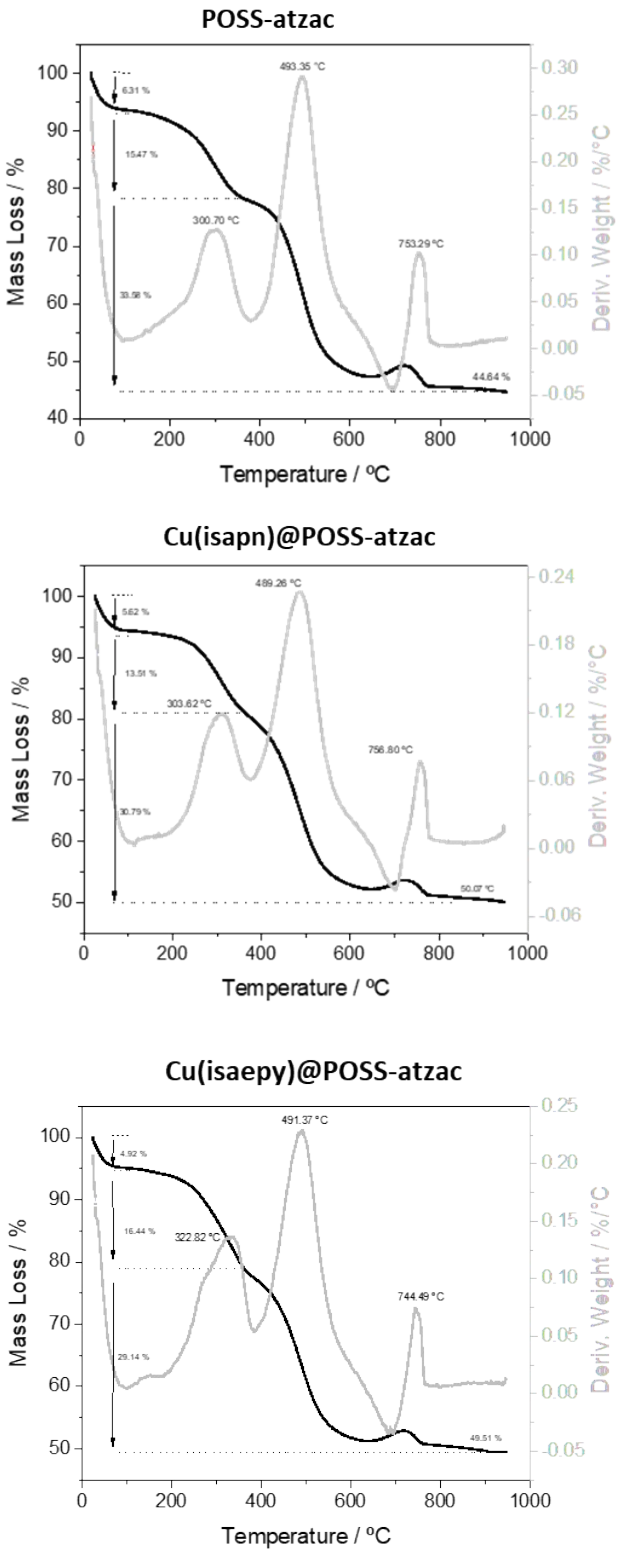

Figure S6. Thermogravimetric curves of POSS-*atzac* loaded with their respective complexes.

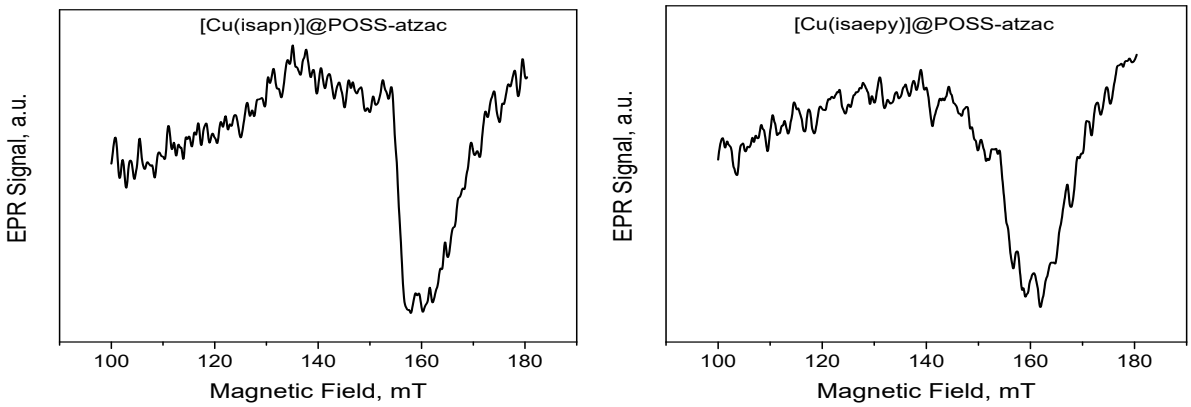

Figure S7. Solid-state X-band EPR spectra of [Cu(isapn)]@POSS-atzac and [Cu(isaepy)]@POSS-atzac, around 160 mT. Experimental conditions: Temperature = 10 K, Frequency = 9.4 GHz, Gain = 50 dB, Power Attenuation = 20 dB, Modulation Amplitude = 10 G, Center Field = 1400 G, Power = 2.145 mW and Modulation Frequency = 100 kHz.

Antiproliferative Assay

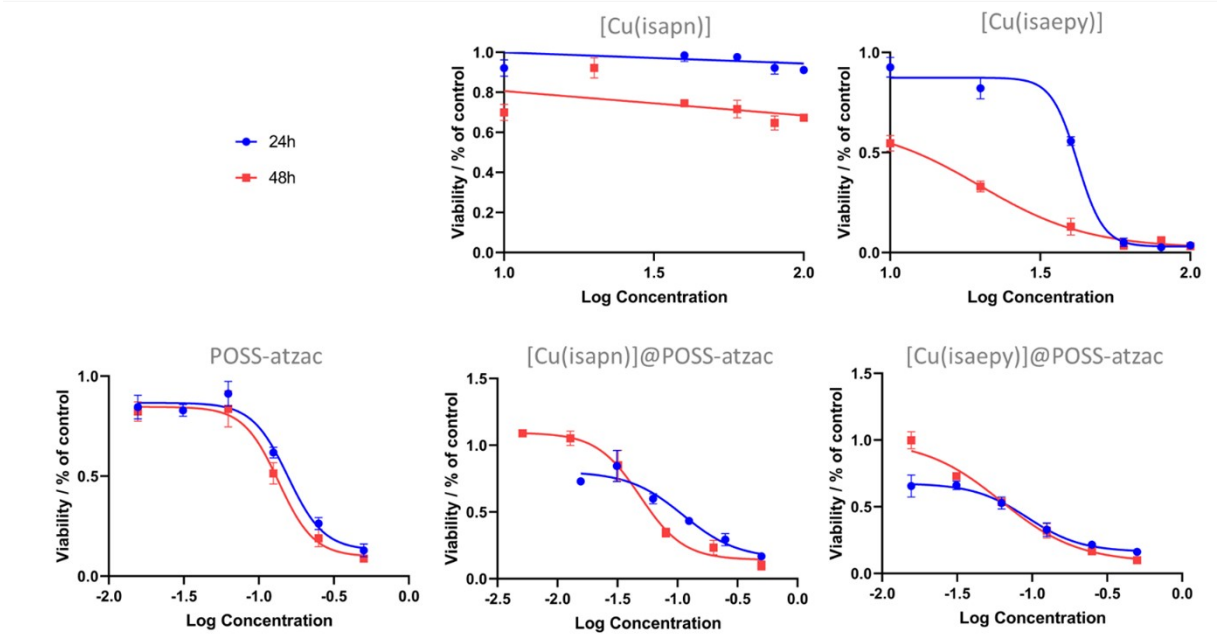

Figure S8. Growth inhibition in percentage on SK-MEL-147 cells induced by the free copper(II) compounds [Cu(isapn)] and [Cu(isaepy)], the free matrices POSS-atzac and the supported compound, after either 24 or 48h exposure times. Concentrations of compounds in the range 10 to 100 μM (metal complexes) or 3 nM to 1 mM (materials).

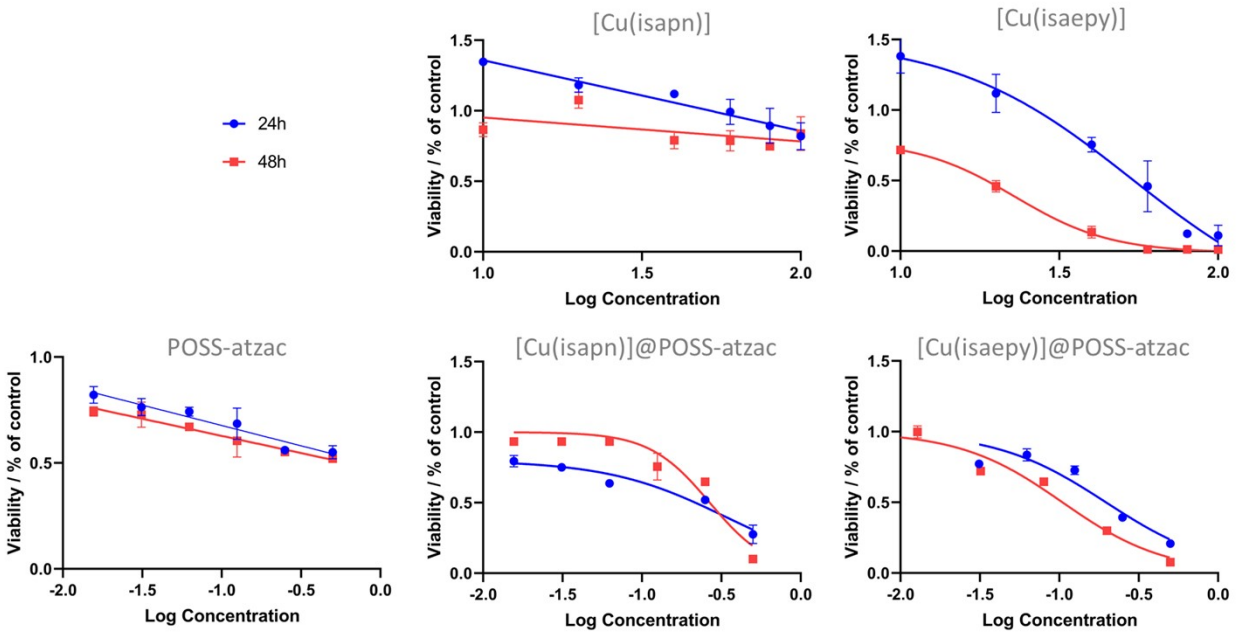

Figure S9. Growth inhibition in percentage on fibroblast P4 cells induced by the free copper(II) complexes [Cu(isapn)] and [Cu(isaepy)], the free matrixes POSS-atzac and POSS-att and the supported compounds, after either 24 or 48h exposure times, in DMEM. Concentrations of compounds in the range 10 to 100 μM (metal complexes) or 10 nM to 1 mM (materials).

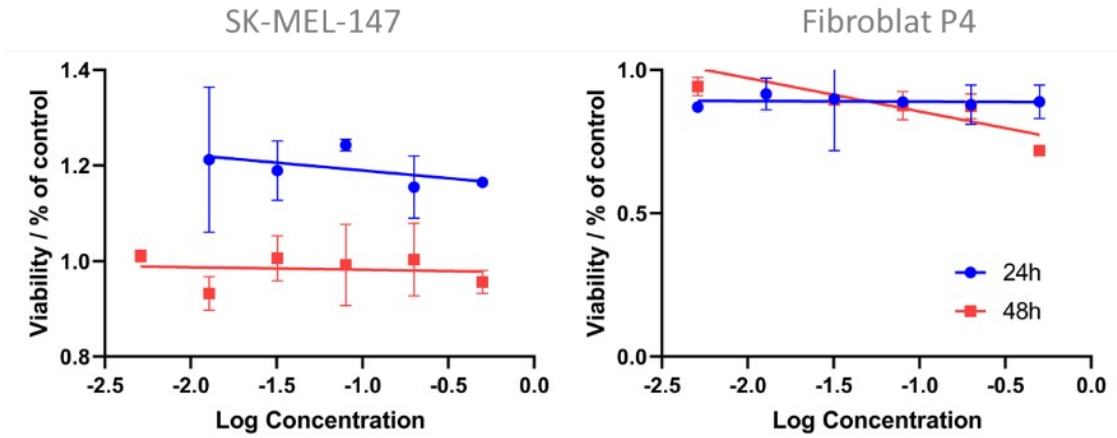

Figure S10. Growth inhibition profiles in percentage on SK-MEL-147 cells induced by the non-functionalized POSS-Cl, after either 24 or 48h exposure times, in DMEM. Concentrations of compounds in the range 3 nM to 1 mM.

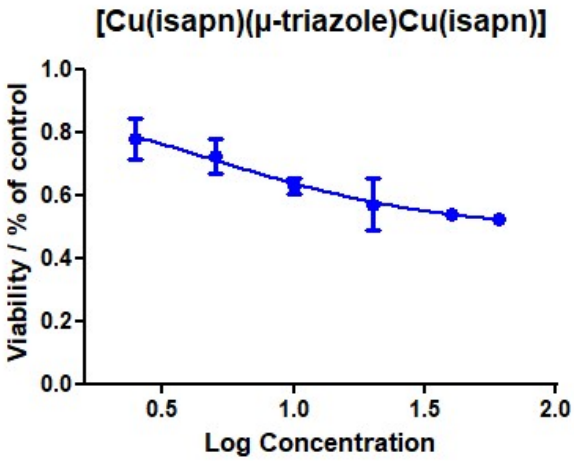

Figure S11. Viability in percentage of SK-MEL-147 cells after 24h incubation with complex [Cu(isapn)(μ-triazole)Cu(isapn)], at 37°C, in DMEM medium. Concentration of compound in the range 2 μM to 100 μM. Data control for fibroblasts were the same as in Figure S10.

Nuclease Activity Assay

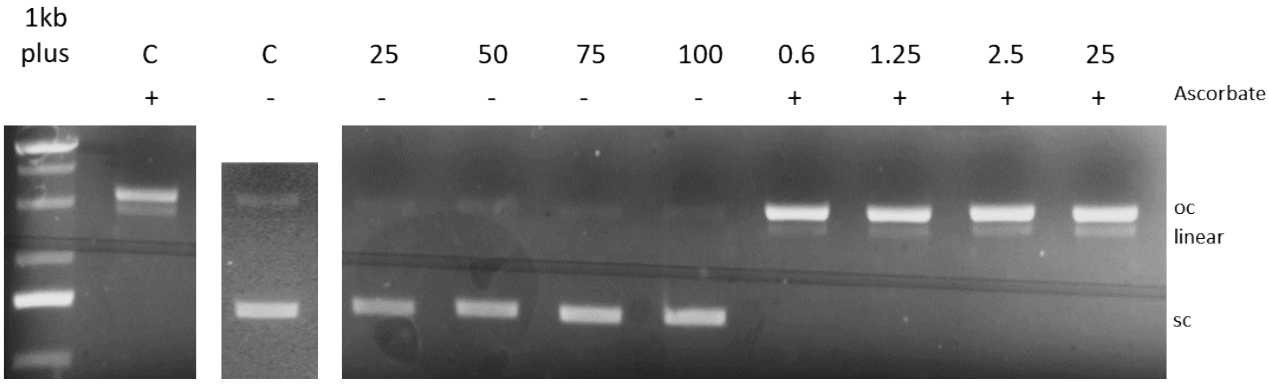

Figure S12. Controls and evaluation of effect of the free matrix POSS-atzac on plasmid DNA. In the ascorbate-free experiment, 200 ng of pBluescript (Bluscript plasmid) were exposed to POSS-atzac 25 to 100 μg mL^{-1} in 1x PBS buffer (10 mM phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4) at 37 °C for 24 hours. **B.** In the ascorbate-activated experiment, 200 ng of pBluescript were exposed to POSS-atzac in concentrations ranging from 0.6 to 25 μg mL^{-1} in the presence of 1 mM ascorbate in 1x PBS buffer at 37 °C for 24 hours. C: control, 200 ng of Bluscript plasmid in absence of in the presence of 1 mM ascorbate.

Oxidative Stress

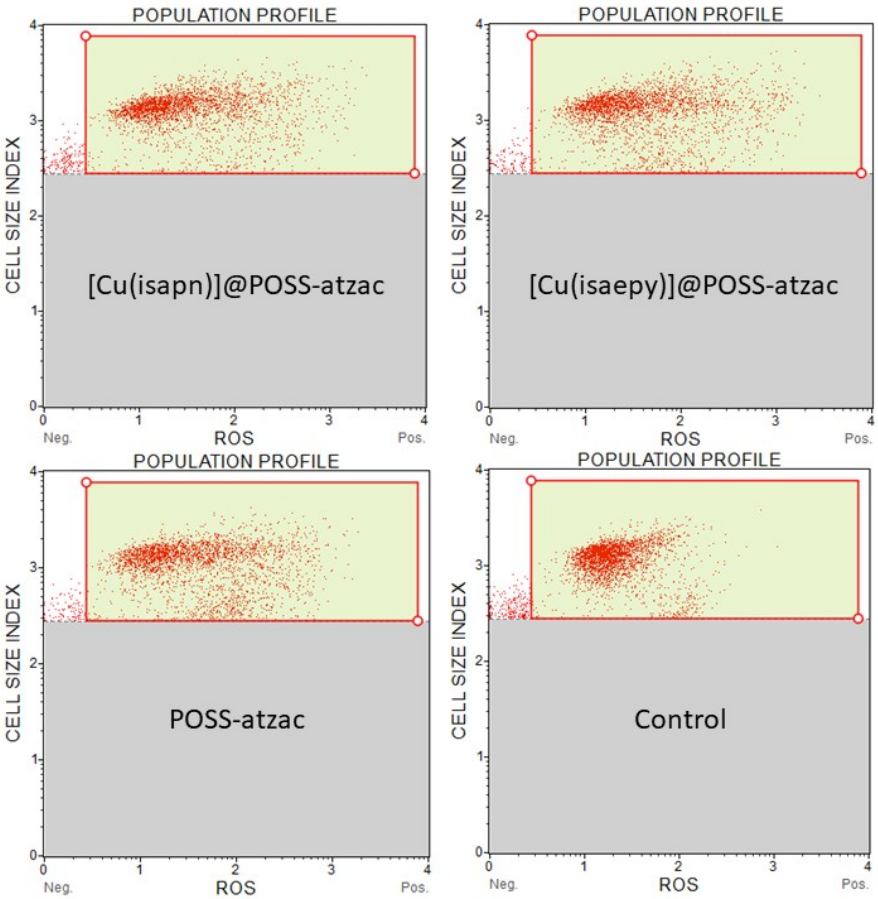

Figure S13. Cell population analysis of the oxidative stress induced on SK-MEL-147 cells upon 24h treatment at IC_{50} concentration of POSS-atzac, [Cu(isapn)]@POSS-atzac and [Cu(isaepy)]@POSS-atzac in comparison to untreated cells (control).

Melanin Oxidation Assay

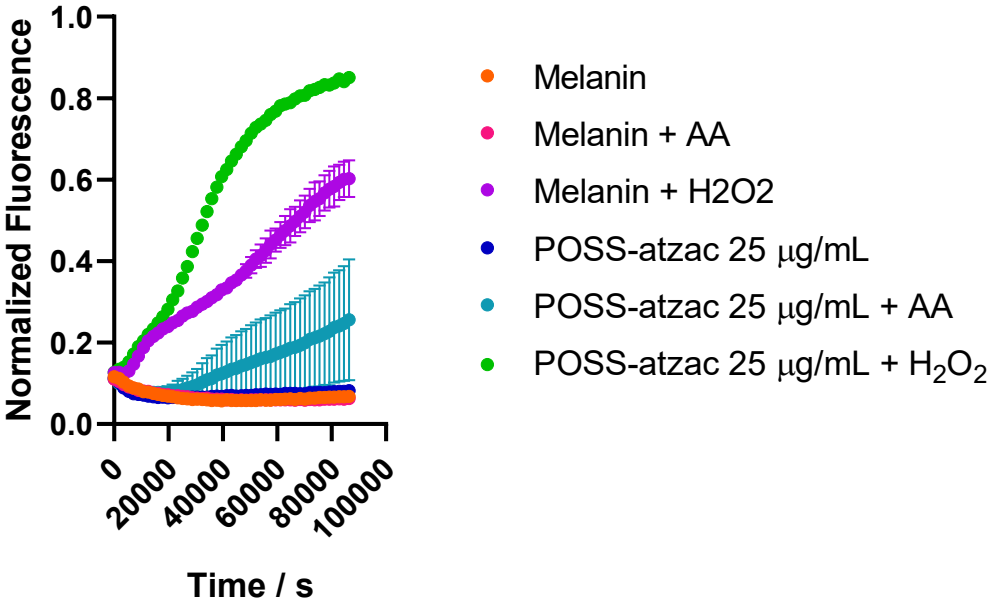

Figure S14. Melanin oxidation assay in PBS 1x pH 7.4, evaluating the free matrix POSS-atzac assayed at 25 μg/mL. The formation of the melanin oxidation product was followed by fluorescence, either in the absence of a Fenton-like reaction starter, or in the presence of ascorbate (AA) or H_2O_2.