Electronic Supplementary Information

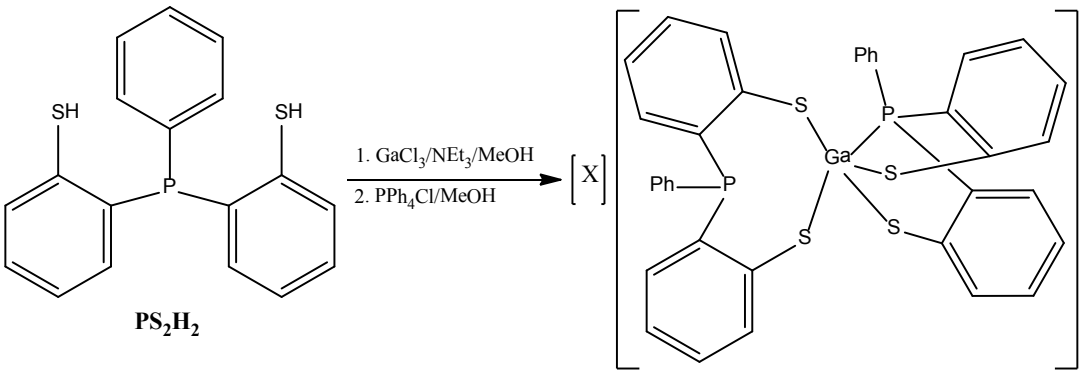
Of the manuscript:

Gallium phosphinoarylbisthiolato complexes counteract drug resistance of cancer cells

Eva Fischer-Fodor[a 1 2], Ana-Maria Vălean[b,d‡] , Petru Ilea[b,c], Corina Tatomir[a], Florica Imre-Lucaci[b], Maria Perde Schrepler[a], Ludovic Tibor Krausz[e], Lucian Barbu Tudoran[f], Calin George Precup[e], Iulia Lupan[g], Piroska Virag[a], Evamarie Hey-Hawkins[d], Luminita Silaghi-Dumitrescu[b]

[a] Oncology Institute "Prof.Dr.I.Chiricuta" Cluj Napoca, RO-400015, Romania
[b] Faculty of Chemistry and Chemical Engineering, "Babes-Bolyai" University, Cluj-Napoca, RO-400084, Romania
[c] Babeş-Bolyai" University, Interdisciplinary Research Institute on Bio Nano Sciences, Cluj Napoca, RO-400271, Romania
[d] Institut für Anorganische Chemie der Universität Leipzig, D-04103, Leipzig, Germany
[e] Pharmacology Department, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, RO-400012, Romania.
[f] Electron Microscopy Center, Department of Molecular Biology and Biotechnology, Babes-Bolyai University, Cluj-Napoca, RO-400084, Romania
[g] Surgery Clinic No 1 - Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, RO-400012, Romania.
[h] Molecular Biology Center, Institute for Interdisciplinary Research on Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, 400271, Romania,

Complexes preparation scheme



PS_2H_2

Where: X=$NHEt_3$ (**1**) and PPh_4 (**2**)

Scheme S1. Synthesis of compound $[NEt_3H][Ga\{PPh(2\text{-}SC_6H_4)_2\text{-}\kappa^3S,S',P\}\{PPh(2\text{-}SC_6H_4)_2\text{-}\kappa^2S,S'\}]$ (**1**). Cation exchange with PPh_4Cl gave the crystalline compound $[PPh_4][Ga\{PPh(2\text{-}$

[1] Corresponding author
[2] These two authors contributed equally to this work.

$SC_6H_4)_2$-κ^3*S,S′,P*}{PPh(2-$SC_6H_4)_2$-κ^2*S,S′*}] (**2**). Molecular weights of compounds are M(1)=820 and M(2)=1057.

Crystal structure of complex 2

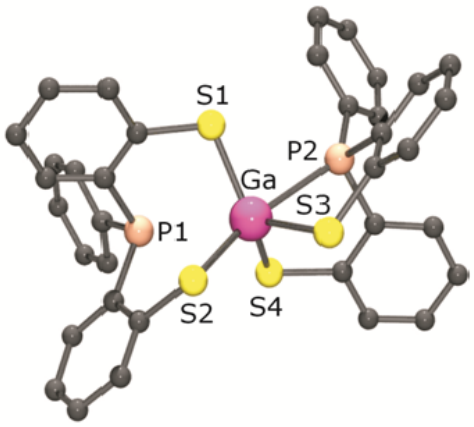


Fig. S1. Molecular structure of [Ga{PPh(2-$SC_6H_4)_2$-κ^3*S,S′,P*}{PPh(2-$SC_6H_4)_2$-κ^2*S,S′*}]; depiction generated with POV-Ray software, according to Valean and co-authors[12]. In complex **2** the [Ga{PPh(2-$SC_6H_4)_2$-κ^3*S,S′,P*}{PPh(2-$SC_6H_4)_2$-κ^2*S,S′*}] anion is stabilized with tetraphenylphosphonium cation and crystal structure was obtained; and complex **1** contains tetraethylammonium cation and does not give rise to crystal structure.

Compounds solubility

Two sets of solutions or fine suspensions of 1 and 2 were prepared for biological tests:

Set 1: Complex **1**: 0.007 g of 1 was dissolved in a mixture of solvents: 2 ml of ethanol and 3 ml of poliethylenglycol 400 (1,707.10-3mol/l)

Complex **2**: 0.019 g of 2 was dissolved in 2 ml of ethanol and 3 ml of polyethylene glycol 400 -very fine suspensions in both cases (3,595.10-3mol/l)

Set 2: Complex **1**: 0.012 g of 1 solvated in 3 ml DMSO (4.87.10-3 mol/l).

Complex **2**: 0.022 g of 2 in 4 ml DMSO (5.20.10-3 mol/l).

Solutions from set 1 were dissolved in PBS to obtain a final stock solution of 1,7 mM for complexes 1 and 2. Set 1 and set 2 were tested on cells, and since no significant differences were obtained in 24-hour antiproliferative effect, in the experiments only the ethanol-polyethylene glycol solutions were used.

Complexes stability towards oxygen and humidity

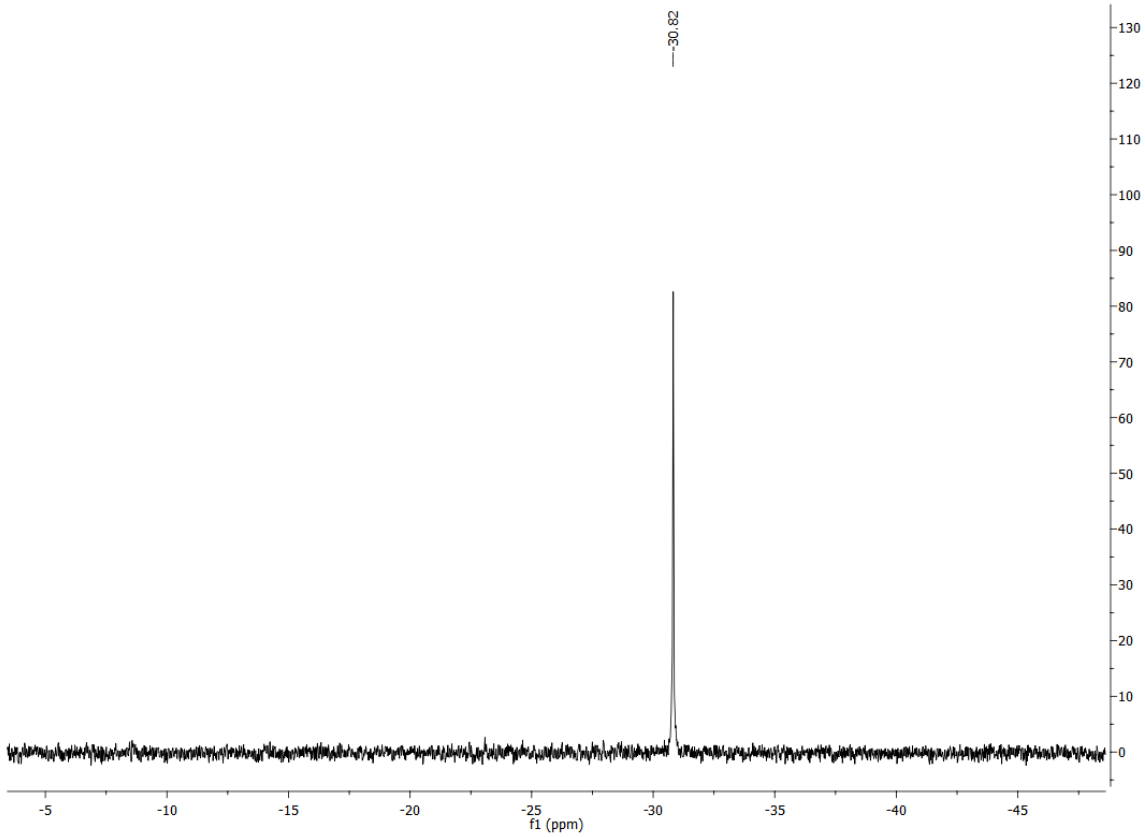


Fig. S. NMR spectra of complex **1** ethanol:polyethyleneglycol solution after 10 minutes.

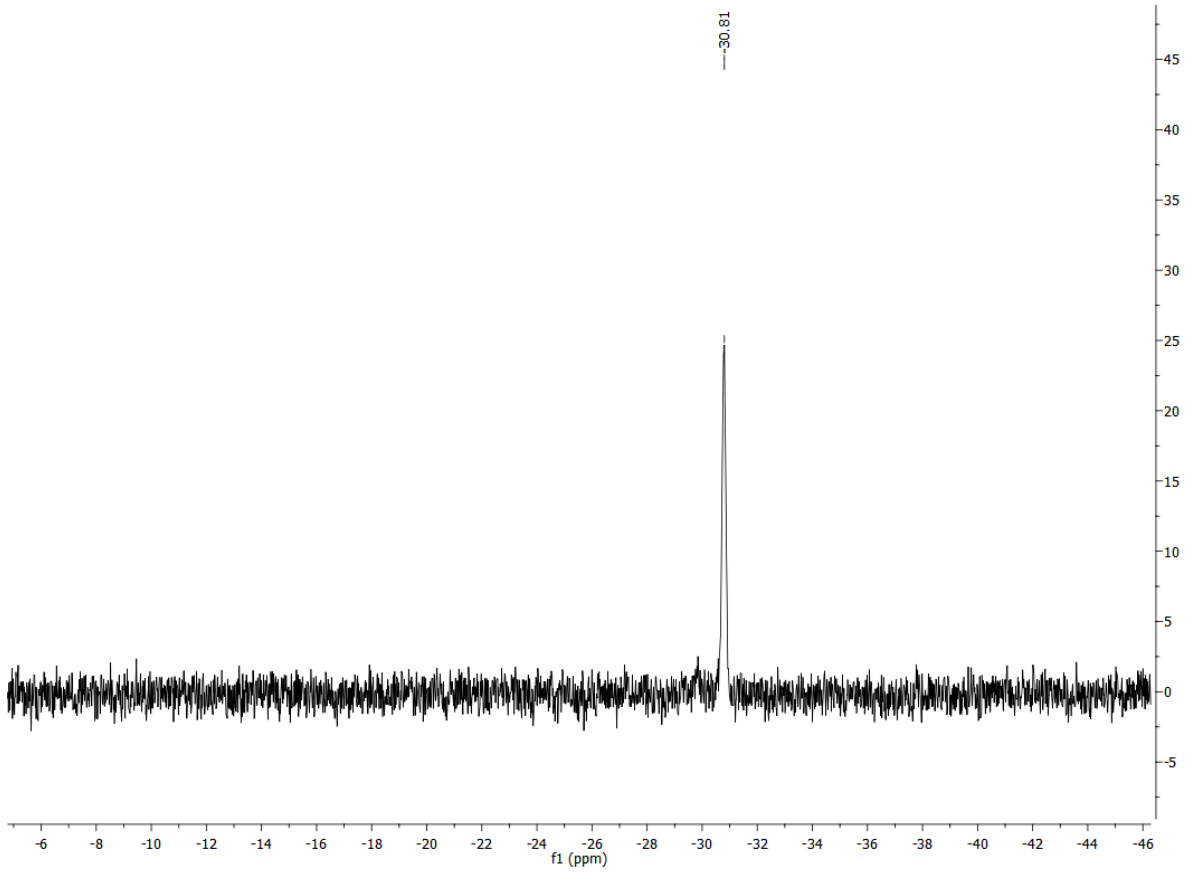


Fig. S2. Complex **1** stability in ethanol : polyethyleneglycol solution after one week monitoring.

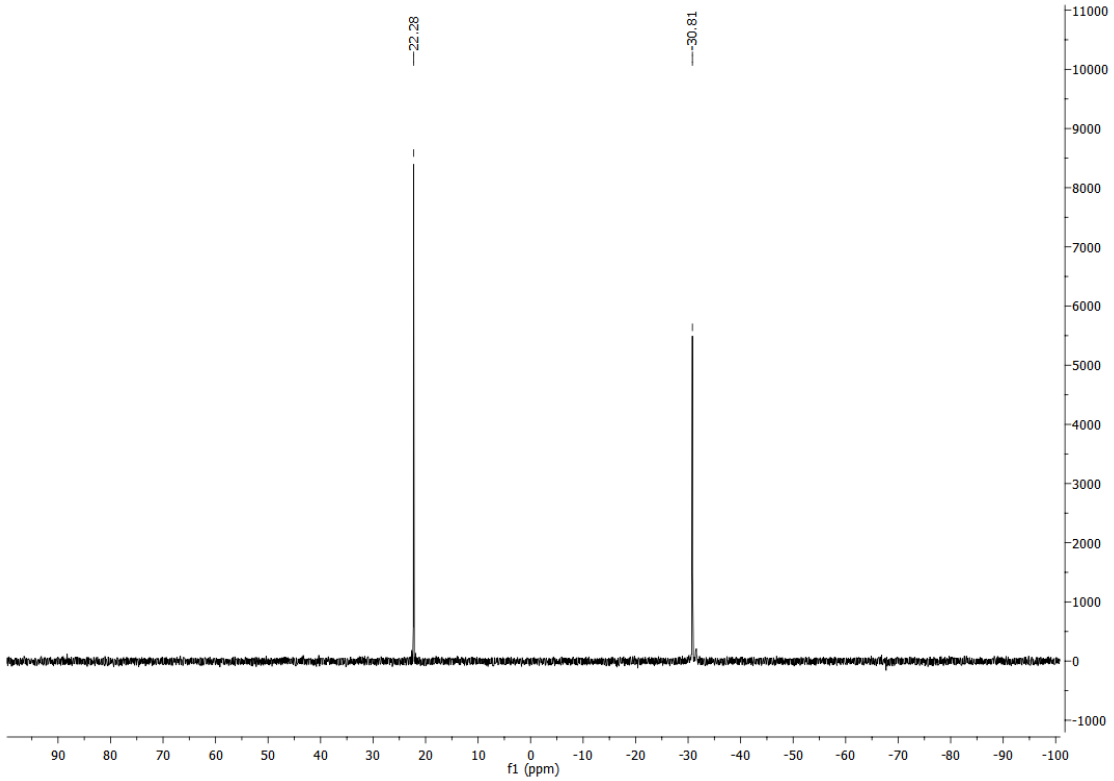


Fig.S3. NMR spectra of complex **2** ethanol: polyethyleneglycol solution after 10 minutes.

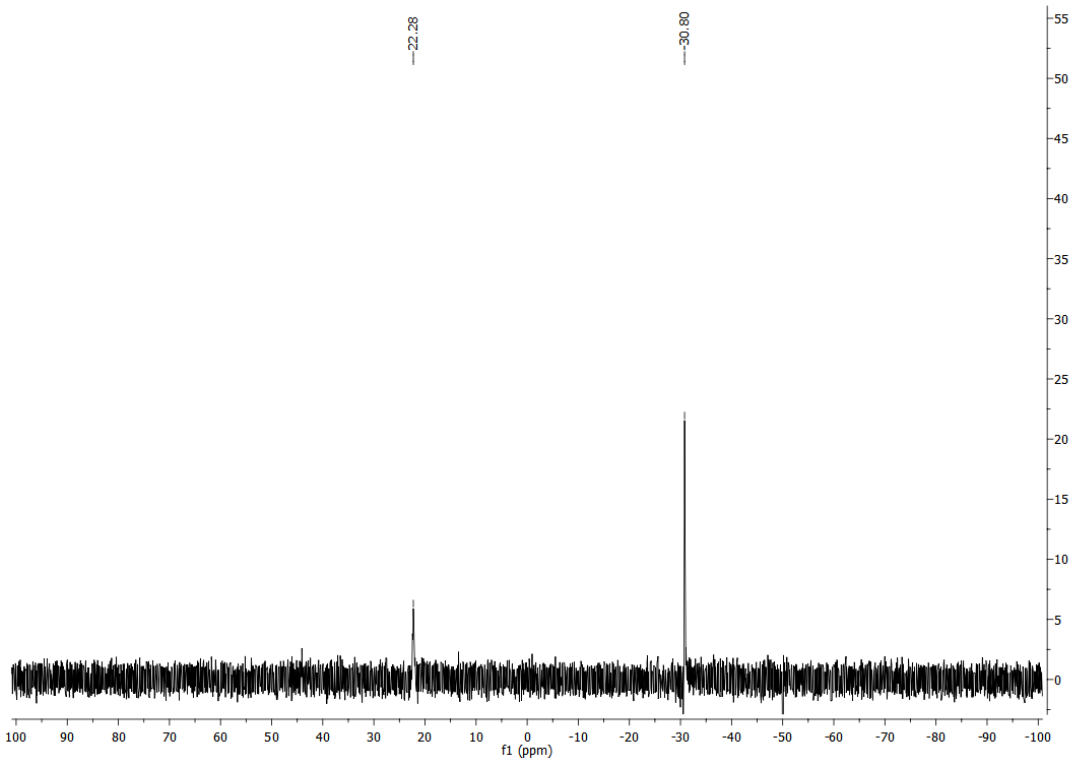


Fig.S4. Complex **2** stability in ethanol: polyethyleneglycol solution after one week monitoring.

Gallium standards preparation for cellular uptake experiment

As Ga atomic spectroscopy standard 1000 mg/liter in 2% HNO_3 acid solution was used (from LGC Standards GmbH,Wesel, Germany) and ultrapure nitric acid (65 %) was purchased from Merck (Darmstadt, Germany). Ga standards, ranged between 20 and 100 µg/liter, were automatically prepared and injected in the graphite furnace using the PAL3000 autosampler. The starting solution of 100 µg/liter Ga was freshly prepared by serial dilutions of the Ga atomic spectroscopy standard stock in ddH_2O (Model Double D Still, Jencons Scientific Ltd., Leighton Buzzard, England) plus 2% HNO_3 acid (v/v). A non-linear calibration curve was generated immediately before each measurement using the "Concentration Least Squares" built-in method, an excellent correlation being obtained (R = 0.9996) for the 0 to 100 µg/liter domain. Each sample was measured in triplicate. The injection volume was 20 µl, of mineralized samples. The furnace program used sequential drying (90°C for 5 s and 110°C for 10 s), charring (300°C for 10 s) and atomization (2600°C for 5 s) phases. Before measurements, the received frozen biological probes were gently mineralized by directly addition of 1 mL of ultrapure nitric acid (65 %) in the freezing containers. The mixtures were stored for 2 h at room temperature and atmospheric pressure and, before measurements, the probes volumes were adjusted to 2 mL using ddH_2O.

Cell cultures morphological evaluation using inverted phase microscopy

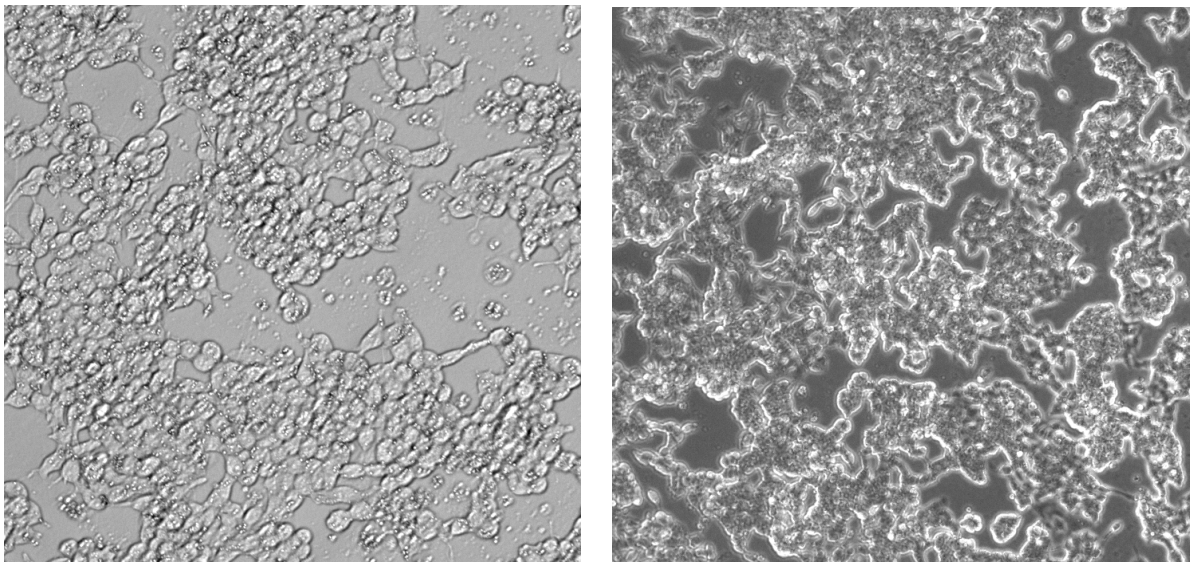


Fig. S5. Untreated A2780(left) and A2780cis cells (right) in subconfluency. Images prevealed using Zeiss D1 Observer microscope with PlasDic objective, magnitude 20X.

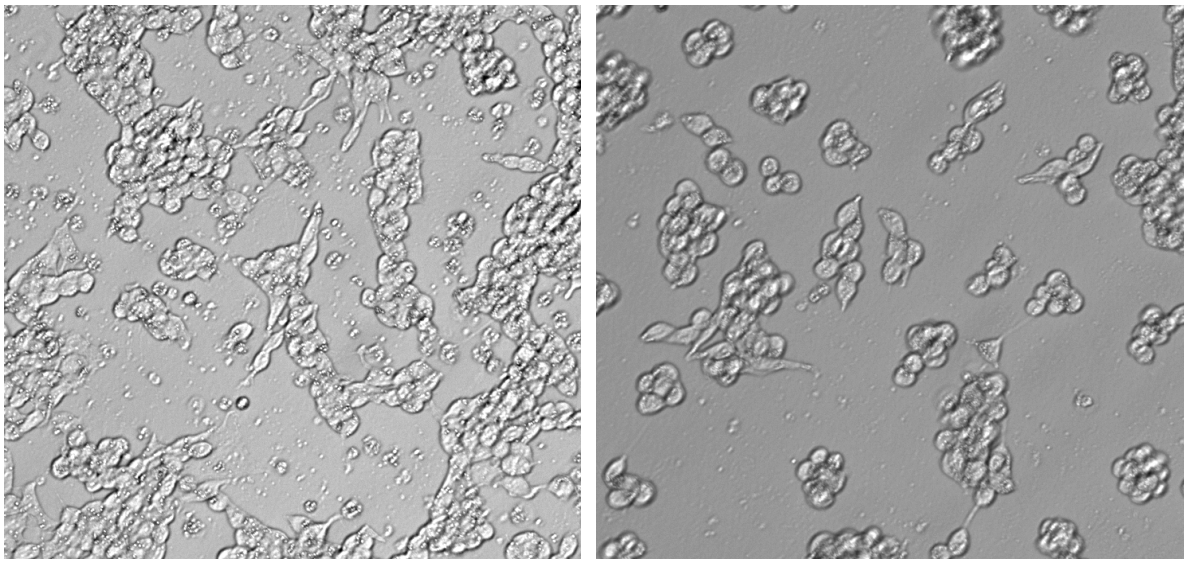


Fig. S6. Inverted phase mircoscope images of A2780 cells treated with complex **1**(left) and complex **2** (right), PlasDic objective, magnitude 20x.

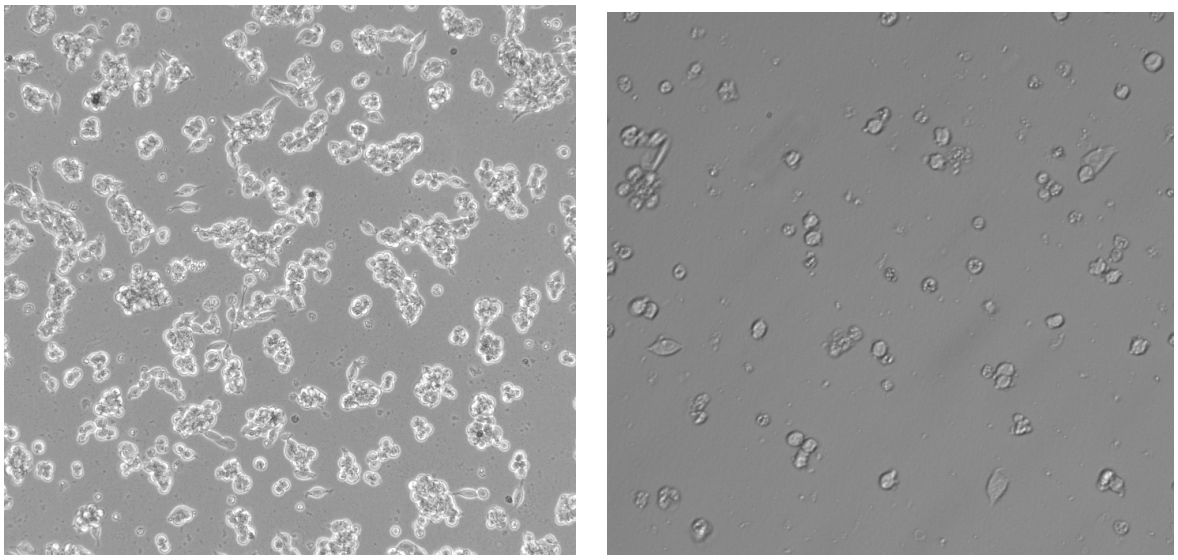


Fig. S7. Inverted phase microscope images of A2780*cis* cells treated with complex **1**(left) and complex **2** (right), PlasDic objective, magnitude 20x.

Ga(III) complexes cytotoxicity- sigmoidal dose-response curves

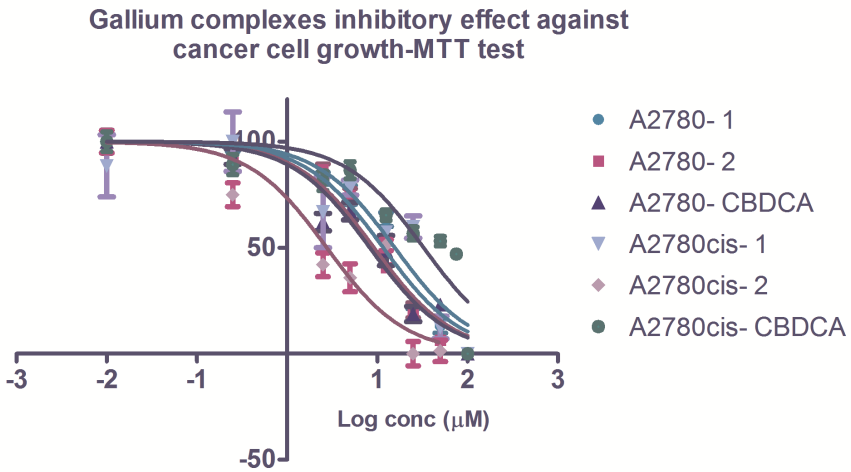


Fig. S8. It is a dose-response relationship in complexes effect against tumor cells; sgmoidal curves provide the IC50 values for each compound.

DNA binding capacity of complexes 1 and 2

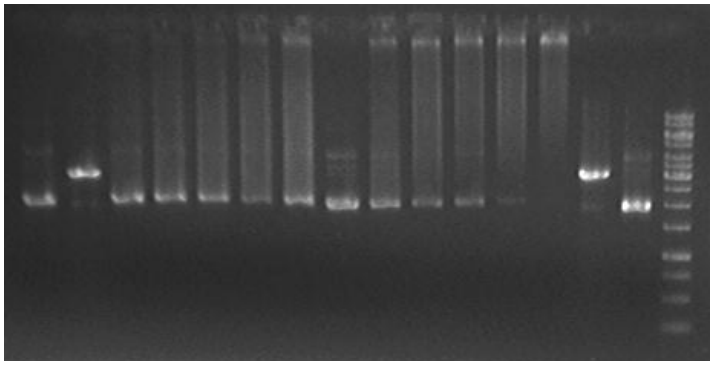


Fig. S8. Interaction of gallium (III) complexes with plasmid pTZ57R DNA. Lanes 1, 8 and 15 – closed circular plasmid DNA without gallium complex; lanes 2 and 14 – linear plasmid DNA (plasmid DNA was digested with *Eco*RI); lanes 3-7 – plasmid DNA with complex **2** at molar ratio (nmol nucleotide / nmol complex 2) 1/2.5, 1/5, 1/7.5, 1/10 and 1/12.5 respectively; lanes 9-13 – plasmid DNA with complex **1** at molar ratio (nmol nucleotide / nmol complex 1) 1/5, 1/7.5, 1/10, 1/20, and 1/50 respectively; lane 16 – GeneRuler 1 kb DNA ladder (Fermentas).

DNA lesions evaluation using the single cell gel electrophoresis

Comet assay protocol for restriction sites identification:

Cell suspension (10^6 cells/ml) was mixed with 1% low melting agarose gel, embedded on agarose-coated microscope slides, and placed overnight in an alkaline lysis buffer (2.5M NaCl, 0.1M EDTA, 0.01M Tris, 1% Triton X-100, 10%DMSO, pH 10). For the modified comet assay, slides were then equilibrated in enzyme buffer (0.04M HEPES, 0.1M KCl, 0.5mM EDTA, 1% FCS, pH 8.0) prior to application of Endo III, FpG or hOGG1. Slides treated with the lesion-specific enzymes were incubated at 37°C in a humidified incubator for 30min. Following after alkaline lysis and enzyme treatment, respectively, the slides were placed in in alkaline buffer (0.3M NaOH, 1mM EDTA, pH 12) at 4°C, to allow DNA unwinding and DNA breakage at alkali labile sites. DNA electrophoresis was performed in an ice-cooled electrophoresis chamber at 25 V and 300 mA for 20 min. Following electrophoresis, the slides were neutralized using 0.4M Tris pH 7.5 and stained with 50µl of 20 µg/ml ethidium bromide. Reagents used were from Sigma Aldrich, except KCl p.a. and NaOH p.a, from Merck (Darmstadt, Germany). Stained slides were digitally analyzed using Eclipse E-1000 UV microscope (Nikon, Tokyo, Japan) and Lucia image analysis software, counting 100 cells per slide. As a result of the experiment, under the microscope cells were visualized as comet-shaped images. Cells were visually assigned a score on a scale of 0-4 (0-undamaged cell, no tail to 4-severely damaged, long diffuse tail) based on percentage of DNA migrated in the comet tail.

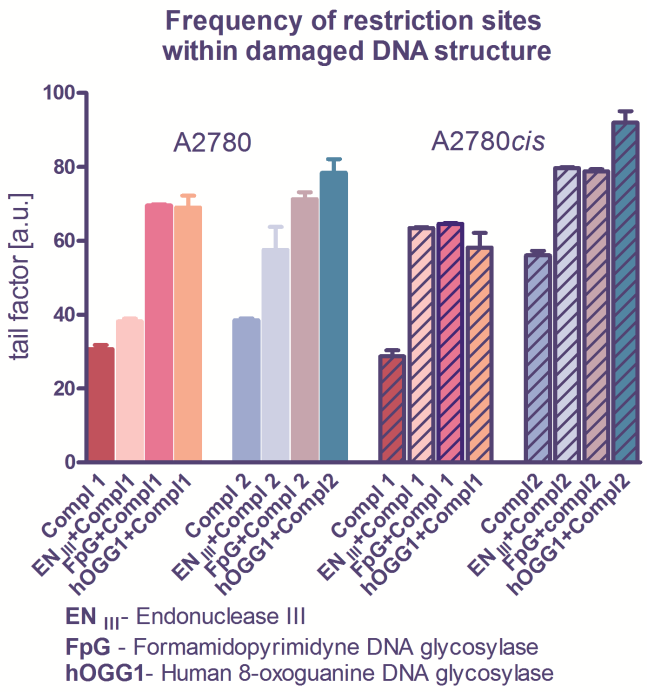


Fig. S9. In concordance with the lesion score evaluation, the tail factor (TF) indicates many loci evidenced by the restriction enzymes. In the places where base pair substitution, deletion or insertion arise, the restriction enzymes provoke supplementary damages in cellular DNA.

Metalloproteases expression

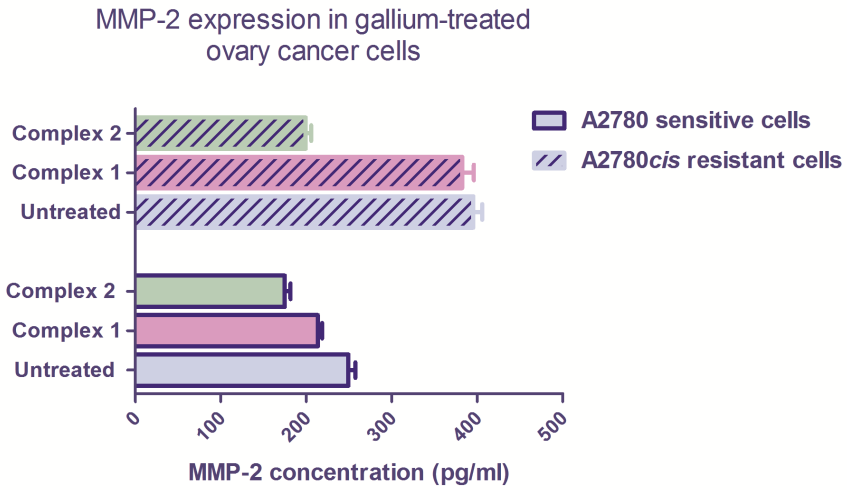


Fig. S10. Quantitative evaluation of matrix metalloprotease 2 (MMP-2) expression in A2780 and A2780*cis* ovary carcinoma cells using multianalyte profiling xMAP technology. MMP-2 is expressed constitutively in both cell lines, prominently in the resistant cells, as expected. Complex **1** is able to restrict MMP-2 secretion in sensitive cells, but in resistant cells his influence is weak, while complex **2** significantly inhibit MMP-2 expression in both cell lines.