Supporting Information

Replacement of the thiosugar of Auranofin with iodide enhances the anticancer potency in a mouse model of ovarian cancer

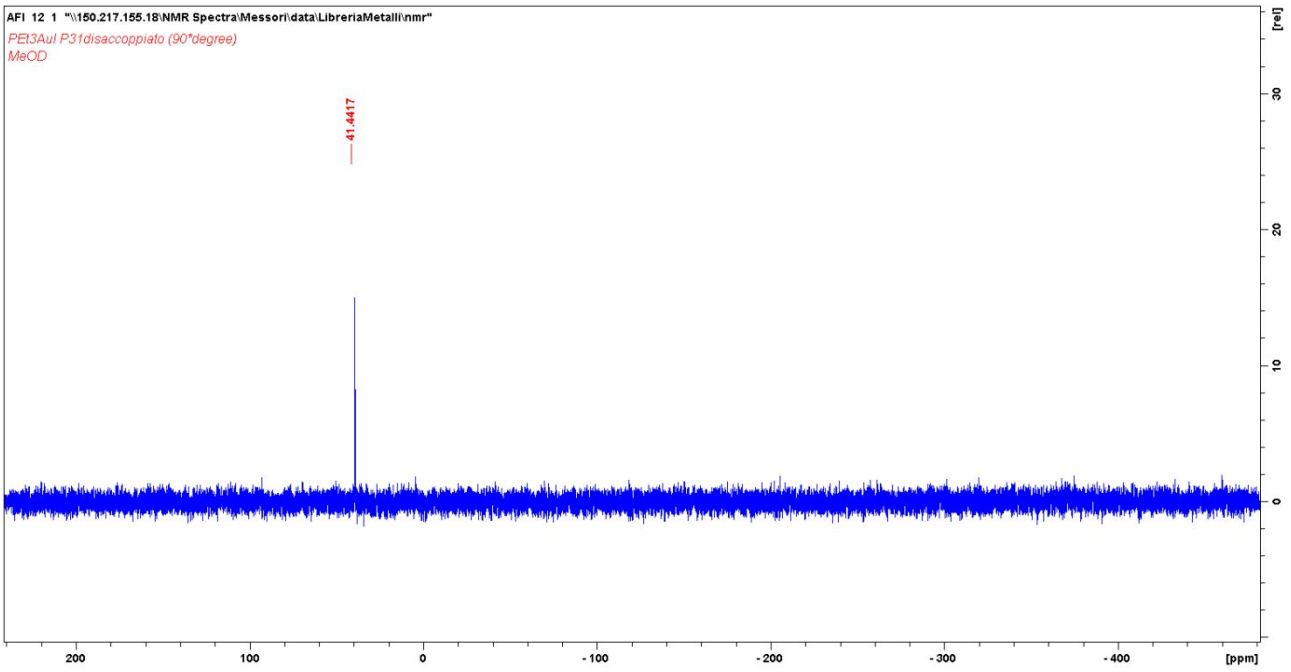
Synthesis and purity of Et_3PAuI (T. Marzo et al. ACS Med. Chem. Lett. 2017, 8, 997−1001)

Auranofin has been purchased from Vinci Biochem srl

Et_3PAuI was synthesized starting from commercially available Et_3PAuCl. First, Et_3PAuCl was treated with an excess of KI in ethanol at 25 °C. After 3 h, the mixture was dried and the resulting white solid kept at −20 °C overnight. The product was then extracted with dichloromethane, and the recombined organic phase was washed with water and dried over MgSO4. After precipitation in pentane, white crystals of Et3PAuI were collected and dried. Yield 74%. The product was characterized through ^{31}P NMR analysis with a Bruker Ultrashield 400 spectrometer (zgpg pulse sequence; ^{31}P transmitter frequency offset -120 ppm; ^{1}H transmitter frequency offset 4 ppm; spectral width 723 ppm; size of fid 65536 points; acquisition time 280 ms; magnetization recovery time 2 sec). The raw data were elaborated through Topspin® 4.0.1 software.

Purity of Et3PAuI was assessed through elemental analysis of C, N and H [calculated C: 16.30%, H: 3.42%, N: 0%, experimental: C: 16.07%, H: 3.18%, N: 0%].

^{31}P NMR (CD3OD, 161.97 MHz, 298 K): 41.44



LogP determination (T. Marzo *et al.* ACS Med. Chem. Lett. 2017, 8, 997−1001)

The octanol–water partition coefficients were determined as follows. Water (50 mL, distilled after Milli-Q purification) and n-octanol (50 mL) were shaken together for 72 h to allow saturation of both phases. Solution of the complexes were prepared in the aqueous phase and an equal volume of octanol was added. Biphasic solutions were mixed for ten minutes and then centrifuged for five minutes at 6000 rpm to allow separation. Concentration in both phases was determined by ICPAES (Au ʎ= 267.595 nm). Reported logP is defined as log[complex]oct/[complex]wat. Final values were reported as the mean of three determinations.

Apoptosis plots

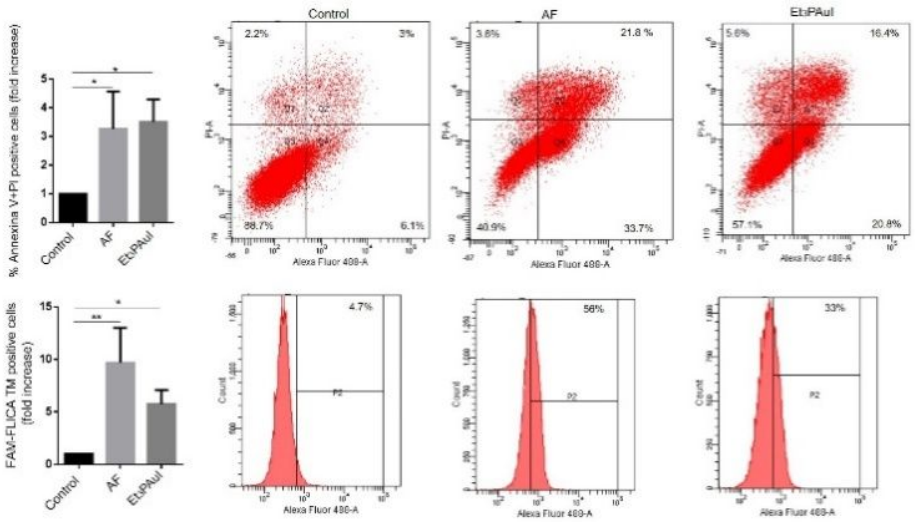


Figure S1: Gold compounds exert apoptosis induction in A2780 cell line. (A) Flow cytometry analysis of annexin V/propidium iodide-stained A2780 cells treated for 72 h with gold compounds at the respective IC_{50} values. The histogram reports the mean fold increase of apoptotic/necrotic cells (A+/PI-, A+/PI+ and A-/PI+ cells) respect to control conditions. Representative dot plots were shown in the panels on the right. (B) Caspase-3 Assay of A2780 cells treated with gold compounds at the respective IC50 values for 72 hours. The histogram reports the fold increase of FAM-FLICATM positive cells (P2) respect to control cells. Representative dot plots were shown in the panels on the right. Four independent experiments were performed for both assays. The statistical analysis was carried out using one-way ANOVA test followed by Tuckey's multiple comparisons test using Graphpad Prism v 6.0 (*p<0.05, **p<0.01).

Assessment of cell death by flow cytometry

Cell death was analysed by TACS Annexin V/PI Kit (Trevigen) according to the manufacturer's instructions. Briefly, control, Auranofin, and Et_3PAuI A2780-treated treated cells for 72 h with 72 h-exposure IC_{50}, were trypsinized, washed with PBS and resuspended in staining solution for 15 min in the dark. Cells were analysed by FACSCanto flow cytometer (BD Biosciences). Gated cells were plotted on a dot-plot showing Annexin-V staining and propidium iodide (PI) staining. Percentage of apoptotic/necrotic cells was determinate adding Annexin V positive (early apoptotic) cells to Annexin/PI positive ones (late apoptotic) and to PI positive cells (necrotic cells).

Assessment of caspase activity by flow cytometry

For Caspase-3 activity determination control, Auranofinand Et_3PAuI A2780-treated cells for 72 h with 72 h-exposure IC_{50}, were trypsinized, washed with PBS and resuspended in FAM-FLICATM Caspases solution (Caspase FLICA kit FAM-DEVD-FMK, ImmunoChemistry Technologies) for 1 h at 37°C, following the manufacturer's instruction. Cells were washed with PBS and analysed by FACSCanto flow cytometer (BD Biosciences).

Cell growth inhibition studies (Sulforhodamine B assay).

The cytotoxic effects of the complexes were evaluated on the growth of A2780 cell line according to the procedure described by Skehan et al.(Skekan, P.; Stroreng, R.; Scudiero, D.; Monks, A.; Mcmahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. J. Natl. Cancer Inst. 1990, 82, 1107−1112). Both compounds were initially diluted in DMSO as stock solutions (20 mM), further dilutions have been performed in PBS (0.5% DMSO present at the higher tested concentration).

Exponentially growing cells were seeded in 96-well microplates at a density of 5×10^3 cell/well. After cell inoculation, the microtiter plates were incubated under standard culture conditions (37°C, 5% CO2, 95% air and 100% relative humidity) for 24 h prior to the addition of study compounds. After 24 h, the medium was removed and replaced with fresh medium containing drug concentrations ranging from 0.003 to 100 µM for a continuous exposure of 24 and 72 h for both study compounds.

For comparison purposes the cytotoxicity effects of cisplatin measured in the same experimental conditions were also determined.

According to the procedure the assay was terminated by the addition of cold trichloroacetic acid (TCA). Cells were fixed *in situ* by 10% TCA and stained by sulforhodamine B (SRB) solution at 0.4% (w/v) in 1% acetic acid. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM tris base, and the absorbance was read on an automated plate reader at a wavelength of 540 nm.

The IC_{50} drug concentration resulting in a 50% reduction in the net protein content (as measured by SRB staining) in drug treated cells as compared to untreated control cells was determined after 72 h of drug exposure.

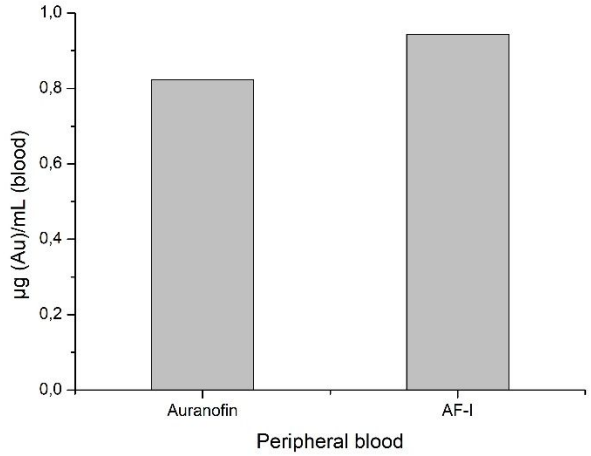
Tolerability studies

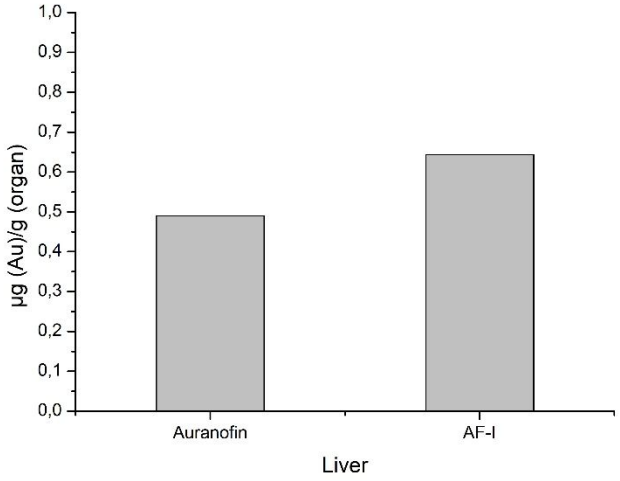
Nude mice were randomly grouped according to their body weight with four mice per test substance, which was administered intraperitoneally using two different doses (20 mg/kg and 40 mg/kg). The complexes have been solubilized in DMSO and then diluted in bidistillated water with 5% DMSO present in the final solution. Only one administration was performed, followed by a three weeks observation period; in this time, we monitored daily possible signs of toxicity including weight loss, mobility reduction, dehydration, hunched posture, ruffled fur, and loss of appetite or lethargy. Animals were euthanized if their body weight loss exceeded 15% within the first 24 h after treatment or 20% at any other point in the study.

Biodistribution studies

For the assessment of biodistribution of Auranofin, Et_3PAuI and, nude mice (female 5 weeks old) were randomly grouped according to their body weight with three mice per test substance, which was administered with intraperitoneal injection using a single dose (10 mg/kg). Mice from each group were anesthetized, bled, and sacrificed, and organs dissected (spleen, liver, kidney, heart) at 24 hours. After blood collection, tissues were weighed and solubilized, and the amount of gold in each tissue was determined through ICP-OES analysis after mineralisation with HNO_3. The determination of gold concentration in the blood or in the organs was performed by a Varian 720-ES Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) equipped with a CETAC U5000 AT+ ultrasonic nebulizer, in order to increase the method sensitivity. Before the analysis, samples were weighed in PE vials and digested in a

thermo-reactor at 80 °C for 24 h with 2 mL of HNO_3 (35 % suprapure grade) 0.5 mL of H_2O_2 suprapure grade. After digestion, the samples were diluted to 6 mL with ultrapure water (≤18 MΩ). 5.0 mL of each sample were spiked with 1 ppm of Ge used as an internal standard and analysed. Calibration standards were prepared by gravimetric serial dilution from a commercial standard solution of Au at 1000 mg L^{-1}. The wavelength used for Au determination was 267.594 nm whereas for Ge the line at 209.426 nm was used. The operating conditions were optimized to obtain maximum signal intensity, and between each sample, a rinse solution of HNO_3 (35 % suprapure grade) was used in order to avoid any "memory effect".





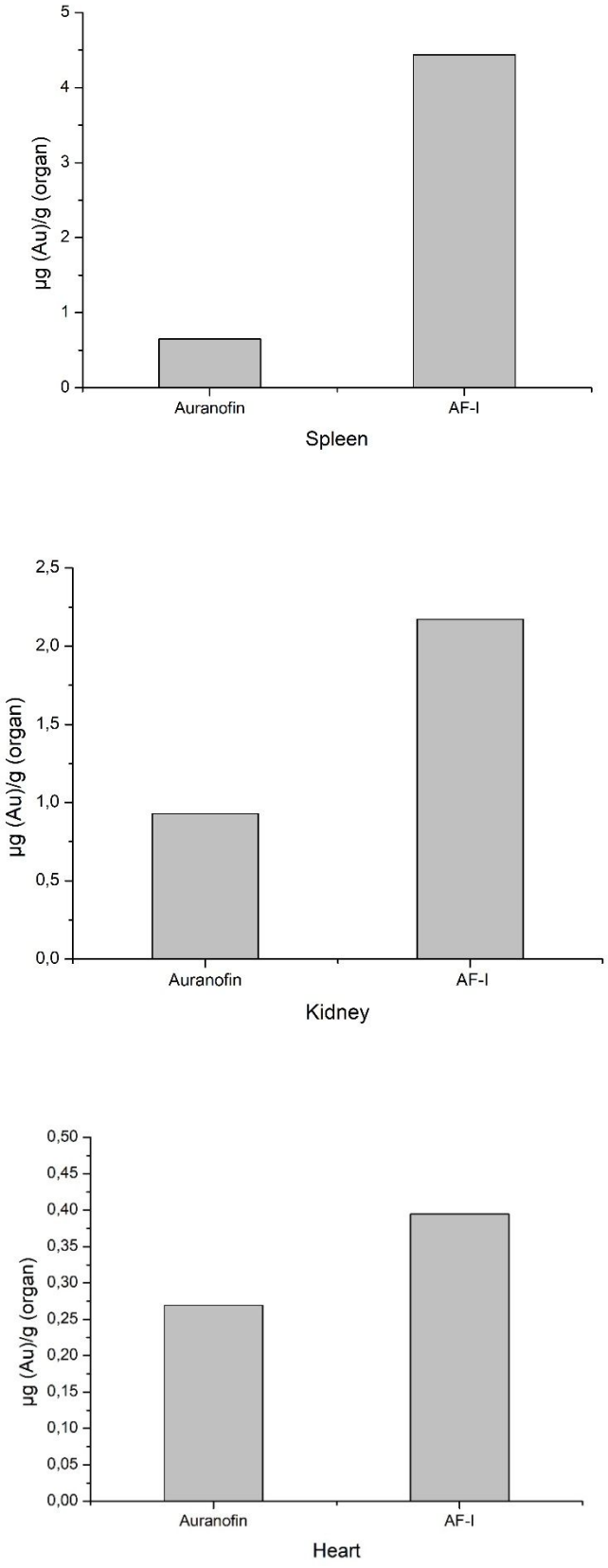


Figure S2: plots of biodistribution pilot study for Auranofin and Et_3PAuI (AF-I)

***In vivo* anticancer activity**

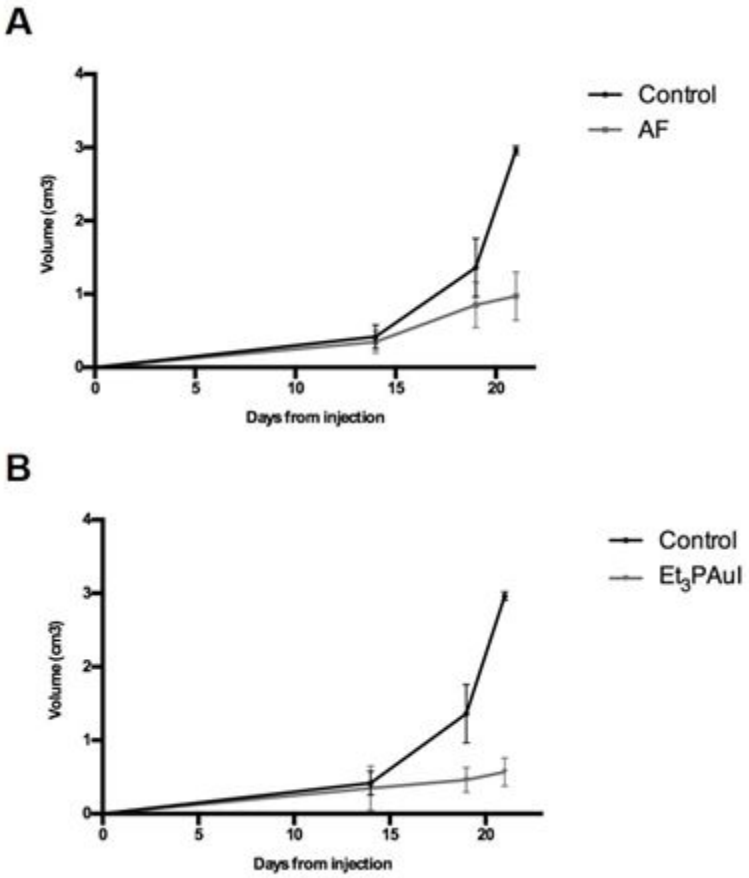


Figure S3 A-B: *In vivo* anticancer activity evaluation of gold compounds in a xenograft subcutaneous (sc) model of ovarian cancer cell line. (A, B) Time course of tumor growth in control, AF, and Et_3PAuI treated mice (4 animals per group), injected with A2780 cells sc. Treatments started when the volume of the masses reached 60 mm^3 (after 7 days from cells injection); the administration was performed by intraperitoneal injections (three times a week for two weeks of treatment) of AF and Et_3PAuI compounds (15 mg/kg). Data are reported as the mean ± SD

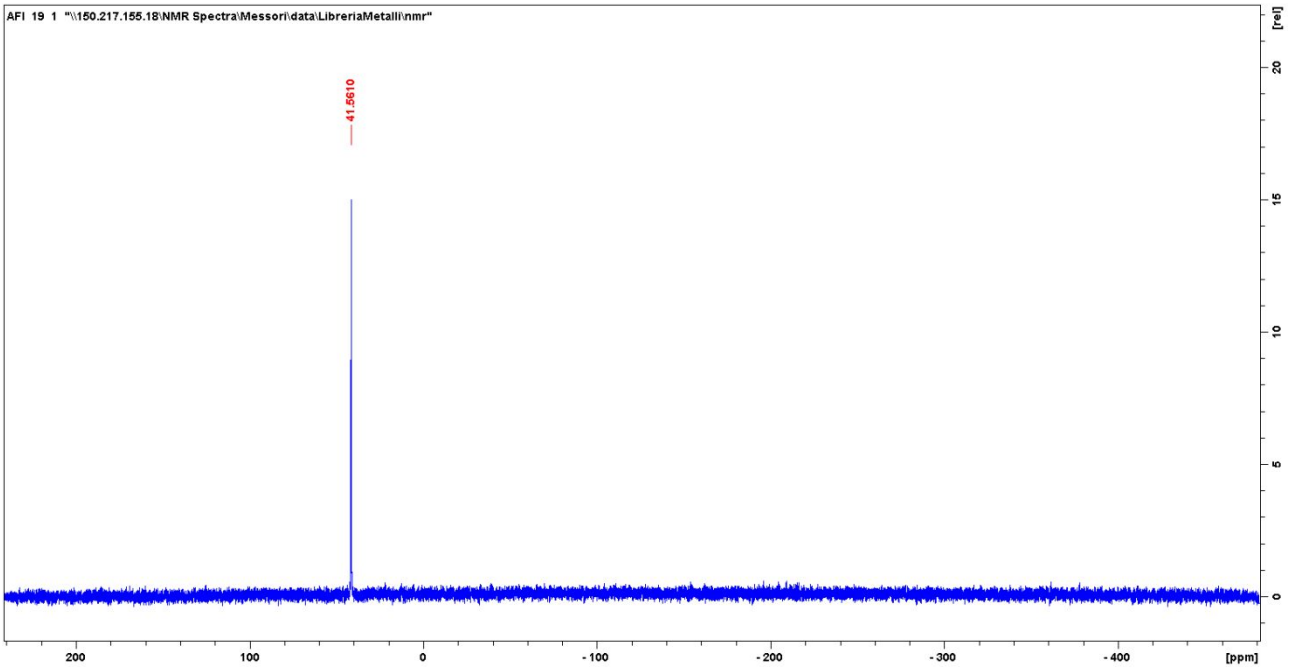


Figure S4: Spectrum recorded after incubation (24h) of $Au(PEt_3)I$ (3.8 mg) in 250µL MeOD + 250µL NaCl 100mM. Signal detected at 41.56 ppm.