

Replacement of the Thiosugar of Auranofin with Iodide Enhances the Anticancer Potency in a Mouse Model of Ovarian Cancer

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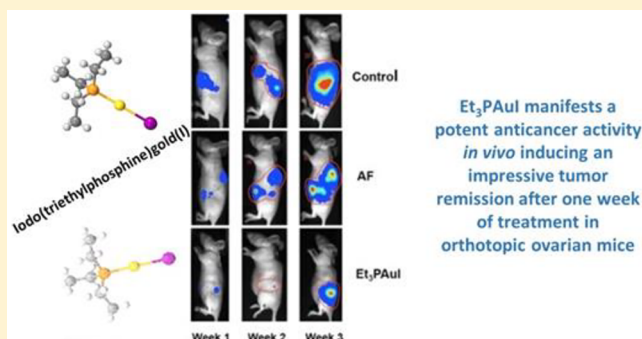
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Supporting Information

ABSTRACT: In recent years, a few successful attempts were made to repurpose the clinically approved antiarthritic gold drug, Auranofin (AF), as an anticancer agent. The present study shows that the iodo(triethylphosphine)gold(I) complex, (Et₃PAuI hereafter)—an AF analogue where the thiosugar ligand is simply replaced by one iodide ligand—manifests a solution chemistry resembling that of AF and exerts similar cytotoxic and proapoptotic effects on A2780 human ovarian cancer cells *in vitro*. However, when evaluated in a preclinical orthotopic model of ovarian cancer, Et₃PAuI produces a far superior anticancer action than AF inducing a nearly complete tumor remission. The highly promising *in vivo* performances here documented for Et₃PAuI warrant its further evaluation as a drug candidate for ovarian cancer treatment.

KEYWORDS: Auranofin, gold compounds, ovarian cancer, metal-based drugs, *in vivo* orthotopic model



Et₃PAuI manifests a potent anticancer activity *in vivo* inducing an impressive tumor remission after one week of treatment in orthotopic ovarian mice

Auranofin, a linear gold(I) complex bearing triethylphosphine and thioglucose tetraacetate as ligands, is an orally administered drug, in clinical use since 1985 for the treatment of rheumatoid arthritis. AF is generally reputed as a reasonably safe drug for human use, even for long chronic treatments.^{1,2} Beyond its clinically established antiarthritic properties, in the frame of the so-called drug repurposing strategies, AF has been extensively tested in recent years as a potential antiparasitic, antimicrobial, and anticancer agent with encouraging results.^{3–5} Of considerable interest are the antiproliferative properties of this compound that have been known since the early 1980s^{5–7} and were later assessed in the NCI 60 cancer cell line panel and in a few *in vivo* models.^{8–10} On the ground of its anticancer properties, AF in the course of the last years has entered three distinct clinical trials, two of them (ovarian and lung cancer) still ongoing and one (leukemia) closed.^{11–13} Results of these trials have not been disclosed yet. At the same time, considerable evidence has been gathered that a few specific biomolecular targets such as the enzyme thioredoxin

reductase, the proteasome system, the NF-κB pathway and other transcription factors, are crucially involved in mediating the cellular effects of AF as well of other gold (I/III) compounds.^{14–18}

It has been hypothesized, and then demonstrated, that the biological and pharmacological properties of AF might be kept and hopefully improved/modulated by replacing the thiosugar ligand with other ligands such as various halides. In principle, replacement of the thiosugar ligand with halide ligands may lead to an increased lipophilicity and an enhanced drug bioavailability and biodistribution. However, this modification should not affect substantially the drug's pharmacological profile, as the invariant [Et₃PAu⁺] moiety is assumed to be the

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“true pharmacophore” whereas the thiosugar ligand is believed to act mainly as a carrier ligand.¹⁸ Accordingly, the actions of iodo(triethylphosphine)gold(I) complex, **Et₃PAuI**, and **AF**, have been comparatively analyzed toward ovarian cancer models both *in vitro* and *in vivo*. **Et₃PAuI** is obtained by replacement of the thiosugar ligand with an iodide ligand according to the reported synthetic procedures¹⁸ (see [Supporting Information](#) for detailed synthesis and characterization).

The chemical structures of **AF** and **Et₃PAuI** are shown in [Figure 1](#).

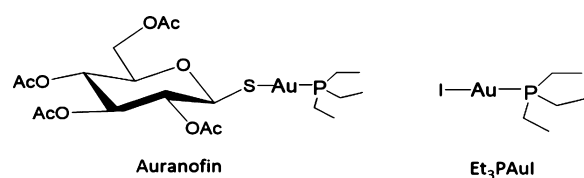


Figure 1. Chemical structures of Auranofin and iodo(triethylphosphine)gold(I).

Replacement of the thiosugar ligand with iodide results into a greatly increased lipophilic character (a logP value of 4.6 has been measured by ICP-OES in the previous work¹⁸) with respect to both **Et₃PAuCl** and **AF** (this latter compound has been previously described and used experimentally for rheumatoid arthritis treatment),¹⁹ for which logP values of 1.7 and 1.6 were measured, respectively.¹⁸ In spite of that, **Et₃PAuI** is still appreciably soluble in aqueous solutions, where it manifests a very high stability as documented by a previous systematic NMR analysis, carried out in different environments including biological media.^{18,20} As reported in the [Supporting Information](#) (see [Figure S4](#)), new ³¹P NMR results clearly demonstrate a high stability of **Et₃PAuI**, with no changes in the structure of the molecule, even in the presence of a large excess of sodium chloride (up to 100 mM). The documented stability in physiological-like conditions makes the compound an ideal candidate for biological testing.

The antiproliferative properties of **Et₃PAuI** compared to **AF** have been investigated *in vitro* toward A2780 human ovarian cancer cells. As displayed in [Table 1](#), both compounds produce

Table 1. Inhibitory Effects of AF, Et₃PAuI, and Cisplatin on A2780 Cells Following 72 h Drug Exposure As Determined by the Sulforhodamine B Assay^a

cell lines	IC ₅₀ (μM) ± SD		
	Et ₃ PAuI	Auranofin	cisplatin
A2780	0.394 ± 0.174	0.754 ± 0.139	1.53 ± 0.66
<i>n</i>	4	4	5
<i>p</i> ^b		0.0138	

^aFour independent experiments were performed. SD, standard deviation; *n*, number of independent experiments. ^b*t* test.

potent cytotoxic effects with IC₅₀ values falling in the high nanomolar range. **Et₃PAuI** is slightly more cytotoxic than **AF** and both result significantly more effective than cisplatin, the well-known anticancer metal compound. These results are consistent with those previously obtained in colon cancer cell lines for the same gold compounds and recently published literature¹⁸ further supporting the concept that the presence of the thiosugar ligand is not an essential requirement for the

cytotoxic action and that substantial differences in the cellular uptake, despite the difference in the logP value, between **Et₃PAuI** and **AF** are unlikely to occur. Remarkably, in agreement with a previous observation,¹⁸ no appreciable cytotoxic effects were detected for **Et₃PAuI** in normal cell lines, *i.e.*, human fibroblasts (HDF cell line) and human embryonic kidney cells (HEK293) over the concentration range 0–5000 nM.

Then, we examined whether **Et₃PAuI** and **AF** are able to induce apoptosis in A2780 cells; accordingly, we performed flow cytometry analysis of annexin V/propidium iodide-stained A2780 cells, treated with these two gold compounds, at the respective IC₅₀ values, for 72 h. As shown in [Figure S1](#), the percentage of apoptotic cells in **AF**- and **Et₃PAuI**-treated samples is significantly increased (control vs **AF**, *p* = 0.0121; control vs **Et₃PAuI**, *p* = 0.0107). In order to evaluate and quantitate apoptosis via caspase activity, we checked the activity of caspase 3 by FAM-FLICA Caspase-3 Assay Kit. The induction of apoptosis observed for **AF** and its iodide analogue may be related with the strong thioredoxin reductase inhibition potency. Indeed, the IC₅₀ values for the inhibition of the enzyme are generally in nice agreement with cytotoxicity values.¹⁸

The results collected evidenced that both gold compounds induce apoptosis through a caspase 3 activation, with **AF** being slightly more effective than **Et₃PAuI** (control vs **AF**, *p* = 0.0037; control vs **Et₃PAuI**, *p* = 0.04, see [Supporting Information](#)).

Et₃PAuI was subsequently evaluated *in vivo* in comparison to **AF** in two different ovarian cancer models, *i.e.*, a xenograft subcutaneous and an orthotopic model. Preliminarily, drug tolerability studies were carried out to assess the *in vivo* safety of **Et₃PAuI**. The single-dose tolerability was evaluated in athymic nude mice over a 3-week observation period. **Et₃PAuI** was administered by intraperitoneal (ip) injection using two different doses (20 and 40 mg/kg). Only a single administration was performed, followed by a 3-week observation period; animals were controlled daily, and no signs of sufferings such as weight loss, mobility reduction and appetite reduction were registered. In addition, a pilot study for gold biodistribution has been carried out and a generalized higher accumulation for **Et₃PAuI**, both in organs and in the peripheral blood, was highlighted (see [Supporting Information](#)). Based on the above observations, we can hypothesize that the detected differences in the *in vivo* activity of the two compounds could be due to their different lipophilic character leading to an enhanced bioavailability and more favorable pharmacokinetics for **Et₃PAuI**.

The xenograft model consisted of 1 × 10⁶ A2780 cells implanted subcutaneously (sc) in both flanks of the animals. The growth of the tumor masses was measured daily with caliper and the treatment started when the volume of the masses reached 60 mm³. The treatment was performed by ip injections (three times a week for 2 weeks of treatment) of **AF** and **Et₃PAuI**, both 15 mg/kg. The growth of the tumor masses was monitored over 21 days and time course is shown in [Figure S3A,B](#) respectively. At the end of the experiments, mice were sacrificed and the explanted masses measured; the volume (cm³) was calculated using the ellipsoid equation.²¹ Remarkably, treatment with both **Et₃PAuI** and **AF** was accompanied by a strong decrease of the tumor volume determined at the sacrifice ([Figure 2](#), control: 2.96 ± 0.06 vs **Et₃PAuI**: 0.56 ± 0.20, *p* < 0.01; control: 2.96 ± 0.06 vs **AF**:

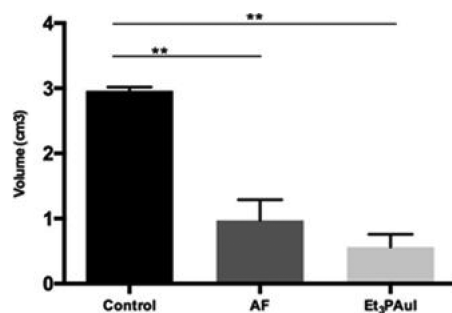


Figure 2. *In vivo* anticancer activity evaluation of gold compounds in a xenograft subcutaneous (sc) model of ovarian cancer. Mean volume of tumor masses obtained at the sacrifice, after 3 weeks from injection of A2780 cells. Data are reported as the mean \pm SD (* $p < 0.05$, ** $p < 0.01$). Further details are reported in the [Supporting Information](#).

0,79 \pm 0,27, $p < 0.01$). The above experimental protocol did not alter the behavior, vitality, or weight of treated mice.

Subsequently, the *in vivo* anticancer activity of Et₃PAuI versus AF was assessed in an orthotopic model of ovarian cancer, a model that better represents the cancer behavior and response to therapy. Thirty female athymic nude mice were injected in the ovarian bursa (5×10^5 cells each mouse) with A2780 cells transfected with the firefly luciferase gene (A2780-luc). Autopsy confirmed that ovarian cancer occurred in all animals and representative images of the procedure and of tumor masses are reported in [Figure 3A](#). The animals were divided in three groups of treatment (10 mice each): controls, AF treated and Et₃PAuI treated. The administration schedule was the following: mice received compounds (15 mg/kg) ip three times a week for 2 weeks. The rationale for such a treatment schedule relied on the schedule reported by Mirabelli et al.⁵ and on the above-reported biodistribution data ([Supporting Information](#)) showing that administration of 15 mg/kg gives rise to a peak peripheral blood concentration of 823.19 and 944.17 ng/mL, for AF and Et₃PAuI, respectively, after 24 h. Mice were monitored daily, and the luciferase activity signal was quantified weekly (representative panels are reported in [Figure 3B](#)).

Both gold compounds effectively reduced the tumor burden ([Figure 3B](#)); notably, Et₃PAuI is already very effective in antitumor activity after few doses (*i.e.*, 1 week of treatment) leading to an almost complete tumor remission, as evidenced by the drastic reduction of the bioluminescent signal (ROI levels) compared to the control group (control: 20260 \pm 4631 vs Et₃PAuI: 4729 \pm 2881; $p < 0,01$) (see graph in [Figure 3C](#)).

The tumor masses of the sacrificed animals after 3 weeks of treatment were collected and measured with caliper; the volume (cm³) was calculated using the ellipsoid equation even for this model.²¹ Most important, the tumor masses of the animals treated with Et₃PAuI showed a very strong reduction of the volume compared both to the control group (control vs Et₃PAuI $p < 0.05$) and to the AF group (AF: 6.86 \pm 1.57 vs Et₃PAuI: 1.00 \pm 0.70, $p < 0.01$) ([Figure 3D](#)). This latter evidence points out that Et₃PAuI is a more effective anticancer drug compared with AF in our animal model.

In conclusion, we have shown here that a specific structural modification of the repurposed gold(I) drug AF, now under consideration in clinical trials for cancer treatment, *i.e.*, the substitution of the thiosugar ligand with iodide, affords a distinct gold(I) complex Et₃PAuI, whose anticancer profile *in vitro* roughly resembles that of AF. This confirms that, even in

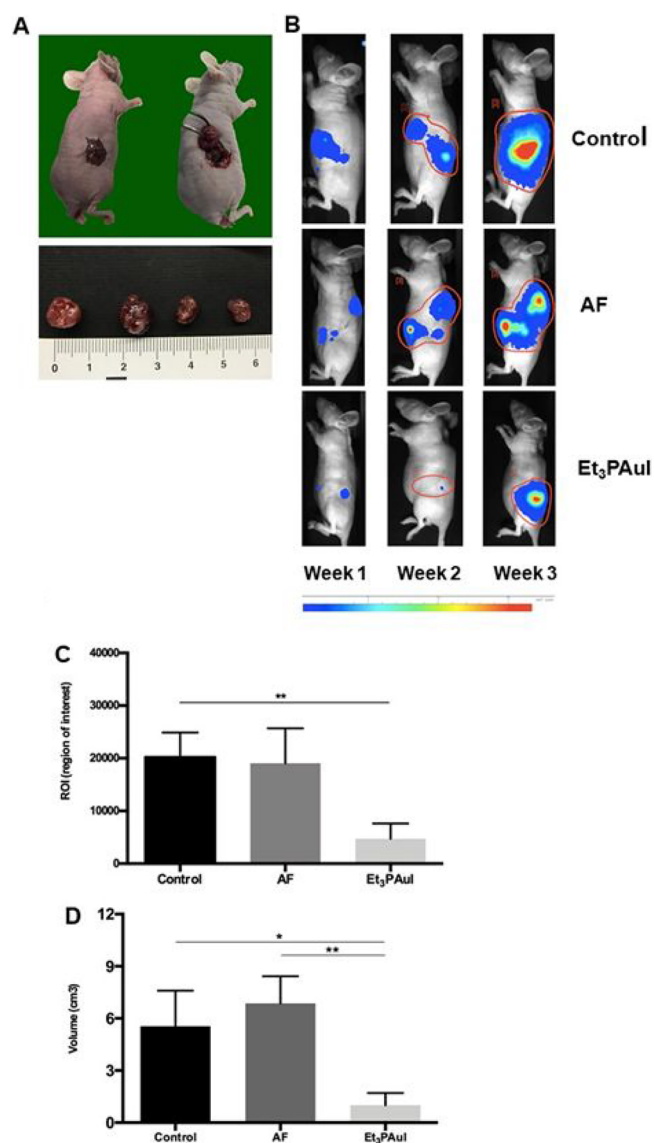


Figure 3. (A) Orthotopic model establishment: injection of 1×10^6 A2780-luc cells in the ovary bursa of nude mice. Representative macroscopic images of ovarian tumor masses obtained are showed in the bottom panels. (B) Representative pseudocolor BLI images tracking A2780-luc cells emission in mice, 7, 14, and 21 days after A2780-luc injection. Color bar represents light intensity levels reported as counts per minute (cpm). (C) Levels of ROI after 1 week of treatment; data are reported as mean of each group ($n = 10$ animals/group) \pm SD (** $p < 0.01$). (D) Volume of tumor masses (mean \pm SD) at the sacrifice after 3 weeks from injection of A2780-luc cells (control group, $n = 8$; AF, $n = 5$; Et₃PAuI, $n = 5$). * $p < 0.05$, ** $p < 0.01$. Further details are reported in the [Supporting Information](#).

the present ovarian cancer model, the thiosugar ligand is not mechanistically indispensable and that the [Et₃PAu]⁺ moiety is likely to be the true pharmacophore. Very interestingly, in our *in vivo* models, Et₃PAuI, has turned out to be far more effective than AF in contrasting tumor growth. Particularly impressive are the results and the differences between AF and Et₃PAuI observed in the orthotopic model of ovarian cancer. Et₃PAuI is found to induce a very large tumor regression after only 1 week of treatment, as pointed out by the decrease of the bioluminescent signal. A conspicuous shrinkage of the tumor

is still evident after 3 weeks. The enhanced anticancer activity observed in this orthotopic model might well arise from the far greater lipophilicity of Et_3PAuI , which might lead to a more favorable pharmacokinetics and biodistribution. Notably, the improved *in vivo* anticancer activity of Et_3PAuI over **AF** does not bring about any increased systemic toxicity, making Et_3PAuI a promising new drug candidate. In view of its far superior efficacy in animal models, Et_3PAuI merits a more extensive preclinical testing as it might be a valuable alternative to **AF** in cancer clinical trials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.9b00007.

Synthesis and purity of Et_3PAuI , logP determinations, apoptosis plots; cell death and caspase activity; cell growth inhibition studies (sulforhodamine B assay); tolerability studies; biodistribution studies; *in vivo* anticancer activity evaluation xenograft subcutaneous models plots; NMR stability (PDF)

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Notes

The authors declare no competing financial interest.

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