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Human Serum Albumin-Delivered $[Au(PEt_3)]^+$ Is a Potent Inhibitor of T Cell Proliferation

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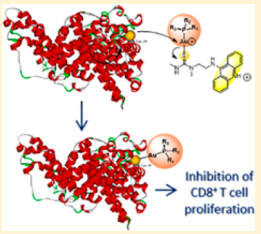
Supporting Information

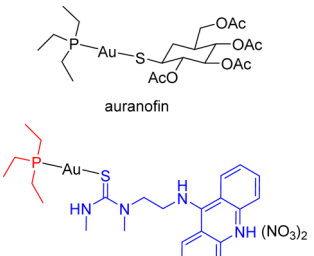
ABSTRACT: Using a modular library format in conjunction with cell viability (MTS) and flow cytometry assays, 90 cationic complexes $[AuPL]^{n+}$ (**P** = phosphine ligand; **L** = thiourea derivative or chloride) were studied for their antiproliferative activity in $CD8^+$ T lymphocyte cells. The activity of the compounds correlates with the steric bulk of the phosphine ligands. Thiourea serves as a leaving group that is readily replaced by cysteine thiol (NMR, ESI-MS). Taking advantage of selective thiourea ligand exchange, the fragments $[Au(PEt_3)]^+$ and [Au(JohnPhos)]$^+$ (JohnPhos = 1,1′-biphenyl-2-yl)di-*tert*-butylphosphine) in compounds **1** and **2** were transferred to recombinant human serum albumin (rHSA). PEt_3 promoted efficient modification of Cys34 in HSA (**HSA-1**), whereas use of bulky JohnPhos as a carrier ligand led to serum protein nonspecifically modified with multiple gold adducts (**HSA-2**) (Ellman's test, ESI-TOF MS). **HSA-1**, but not **HSA-2**, strongly inhibits T cell proliferation at nanomolar doses. The potential role of HSA as a delivery vehicle in gold-based autoimmune disease treatment is discussed.



KEYWORDS: *Gold phosphine complexes, human serum albumin, inhibitor screening, T cell proliferation*

Gold(I)-based inorganic salts and coordination compounds have a long history of medical uses, such as treatment of tuberculosis (TB) (sanocrysin) and rheumatoid arthritis (RA, auranofin).[1,2] Because of their poor tolerability, injectable and oral gold(I) drugs are now only administered as second-line disease-modifying antirheumatic drugs (DMARD) in RA patients who have not responded to prior anti-inflammatory treatments.[1] Gold(I) drugs are reactive electrophiles that readily undergo ligand exchange reactions with proteins and enzymes, primarily with cysteine (Cys) and selenocysteine (Sec) residues.[1,3] Binding to, and inhibition of, multiple targets have been implicated in the mechanism of gold(I) drugs during various stages of RA (initiation, inflammation, tissue injury). These include lysosomal and extracellular cathepsin proteases involved in antigen processing and matrix degradation in diseased tissues, respectively.[4,5] Gold(I) complexes, especially those bearing lipophilic phosphine (PR_3) ligands, have also shown potent antiproliferative activity in cancer cell lines and antitumor activity in vivo. Auranofin (Chart 1) is currently being evaluated for the treatment of chronic lymphocytic leukemia (CLL) in phase II clinical trials.[6] The mechanism of action of gold−phosphine compounds is thought to involve inhibition of the mitochondrial and cytoplasmic thioredoxin/thioredoxin reductase (Trx/TrxR) systems, triggering oxidative stress and cell death.[7,8] Like other metallopharmaceuticals, these compounds often have a narrow therapeutic window because they fail to discriminate between cancer cells and normal cells. To address the unfavorable drug-like properties of gold(I) compounds, more tolerable entities based on new ligand and prodrug designs, as well as targeted forms of delivery have been devised.[7,9]

Chart 1. Structures of Gold−Phosphines



To identify new suppressors of T lymphocyte activation, proliferation, and function during undesired autoimmune

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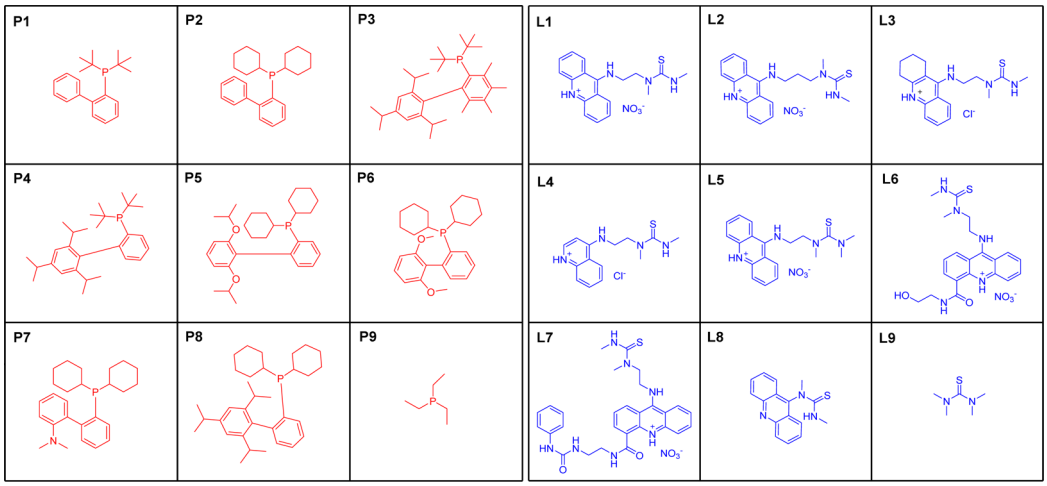
Chart 2. Phosphines and Thioureas Used to Assemble a Modular Library of Gold(I) Complexes

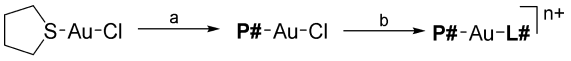


response, such as systemic lupus erythematosus (SLE), we recently investigated the immunosuppressive activity of mixed gold(I)−phosphine−thiourea complexes.[10] In these compounds gold is linearly coordinated by the thiourea sulfur of a 9-aminoacridine ligand or structurally related derivatives, and a trans-ligand including (pseudo)halides and phosphines.[11] Gold(I) complexes of the acridinylthiourea derivative 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea (ACRAMTU) were originally designed as derivatives of Pt-ACRAMTU, the prototype of a potent class of DNA-targeted platinum−acridine hybrid anticancer agents.[12,13] The dicationic phosphine-modified complex, $[Au(PEt_3)(ACRAMTU)](NO_3)_2$ (**1**), inhibited T cell proliferation at concentrations an order of magnitude lower than had previously been observed in human cancer cells by disrupting the mitochondrial Trx/TrxR system.[10] It also demonstrated potent immunosuppressive activity in mice but was mildly toxic to the test animals when injected intraperitoneally.[10] Targeting the redox balance of T cells with gold-based therapies to treat autoimmune disorders is an unexplored concept. In a search for new derivatives with improved selectivity and reduced toxicity, we have now investigated structure−activity relationships of this pharmacophore by screening 90 gold(I)−phosphine analogues using a modular library format. Specifically, we wanted to assess how bulky phosphines affect the reactivity and antiproliferative activity of this chemotype. We discovered that transfer of $[Au(PEt_3)]^+$ in one of the most potent derivatives to Cys34 in human serum albumin (HSA) via thiourea−thiol exchange produced a metal-modified protein that strongly inhibited T cell proliferation.

Using a previously established synthetic scheme,[10] we generated mixed gold(I)−phosphine−thiourea complexes from nine phosphine ligands (**P**) and nine thiourea ligands (**L**) (Chart 2). The reactions involved substitution of the tetrahydrothiophene (tht) and chloro ligands in [AuCl(tht)] in two consecutive reactions steps by **P** and **L** (Scheme 1).

The nine precursor complexes [AuCl**P#**] were isolated and fully characterized prior to assembling the second step (Scheme 1, step *b*) on 96-well plates using microscale reactions. Complete conversion to the desired complexes $[Au\mathbf{P\#L\#}]^{n+}$ (# = 1−9; *n* = 1, 2; denoted below as **P#−L#**) was confirmed by ^{31}P NMR spectroscopy for selected samples. Reactions were used directly as stock solutions in a colorimetric cell viability assay. To produce the desired structural diversity in this set of

Scheme 1. Library Assembly for Screening Gold(I)−Phosphine−Thiourea Complexes[a]



81 micro-scale reactions

[a]Reagents and conditions: (a) 1.1 equiv of **P1−P9**, DCM, r.t., 1 h; (b) 1.1 equiv of **L1−L9**, DMF, r.t., 30 min.

compounds, eight electron-rich dialkyl−aryl phosphines[14] of varying steric hindrance ("Buchwald phosphines", **P1−P8**) were introduced in place of PEt_3 (**P9**). The goal of replacing PEt_3 in compound **1** with bulkier phosphine ligands was to enhance the metal's selectivity (and potentially tolerability) by reducing its chemical reactivity. As ligands trans to phosphine, eight thioureas containing *N*-heterocyclic chromophores of varying basicity, borrowed from our extensive library of ACRAMTU derivatives, were introduced: 9-aminoacridines **L1**, **L2**, **L5** ($pK_a \approx 9-10$, DNA intercalating),[15,16] 1,2,3,4-tetrahydro-9-aminoacridine (tacrin) **L3** and -4-aminoquinoline **L4** ($pK_a \approx 9-10$, nonintercalating),[17,18] 9-aminoacridine-4-carboxamides **L6** and **L7** ($pK_a \approx 8$),[19] and 9-substituted **L8** ($pK_a \approx 4$, nonintercalating).[20] In addition, the simple derivative 1,1,3,3-tetramethylthiourea (tmtu, **L9**) was also studied. The rationale behind testing the current set of thioureas was to establish the structural requirements for **L** as a carrier group. While ACRAMTU-based ligands alone proved to be significantly less cytotoxic than their Pt and Au complexes in all solid and hematological tumor models studied,[11,16] one goal of the screen was to establish whether the intercalator can be replaced with non-DNA binding, less genotoxic moieties. For comparison, the nine chlorogold(I) precursors, [AuCl**P#**] (with **L10** = chloride), were also included in the screen.

To assess the inhibitory effect of the 90 gold(I)−phosphine analogues, purified naïve $CD8^+$ T cells were treated with test compounds at concentrations of 150 and 600 nM for 60 min at 37 °C, stimulated with αCD3 and αCD28 antibodies, and incubated for another 72 h. The specific increase in T cell proliferation post stimulation in the presence of test compound relative to untreated control was then determined using a colorimetric (MTS) cell viability assay. Analysis of the inhibition data across the entire library shows that the activity of the compounds is dominated by the nature of the phosphine

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ligands (**P**). At a concentration of 150 nM (Figure 1), PEt_3 (**P9**) and the sterically least hindered phosphines among the

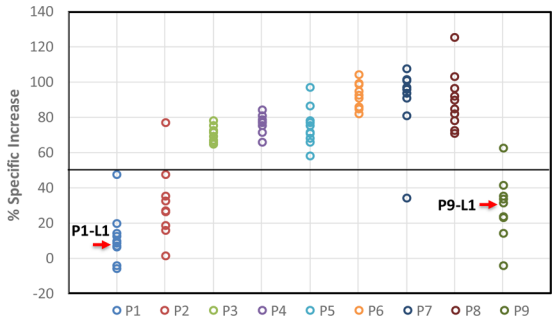

Figure 1. Prescreening results for the 90 gold(I)−phosphine−thiourea complexes at a fixed concentration of 150 nM of test compound in a modular high-throughput format in $CD8^+$ T cells. Compound activity is expressed as percent increase in metabolic activity relative to isotype vehicle-treated control cells. Inhibition data has been sorted by phosphine component. Each circle represents a specific library member, **P#−L#**. The two compounds selected for further study, **P1−L1** and **P9−L1**, are highlighted with arrows.

dialkyl−aryl derivatives, **P1** and **P2**, produced the most active complexes with inhibition levels of greater than 50% compared to the vehicle-treated control. By contrast, the compounds modified with **P3−P8**, which contain substituted biphenyl moieties, showed significantly reduced activity. Within each of the nine sets of complexes the inhibitory activity varies depending on the nature of **L**, suggesting that the thiourea ligands modulate, to some extent, the activity of the complexes (Figure 1). However, no trends in the relative contribution to the overall activity from **L** across the nine sets of complexes seem to exist and no collectively applicable structure−activity relationships (SAR) emerged. At 600 nM test compound, all derivatives except for those containing the bulkiest phosphines (**P3**, **P4**, **P5**, and **P8**) lead to complete suppression of T cell proliferation (data not shown). By contrast, complexes based on the latter four phosphines showed no dose response in this assay.

To shed light on the reactivity of the pharmacophore with biologically relevant thiols, we studied the interactions of two derivatives with glutathione (GSH): **P9−L1** (compound **1**), which was confirmed to be highly active at submicromolar inhibitory concentrations consistent with those reported previously,[10] and **P1−L1** (compound **2**) containing 1,1′-biphenyl-2-yl)di-*tert*-butylphosphine ("JohnPhos") ligand, which also demonstrated exquisite activity in the prescreen (Figure 1). (**P1−L1** was resynthesized on a preparative scale and fully characterized; see the Supporting Information.) ^{31}P NMR spectra recorded of the reactions of **P9−L1** and **P1−L1** with 2 equiv (excess) of GSH in PBS buffer (pH 7.6) show a single new peak for each complex at 38.4 and 63.5 ppm, respectively, consistent with a mixed phosphine/thiol coordination of gold(I) in both cases.[21] Likewise, positive-ion electrospray ionization mass spectra (ESI MS) of the reaction mixtures confirm that the thiourea ligands in **P9−L1** and **P1−L1** have been replaced by GSH sulfur (Supporting Information). Only in reactions of **P9−L1** were trace amounts of free phosphine (oxide) observed, corroborating the selectivity of the ligand exchange reaction.

Next, we studied the reactions of compounds **1** and **2** with recombinant human serum albumin (rHSA). HSA, which contains a single reactive free thiol, Cys34, is now being used as a versatile injectable delivery platform to improve the safety and efficacy of therapeutics and diagnostics.[22] Because of its well-documented high affinity for gold(I) agents,[1] we wanted to address whether the serum protein is able to mediate the inhibitory properties of our gold(I)−phosphines in T cells, or if it merely serves as a nucleophilic sink that traps the thiophilic metal. The procedure involved (i) treatment of rHSA with gold compound, (ii) determining the level of gold-modified cysteine using Ellman's test, (iii) size-exclusion chromatography to remove released ACRAMTU ligand and desalt the sample, (iv) quantification of total protein recovered using Bradford assay, and (v) characterization of the products by electrospray ionization time-of-flight mass spectrometry (ESI-TOF MS, Figure 2). Compound **1**, containing the sterically less hindered

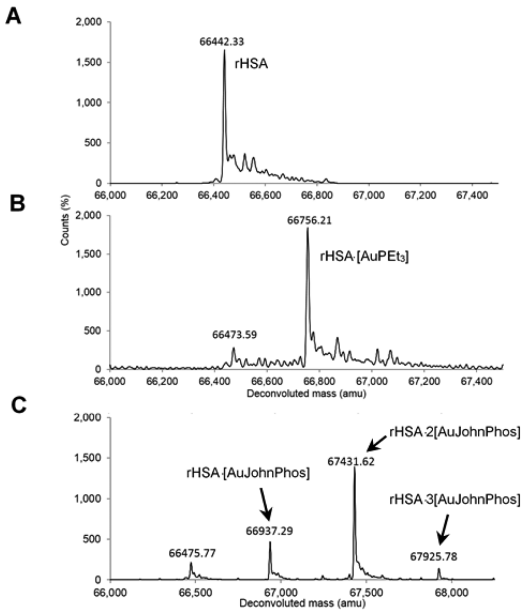

Figure 2. Deconvoluted ESI-TOF mass spectra of rHSA (calculated molecular weight based on amino acid sequence is 66 437 Da) (A), and rHSA reacted with 1.33 equiv of $[Au(PEt_3)]^+$ (MW 315.13) (B) or 1.75 equiv of $[Au(JohnPhos)]^+$ (MW 495.38) (C).

phosphine ligand PEt_3, was able to modify >85% of the Cys34 free thiol groups present in the sample of rHSA. Under the same conditions, compound **2** containing bulky "JohnPhos" produced a maximum of 40% gold-phosphine modified Cys34. Attempts to increase the yield of the latter adduct by applying a larger excess of **2** were unsuccessful and instead led to rHSA molecules containing multiple $[AuJohnPhos]^+$ fragments (Figure 2C). These findings suggest that reaction of the bulky $[Au(JohnPhos)]^+$ moiety in compound **2** with Cys34 may be sterically disfavored and result in nonselective transfer of gold to alternative, more accessible amino acid residues, such as methionine or histidine.[23]

Using carboxyfluorescein succinimidyl ester (CFSE) labeled $CD8^+$ cells in conjunction with flow cytometry analysis, we compared the effects of compound **1**, compound **2**, rHSA·$[Au(PEt_3)]$ (**HSA-1**), and rHSA·*n*[AuJohnPhos] (**HSA-2**) on T cell proliferation. Tetrazolium-to-formazan reduction in colorimetric cell viability assays measures cell metabolic activity and often under- or overestimates the antiproliferative potential of drugs.[24] To account for this shortcoming and to determine the true number of viable, proliferating T cells, we used an assay

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based on direct cell counting. Purified, labeled $CD8^+$ cells were treated with vehicle, rHSA, gold complexes, or the corresponding modified protein samples for 60 min, activated with the appropriate antibodies for 72 h, and analyzed for CFSE fluorescence (Figure 3). When treated with 400 nM compound

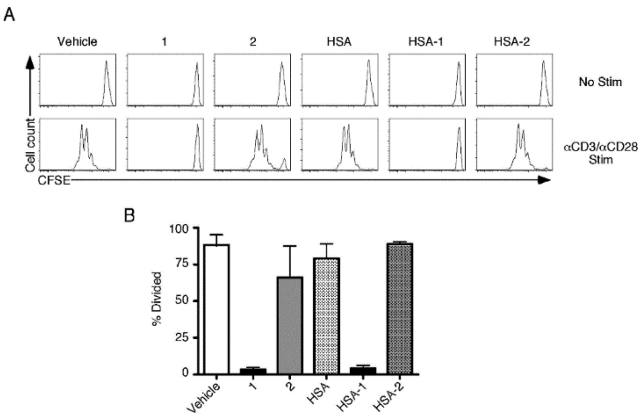

Figure 3. Pretreatment with compound **1** or **HSA-1** effectively inhibits T cell activation and proliferation. (A) Results of the flow cytometry experiments. Effect of 400 nM **1**, **2**, HSA, **HSA-1**, and **HSA-2** on CFSE fluorescence in unstimulated $CD8^+$ cells and $CD8^+$ cells activated with αCD3 and αCD28 antibodies. (B) Percent proliferating cells based on loss of CFSE fluorescence in histograms for vehicle and treatment groups. The mean and standard deviation for three independent experiments are plotted. For microscopic images of cells treated with **HSA** and **HSA-1**, see the Supporting Information.

1 or **HSA-1** at the same concentration (based on protein), virtually no change in the fluorescence signal was observed after stimulation of the cells with antibodies, indicating that proliferation of activated T cells was completely inhibited. By comparison, compound **2** was a significantly weaker inhibitor than expected from the prescreening data, potentially confirming the limitations of the MTS assay. **HSA-2** and HSA alone had no effect on cell proliferation.

The current study sheds light on the SAR of a class of mixed-ligand gold(I) complexes and on their mechanism of action. Using a modular screening platform, we demonstrated that the ability of the pharmacophore to reduce T cell viability depends on the steric hindrance of the phosphine ligand. $[Au(PEt_3)]^+$, the classical mitochondriotropic cation in gold(I)-based drugs, inhibits proliferation at the submicromolar concentrations tested, both as a component of compound **1** and when delivered as **HSA-1**. ACRAMTU appears to serve as a readily exchangeable carrier ligand, similar to the thioglucose ligand in auranofin,[25] and does not appear to be essential for the inhibitory activity of **1** in $CD8^+$ cells. We took advantage of the thiourea−thiol ligand exchange chemistry to modify cysteine thiol in rHSA with $[Au(PEt_3)]^+$. ACRAMTU proves to be an ideal *transfer ligand* for generating gold(I)-modified protein: (i) the acridine derivative is protonated at physiological pH, rendering the compounds 2+ charged and highly water-soluble; (ii) ACRAMTU has a UV−vis signature at 413 nm, which allows easy detection and precise spectrophotometric quantification; (iii) $[Au(PEt_3)]^+$ transfer from ACRAMTU to cysteine thiol proceeds quantitatively and without generating side products. Unlike auranofin's thioglucose ligand, ACRAMTU released in the process does not compete with thiol binding nor reverse it, or cause decomposition of the $[Au(PEt_3)]^+$ moiety.[26]

One important role of the lipophilic phosphine carrier ligands is to facilitate uptake across membranes and, as a component of cationic Au complex ions, lead to gold accumulation in the mitochondria. The extent to which gold drugs are mobilized from plasma proteins, with which they rapidly associate while in circulation, and the exact mechanisms of intracellular gold trafficking and targeting of Sec residues in TrxR are unknown. A critical observation in our system is that **HSA-1** maintains the same high activity as the parent compound, **1**. This is an important finding since previous clonogenic cell culture assays reported by Snyder et al. have shown that serum albumin reduces auranofin's cytotoxicity,[27] presumably by sequestering the drug's active gold component. However, the same study also provided support for a mechanism by which the $[Au(PEt_3)]^+$ moiety of auranofin is exchanged between, and transported by, multiple membrane-bound and cytoplasmic Cys-containing proteins until it reaches its pharmacological target. Prior work by Pellom et al.[28] has demonstrated that activated T cells upregulate surface free thiols (SFT) following stimulation prior to entering S phase in the cell cycle. Thus, a shuttle mechanism involving membrane thiols may provide a cellular uptake mechanism for $[Au(PEt_3)]^+$ in **HSA-1** that would not require endocytosis of HSA-gold. Inefficient transfer of gold from ACRAMTU to Cys34 in HSA, and from HSA to SFT, caused by the bulky JohnPhos group may explain why compound **2** and **HSA-2** are relatively less effective mediators of T cell inhibition. Mobilization of $[Au(PR_3)]^+$ from HSA and transfer to cellular targets appears to be an important prerequisite for antiproliferative activity in T cells. The results from this study suggest that HSA may not only play an important role as an endogenous mediator of T cell inhibition but may also have a potential use as a biocompatible delivery system for the systemic treatment of autoimmune diseases with gold-based drugs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00142.

Experimental procedures, details of product characterization, and supplementary figures (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Berners-Price, S. J.; Filipovska, A. Gold compounds as therapeutic agents for human diseases. *Metallomics* **2011**, *3*, 863−873.
(2) Benedek, T. G. The history of gold therapy for tuberculosis. *J. Hist. Med. Allied Sci.* **2004**, *59*, 50−89.
(3) Zou, T. T.; Lum, C. T.; Lok, C. N.; Zhang, J. J.; Che, C. M. Chemical biology of anticancer gold(III) and gold(I) complexes. *Chem. Soc. Rev.* **2015**, *44*, 8786−8801.
(4) Burmester, G. R. Molecular mechanisms of action of gold treatment in rheumatoid arthritis - an update. *Z. Rheumatol.* **2001**, *60*, 167−173.
(5) Han, S.; Kim, K.; Kim, H.; Kwon, J.; Lee, Y. H.; Lee, C. K.; Song, Y.; Lee, S. J.; Ha, N.; Kim, K. Auranofin inhibits overproduction of pro-inflammatory cytokines, cyclooxygenase expression and PGE(2) production in macrophages. *Arch. Pharmacal Res.* **2008**, *31*, 67−74.
(6) Identifier NCT01419691; Phase I and II Study of Auranofin in Chronic Lymphocytic Leukemia (CLL). https://clinicaltrials.gov/ct2/show/NCT01419691.
(7) Gandin, V.; Fernandes, A. P. Metal- and Semimetal-Containing Inhibitors of Thioredoxin Reductase as Anticancer Agents. *Molecules* **2015**, *20*, 12732−12756.
(8) Gandin, V.; Fernandes, A. P.; Rigobello, M. P.; Dani, B.; Sorrentino, F.; Tisato, F.; Bjornstedt, M.; Bindoli, A.; Sturaro, A.; Rella, R.; Marzano, C. Cancer cell death induced by phosphine gold(I) compounds targeting thioredoxin reductase. *Biochem. Pharmacol.* **2010**, *79*, 90−101.
(9) Kouodom, M. N.; Ronconi, L.; Celegato, M.; Nardon, C.; Marchio, L.; Dou, Q. P.; Aldinucci, D.; Formaggio, F.; Fregona, D. Toward the selective delivery of chemotherapeutics into tumor cells by targeting peptide transporters: tailored gold-based anticancer peptidomimetics. *J. Med. Chem.* **2012**, *55*, 2212−2226.
(10) Langston, P. K.; Yang, M.; Bierbach, U.; Parsonage, D.; Poole, L. B.; Price, M. J.; Grayson, J. M. Au-ACRAMTU-PEt3 Alters Redox Balance To Inhibit T Cell Proliferation and Function. *J. Immunol.* **2015**, *195*, 1984−1994.
(11) Eiter, L. C.; Hall, N. W.; Day, C. S.; Saluta, G.; Kucera, G. L.; Bierbach, U. Gold(I) analogues of a platinum-acridine antitumor agent are only moderately cytotoxic but show potent activity against Mycobacterium tuberculosis. *J. Med. Chem.* **2009**, *52*, 6519−6522.
(12) Liu, F.; Suryadi, J.; Bierbach, U. Cellular Recognition and Repair of Monofunctional-Intercalative Platinum-DNA Adducts. *Chem. Res. Toxicol.* **2015**, *28*, 2170−2178.
(13) Suryadi, J.; Bierbach, U. DNA Metalating-Intercalating Hybrid Agents for the Treatment of Chemoresistant Cancers. *Chem. - Eur. J.* **2012**, *18*, 12926−12934.
(14) Surry, D. S.; Buchwald, S. L. Dialkylbiaryl Phosphines in Pd-Catalyzed Amination: A User's Guide. *Chem. Sci.* **2011**, *2*, 27−50.
(15) Ackley, M. C.; Barry, C. G.; Mounce, A. M.; Farmer, M. C.; Springer, B. E.; Day, C. S.; Wright, M. W.; Berners-Price, S. J.; Hess, S. M.; Bierbach, U. Structure-activity relationships in platinum-acridinylthiourea conjugates: effect of the thiourea nonleaving group on drug stability, nucleobase affinity, and in vitro cytotoxicity. *JBIC, J. Biol. Inorg. Chem.* **2004**, *9*, 453−461.
(16) Martins, E. T.; Baruah, H.; Kramarczyk, J.; Saluta, G.; Day, C. S.; Kucera, G. L.; Bierbach, U. Design, synthesis, and biological activity of a novel non-cisplatin-type platinum-acridine pharmacophore. *J. Med. Chem.* **2001**, *44*, 4492−4496.
(17) Pickard, A. J.; Liu, F.; Bartenstein, T. F.; Haines, L. G.; Levine, K. E.; Kucera, G. L.; Bierbach, U. Redesigning the DNA-targeted chromophore in platinum-acridine anticancer agents: a structure-activity relationship study. *Chem. - Eur. J.* **2014**, *20*, 16174−16187.
(18) Takla, P. G.; Dakas, C. J. A Study of Interactions of Chloroquine with Ethanol, Sugars and Glycerol Using Ultraviolet Spectrophotometry. *Int. J. Pharm.* **1988**, *43*, 225−232.
(19) Guddneppanavar, R.; Saluta, G.; Kucera, G. L.; Bierbach, U. Synthesis, biological activity, and DNA-damage profile of platinum-threading intercalator conjugates designed to target adenine. *J. Med. Chem.* **2006**, *49*, 3204−3214.
(20) Baruah, H.; Rector, C. L.; Monnier, S. M.; Bierbach, U. Mechanism of action of non-cisplatin type DNA-targeted platinum anticancer agents: DNA interactions of novel acridinylthioureas and their platinum conjugates. *Biochem. Pharmacol.* **2002**, *64*, 191−200.
(21) Ahmad, S.; Isab, A. A. ^{13}C, ^{31}P and ^{15}N NMR studies of the ligand exchange reactions of auranofin and chloro(triethylphosphine)-gold(I) with thiourea. *J. Inorg. Biochem.* **2002**, *88*, 44−52.
(22) Elsadek, B.; Kratz, F. Impact of albumin on drug delivery - New applications on the horizon. *J. Controlled Release* **2012**, *157*, 4−28.
(23) Zou, J.; Taylor, P.; Dornan, J.; Robinson, S. P.; Walkinshaw, M. D.; Sadler, P. J. First crystal structure of a medicinally relevant gold protein complex: Unexpected binding of $[Au(PEt_3)]^+$ to histidine. *Angew. Chem., Int. Ed.* **2000**, *39*, 2931−2934.
(24) Stepanenko, A. A.; Dmitrenko, V. V. Pitfalls of the MTT assay: Direct and off-target effects of inhibitors can result in over/underestimation of cell viability. *Gene* **2015**, *574*, 193−203.
(25) Ecker, D. J.; Hempel, J. C.; Sutton, B. M.; Kirsch, R.; Crooke, S. T. Reactions of the Metallodrug Auranofin [(1-Thio-Beta-D-Glucopyranose-2,3,4,6-Tetraacetato-S) (Triethylphosphine)Gold] with Biological Ligands Studied by Radioisotope Methodology. *Inorg. Chem.* **1986**, *25*, 3139−3143.
(26) Shaw, C. F.; Isab, A. A.; Hoeschele, J. D.; Starich, M.; Locke, J.; Schulteis, P.; Xiao, J. Oxidation of the Phosphine from the Auranofin Analog, Triisopropylphosphine(2,3,4,6-Tetra-O-Acetyl-1-Thio-Beta-D-Glucopyranosato-S)Gold(I), Via a Protein-Bound Phosphonium Intermediate. *J. Am. Chem. Soc.* **1994**, *116*, 2254−2260.
(27) Snyder, R. M.; Mirabelli, C. K.; Crooke, S. T. Cellular-Association, Intracellular-Distribution, and Efflux of Auranofin Via Sequential Ligand-Exchange Reactions. *Biochem. Pharmacol.* **1986**, *35*, 923−932.
(28) Pellom, S. T.; Michalek, R. D.; Crump, K. E.; Langston, P. K.; Juneau, D. G.; Grayson, J. M. Increased cell surface free thiols identify effector CD8+ T cells undergoing T cell receptor stimulation. *PLoS One* **2013**, *8*, e81134.

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