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A cytotoxic and cytostatic gold(III) corrole

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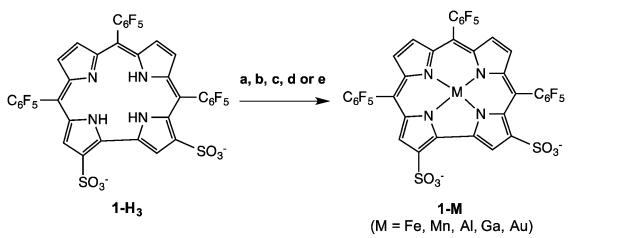
We have synthesized and characterized a water-soluble gold(III) corrole (1-Au) that is highly toxic to cisplatin-resistant cancer cells. Relative to its 1-Ga analogue, axial ligands bind only weakly to 1-Au, which likely accounts for its lower affinity for human serum albumin (HSA). We suggest that the cytotoxicity of 1-Au may be related to this lower HSA affinity.

The seminal report on the scalable synthesis of the first corrole that is stable in its free-base form, 5,10,15-trispentafluorophenylcorrole,[1] was followed one year later by the introduction of a positively charged derivative as an anti-cancer agent.[2] More recent health-related research has focused on the iron(III), manganese(III), aluminium(III), and gallium(III) complexes of 2,17-bis-sulfonato-5,10,15-trispentafluorophenylcorrole (Scheme 1: **1-Fe**, **1-Mn**, **1-Al**, and **1-Ga**).[3–5]

These well characterized amphipolar complexes form tight conjugates with a variety of proteins that have been used as trafficking and cell penetrating vehicles in physiologically relevant media.[6] Closed-shell metallocorroles have been employed for optical imaging, whereas certain others exhibit pro-oxidant, anti-oxidant, and cell-killing properties, as follows: **1-Mn** catalyzes the peroxidase-like enantioselective oxidation of sulfides by hydrogen peroxide,[7] **1-Fe** is the best synthetic catalyst reported to date for the catalase-like decomposition of hydrogen peroxide to water and molecular oxygen,[8] **1-Al** is a versatile optical imaging agent,[9] and **1-Ga** is cytotoxic to breast cancer cells.[10] What distinguishes corroles from most other ligands is that none of its metal-ion derivatives is demetallated under physiologically relevant conditions.[11]



		Ref.
a) $FeCl_2$, pyridine, reflux	1-Fe	18
b) $Mn(OAc)_2$, DMF, reflux	1-Mn	19
c) $AlMe_3$, toluene/pyridine, 0°C	1-Al	20
d) $GaCl_3$, pyridine, reflux	1-Ga	19
e) $Au(OAc)_3$, pyridine, 25°C	1-Au	this work

Scheme 1 Synthesis of **1-Au** and previously reported metal complexes of **1-H$_3$**.

During the last decade, several gold(I) and gold(III) compounds were reported to manifest impressive antitumor properties, making up a promising class of experimental anticancer agents.[12] Among these agents, a cytotoxic gold(III) porphyrin complex, [Au(TPP)]Cl, was found to be particularly active, as it blocked the self-renewal ability of cancer stem-like cells.[13] Most notably, [Au(TPP)]Cl was neither demetallated nor reduced during circulation in biological media. This key finding stimulated our interest in gold(III) corroles, which are redox-stable, low-spin square planar complexes, and similar to gold(III) porphyrins and platinum(II) anticancer drugs such as cisplatin.[14]

Here we report a water-soluble gold(III) complex of 2,17-bis-sulfonato-5,10,15-trispentafluorophenylcorrole (**1-Au**): we have studied its association with human serum albumin (HSA), the most abundant (and potential drug transporting) protein present in plasma.[15] In the course of our investigations, we found **1-Au** to be cytotoxic and cytostatic to cisplatin-resistant cancer cell lines.

Two methods for the preparation of gold(III) corroles have been reported: [ClAu(tht)] (tht = tetrahydrothiophene) was used for insertion into a β-brominated corrole in 85% yield,[16] whereas $Au(OAc)_3$ was utilized for the synthesis of several β-unsubstituted gold(III) triarylcorroles in 25–35% yield.[17] The above reagents, as well as chloroauric acid and its salts were tested in an attempted synthesis of **1-Au**, but only gold(III) acetate led to the desired complex. The treatment of 2,17-bis-sulfonato-5,10,15-trispentafluorophenylcorrole (**1-H$_3$**) with excess of $Au(OAc)_3$ in pyridine at room temperature allowed the isolation of **1-Au** in 25% yield (Scheme 1).[21]

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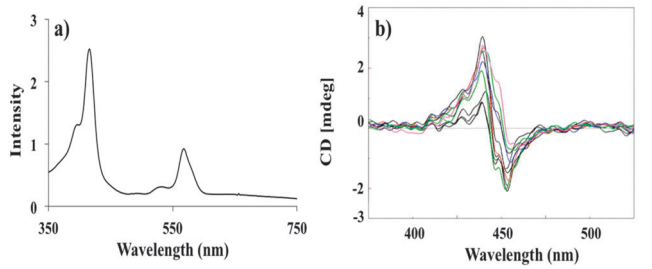

Fig. 1 (a) Absorption spectrum of **1-Au**. (b) Changes in the CD spectra of **1-Au**, with increasing amounts of HSA. [**1-Au**] = 5.0 × 10^{-5} M, [HSA] = 0–5 × 10^{-5} M.

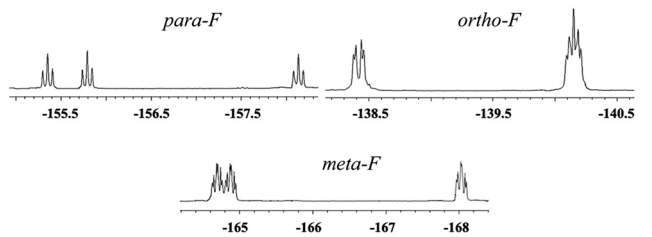

Fig. 2 ^{19}F NMR spectrum of **1-Au**, in CD_3OD.

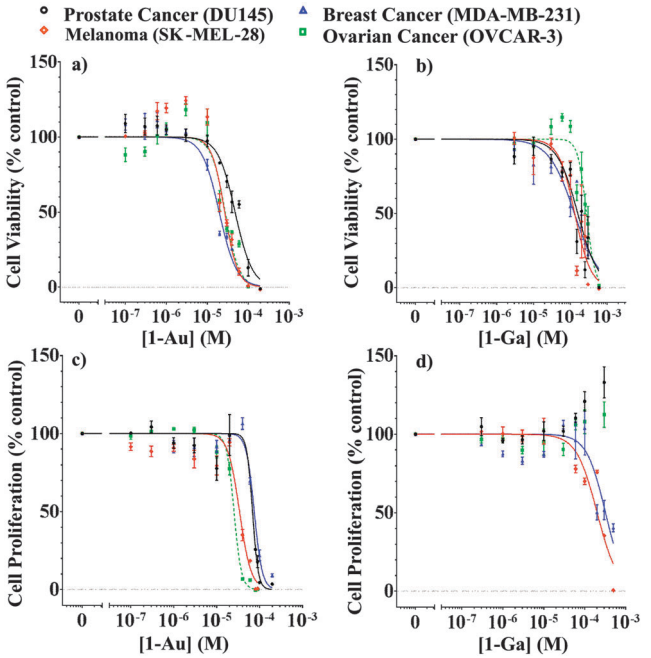

Fig. 3 Dose–response cytotoxicity and cytostaticity curves for **1-Au** (a, c) and **1-Ga** (b, d), presented as percentages relative to cells treated in a corrole-free medium with the same concentration of DMSO (1%) used for dissolving the corroles.

The electronic spectrum of **1-Au** is similar to those of other metal complexes of the same corrole (Fig. 1a); and, as expected, the ^{1}H NMR spectrum displays two singlets (at 10.19 and 9.16 ppm), assigned to the C3–H and C18–H protons adjacent to the sulfonic acid moieties. Analysis of the ^{19}F-NMR spectrum (Fig. 2) reveals that the F atoms above and below the macrocycle plane experience identical magnetic environments: each of three different C_6F_5 groups contributes only one chemical shift for the *ortho*- and *meta*-F's that it contains. The apparent relatively high symmetry and the diamagnetism of **1-Au** are fully consistent with expectations for the existence of a square planar low-spin $5d^8$ gold(III) complex. MS spectroscopy further confirmed the structure and the low affinity of axial ligands, as indicated by the molecular mass without any additives from coordinating solvents (pyridine, methanol, ethyl acetate) that were used in the synthesis and for chromatographic purification.

Examination of **1-Au** in aqueous PBS and PBS/glutathione solutions revealed no changes in the far visible spectrum with time, which is one indication that it has the stability required to be considered as a drug candidate. Four NCI60 cell lines, representative of breast, prostate, skin, and melanoma cancers, were selected for investigations of **1-Au** treatments for their survival (by the MTS assay) and proliferation (by the BrdU assay).[22] Interestingly, **1-Au** was found to possess 3.3 to 10.0-fold greater cytotoxicity than that of **1-Ga** (Fig. 3a and b) across all four cancer cell lines. The cytotoxicity of **1-Au** also was greater than that of cisplatin in the prostate, skin, and melanoma cancer cell lines (Table 1). Cytostatic activity also was examined for the two corroles: that of **1-Au** was 4.3 to more than 13.8-fold larger than that for **1-Ga** across all four cancer cell lines (Fig. 3c and d and Table 2), clearly showing that **1-Au** inhibits DNA replication far more than **1-Ga.** The striking similarity in the MTS and BrdU IC50 values for **1-Au** further suggests that the dominant mechanism of cell-killing by **1-Au** is through cell cycle arrest, a result of DNA replication inhibition. Our proposed mechanism for **1-Au** is well-supported by our previous work that showed late M cell cycle arrest across several different cell lines by **1-Ga**, **1-Al** and **1-Mn**.[23]

Table 1 Comparison of IC_{50} cytotoxicity values of **1-Au**, **1-Ga** and cisplatin in human cancer cell lines

Cell line		DU145	SK-MEL-28	MDA-MB-231	OVCAR-3
Tumor type		Prostate	Melanoma	Breast	Ovarian
IC_{50} (μM)	**1-Au**	47.9	26.8	19.7	27.5
	1-Ga	158.9	131.4	129.2	274.2
	Cisplatin	81.0	36.8	44.8	22.8

Table 2 Comparison of IC_{50} cytostaticity values for **1-Au** and **1-Ga**

Cell line		DU145	SK-MEL-28	MDA-MB-231	OVCAR-3
IC_{50} (μM)	**1-Au**	68.5	35.6	75.1	25.4
	1-Ga	>350.0	201.9	322.5	>350.0

Extensive studies conducted on **1-Ga** demonstrated the importance of its spontaneous noncovalent conjugation with proteins (owing to the amphipolar nature of the macrocycle), which serves for both its circulation and assisted cell membrane penetration. In order to gain insight into why the gold(III) corrole performs better than its gallium analogue, we examined its interaction with human serum albumin. First we employed circular dichroism (CD), since a signal in the visible range will only be obtained if **1-Au** conjugates with HSA and thus experiences a chiral environment. A strong signal was obtained upon titration of **1-Au** with HSA, with a positive Cotton effect and ellipticity inversion at 420 nm (Fig. 1b).[24] No change in the shape of the signal accompanied

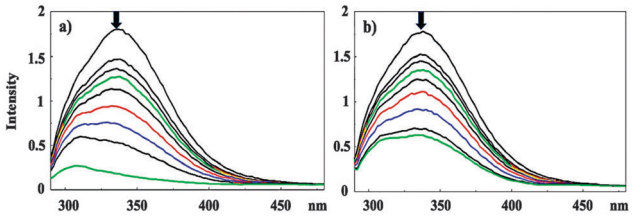

Fig. 4 Quenching of HSA Trp^{214} emission (λ_{exc} = 340 nm) upon titration with (a) **1-Ga** or (b) **1-Au**, with [corrole] : [HSA] ratios of 0, 0.6, 0.7, 1:, 1.4, 2, 2.8, 4.1, and 6.8.

the titration, quite unlike our observations in related CD experiments where we found that 8–10 equivalents of **1-Ga** bind to HSA.[15]

Additional evidence for marked differences in binding affinities of the two corroles was obtained upon quenching of the fluorescence of the only tryptophan residue present in HSA by **1-Au** and **1-Ga** (Fig. 4a and b, respectively).

The comparison clearly demonstrates that the affinity of **1-Au** for HSA (or more precisely to binding sites that are close to Trp^{214}) is much lower than that of **1-Ga**. At the highest corrole/HSA ratio, the Trp^{214} emission was completely quenched for **1-Ga** (the residual emission is due to tyrosines), but not for **1-Au**.

Another qualitative indication of the weaker binding of **1-Au** was obtained by mass spectroscopy.[25] Analysis of the ESI-MS spectra of 1 : 6 solutions of HSA and the corrole revealed that molecular clusters that correspond to HSA-bound conjugates were much more pronounced for **1-Ga** than **1-Au** (Fig. 5). The relatively weak binding of **1-Au** to albumin (present in the *in vitro* assays used for evaluating its cytotoxicity and cytostaticity) could be one of the factors contributing to its higher activity relative to **1-Ga**. The low affinity of **1-Au** for basic amino acid residues means that a more uncomplexed agent will likely be available for intervention in intracellular processes. In support of this proposal, several investigators have shown that the interactions of many metallodrugs (including cisplatin and gold(III) pyridyls) with albumin are a major influence on their biological activities.[26–28]

We have discovered that **1-Au** is a more potent anticancer agent than **1-Ga**, with three to ten-fold greater cytotoxicity and four to more than ten-fold greater cytostaticity in four cancer cell lines. In our hands, **1-Au** also exhibited greater cytotoxicity

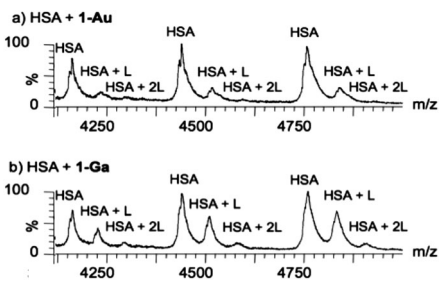

Fig. 5 ESI-TOF mass spectra obtained from aqueous solutions of 5 μM HSA that were incubated with 30 μM of (a) **1-Au** and (b) **1-Ga**. HSA represents the free protein, while HSA + L and HSA + 2L represent HSA conjugated with the respective metallocorroles in a 1 : 1 and 1 : 2 ratios, respectively. The *m*/*z* values for HSA in each of the traces represent different ionization states (*z*), from +14 to +16.

than cisplatin in three out of the four cell lines examined. We suggest that the diminished binding of **1-Au** to HSA relative to **1-Ga** is responsible for its greater cell killing ability. The correlation between cytotoxicity and cytostaticity further suggests that the main mechanism of action is *via* mitotic arrest, as established for other metal complexes of the same corrole.[23] Although the anticancer potency of **1-Au** is not as great as that of [Au(TPP)]Cl, it is substantially more cytotoxic than water-soluble gold(III) porphyrins.[29] The take-home lesson is clear: we must place much more emphasis on investigations of less hydrophilic corroles in our search for better anticancer agents. More in-depth investigations of the mechanism of action of the most active gold corroles, as well as exploration of their potential for photodynamic therapy,[30] will be pursued in these derivatives.

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21 Synthesis of **1-Au**. **1-H$_3$** (37 mg, 0.046 mmol) was added to a solution of gold acetate (52 mg, 0.14 mmol) in pyridine under $N_{2(g)}$ atmosphere. The reaction mixture was stirred overnight, after which solvent was evaporated under reduced pressure without heating. The product was purified by silica-gel chromatography (silica gel, MeOH : EA 1 : 15 as eluent), affording **1-Au** as a violet solid in 25% yield. **1-Au**: UV/Vis (CH_3CN) λ_{max} ($\varepsilon \times 10^4$): 418 nm (1.9), 531 nm (0.22), 568 nm (0.67). ^{1}H-NMR (CD_3OD, 400 MHz): δ = 10.19 (s, 1H, pyrrole), 9.16 (s, 1H, pyrrole),

9.00 (d, J = 5.1 Hz, 2H, pyrrole), 8.97 (d, J_1 = 5.1 Hz, 2H, pyrrole). ^{19}F-NMR (376.75 MHz, CD_3OD): δ = −138.41 (dd, J_1 = 6.5 Hz, J_2 = 23.0 Hz, 2 *ortho*-F), −140.15 (m, 4 *ortho*-F), −155.35 (t, J = 20.8 Hz, 1 *para*-F), −155.14 (t, J_1 = 19.1 Hz, 1 *para*-F), −158.13 (t, J = 19.1 Hz, 1 *para*-F), −164.69 (dt, J_1 = 7.15 Hz, J_2 = 22.1 Hz, 2 *meta*-F), −164.87 (dt, J_1 = 7.3 Hz, J_2 = 22.1 Hz, 2 *meta*-F), −168.03 (dt, J_1 = 6.7 Hz, J_2 = 22.77 Hz, 2 *meta*-F). MS-ES$^-$ (TOF ES$^-$): *m/z*: 1148.77 (calcd for $[C_{37}AuH_7F_{15}N_4O_6S_2]^-$: 1149.10). Purity was further confirmed by analytical HPLC, performed on a 150 × 4.6 mm Kromasil C18 reverse phase column and H_2O/CH_3CN as the eluent.

22 Cells were grown in RPMI 1640 medium (Mediatech, Inc.) supplemented with 10% fetal bovine serum (Omega Scientific, Inc., Tarzana, CA), and maintained at 37 °C under 5% CO_2 in a humidified incubator. Cytotoxicity was determined by cell viability measurements using the CellTiter 96 Aqueous One Cell Proliferation Assay (MTS) from Promega (Madison, WI). Cytostatic activity was measured by the BrdU incorporation assay using the Cell Proliferation ELISA, BrdU (colorimetric assay) from Calbiochem (San Diego, CA). Cells were plated in seven 96-well dishes (5.6×10^4 cells per well; 90 μL per well) 24 h prior to treatment. Drug treatment was initiated by adding 10 μL of fresh media containing the respective concentrations of metallocorrole or cisplatin in DMSO to make up 0.1 mL per well, followed by incubation for 72 h at 37 °C, 5% CO_2. For control wells, the same volume (10 μL) of fresh media containing 10% DMSO was added to make up 0.1 mL per well. The final amount of DMSO per well was kept at 1%. The MTS and BrdU assays were performed according to the manufacturers' directions. All drug treatments were performed in the dark to minimize the effects of metallocorrole photochemistry. Experiments were performed in triplicate. The means from triplicate spectrophotometric data were analyzed by nonlinear regression analysis using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA) to obtain a growth equation for each treatment. The rate of cell proliferation (BrdU) or growth (MTS) of corrole-treated cells relative to control cells is reported as percent of untreated cells.

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25 A 10 mM NH_4OAc buffer was filtered through a 47 mm MF-Millipore membrane filter with 0.22 μM pore size (EMD Millipore, US) and used for sample preparation. The concentration of metallocorrole and HSA were kept at 30 μM and 5 μM, respectively. **1-Au** and **1-Ga** were allowed to incubate with HSA for 60–90 min at 25 °C. A Waters (Manchester, UK) Synapt G2 HDMS high definition mass spectrometer with ion mobility MS equipped with a Waters nanoAcquity 2D UHPLC system was used for measurements.

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