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New Gold(I) Organometallic Compounds with Biological Activity in Cancer Cells

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Abstract

N-Heterocyclic carbene gold(I) complexes bearing a fluorescent coumarin ligand were synthesized and characterized by various techniques. The compounds were examined for their antiproliferative effects in normal and tumor cells *in vitro*; they demonstrated moderate activity and a certain degree of selectivity. The compounds were also shown to efficiently inhibit the selenoenzyme thioredoxin reductase (TrxR), whereas they were poorly effective towards the glutathione reductase (GR) and glutathione peroxidase enzymes. Notably, {3-[(7-methoxy-2-oxo-2*H*-chromen-4-yl)methyl]-1-methylimidazol-2-ylidene}(tetra-*O*-acetyl-1-thio- β -D-glucopyranosido)gold(I) (**3**) showed a pronounced inhibition of TrxR also in cell extracts, and it appeared to activate GR. Mechanistic information on the system derived from biotin-conjugated iodoacetamide assays showed selective metal binding to selenocysteine residues. Preliminary confocal fluorescence microscopy experiments proved that **3** enters tumor cells, where it reaches the nuclear compartment.

Introduction

Following the clinical success of cisplatin, many platinum and non-platinum metallodrugs are currently investigated as experimental antitumor agents with different mechanisms of action and improved pharmacological properties with respect to those of existing drugs.¹ However, major challenges must be faced to reach such a goal, including the identification of the actual biological targets for metal compounds, as well as the determination of their distribution in tissues, cells, and subcellular compartments to achieve an understanding of the possible mechanisms of biological activity.²

Noteworthy is that gold complexes belonging to various families have drawn attention in the last years as a new generation of experimental anticancer agents, and they have been shown to possess anticancer properties *in vitro* and *in vivo*.³ Notably, various organometallic gold complexes were synthesized in which the presence of a direct carbon–gold bond greatly stabilizes the gold oxidation state and guarantees more controlled chemical speciation in biological systems. In general, both organometallic gold(I) and gold(III) compounds have increased stability with respect to classical gold-based coordination complexes, which has allowed the design of compounds in which the redox properties and ligand exchange reactions can be modulated to achieve selective activation in diseased cells.

Mechanistic studies showed that interactions of gold complexes with DNA are not as tight as those found for platinum(II) drugs, which suggests the occurrence of different pathways to cytotoxicity.⁴ Indeed, several studies support the idea that mitochondria and pathways of oxidative phosphorylation are among the primary intracellular targets.⁵ Moreover, inhibition of the selenoenzyme thioredoxin reductase (TrxR) appears as a common mechanistic trait to explain (at least partially) the cytotoxic actions of gold complexes, as strong TrxR inhibition may eventually lead to apoptosis through a mitochondrial pathway.⁶ TrxRs are large homodimeric proteins that play a crucial role in the intracellular redox balance.⁷ Two major isoforms are known, a cytosolic one (TrxR1) and a mitochondrial one (TrxR2); their main function is the reduction of the 12 kDa disulfide protein thioredoxin (Trx) to its dithiolic form.^{6a} Interestingly, the view that TrxRs are effective “druggable” targets for inorganic

compounds is supported, for example, by studies of arsenic trioxide, a potent TrxR inhibitor now approved for promyelocytic leukemia treatment.[8](#)

Within this frame, in the last years, N-heterocyclic carbenes (NHCs) gold(I) have been transformed from niche compounds into some of the most popular scaffolds in medicinal inorganic chemistry.[9](#) For example, studies by our group and others confirmed that many NHC gold(I) complexes with 1,3-substituted imidazol-2-ylidene and benzimidazol-2-ylidene ligands of the type NHC–Au–L (L = chlorido or 2-pyrimidinthiolato) can potently inhibit both the cytosolic and mitochondrial isoforms of the TrxR enzyme.[10](#) The compounds showed potent and selective TrxR inhibition properties, in particular in cancer cell lines.

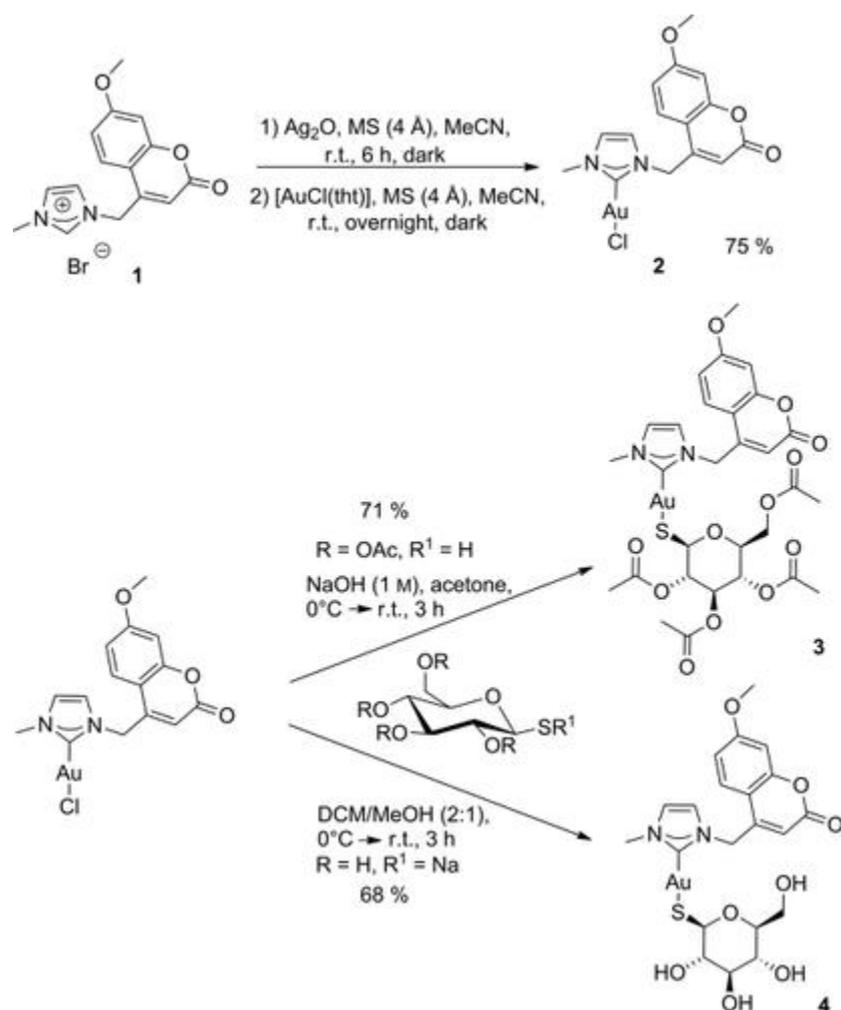
On the basis of these promising results, we report herein on the synthesis and characterization of three new NHC gold(I) complexes bearing a coumarin moiety. This functionalization was chosen, because coumarin derivatives are some of the most studied fluorophores for in vitro imaging. They display good chemical and photochemical stability, relatively high absorption coefficients and quantum yields, and are very easily available. Moreover, two of the new derivatives bear 1-thio- β -D-glucose-type ligands, which may affect the uptake of the compounds, as previously observed for other gold(I) complexes.[11](#)

All compounds were tested in vitro against different human cancerous cell lines (i.e., A2780, MCF-7, and A549) along with noncancerous cells (i.e., HEK-293 T). To gain preliminary mechanistic insight, we screened the interactions of compounds **1–4** with TrxR. The compounds were also screened for inhibition of glutathione reductase (GR), a pyridine disulfide oxidoreductase able to maintain glutathione in its reduced state, and for inhibition of the selenoenzyme glutathione peroxidase (Gpx). Additional complementary information regarding the enzyme metalation process and possible binding sites was obtained through the application of a specific biochemical assay that relies on the thiol-tagging reagent BIAM (biotin-conjugated iodoacetamide). Furthermore, fluorescence confocal microscopy was used to study the uptake of the compounds into cancer cells.

Results and Discussion

Synthesis

The carbene(chlorido)gold complex was efficiently synthesized in three steps by adapting literature procedures (Scheme [1](#)).[12](#) Thus, 1-methylimidazole was alkylated by using commercially available 4-(bromomethyl)-7-methoxycoumarin to obtain imidazolium salt **1** in 95 % yield. Afterwards, **1** was treated with silver oxide at room temperature in the dark to generate the (carbene)silver complex, which was transferred to gold by reaction with the precursor [AuCl(tht)] (tht = tetrahydrothiophene). The formation of (carbene)gold complex **2** was assessed by ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectroscopy. The singlet corresponding to the imidazolium proton disappeared, and a significant shift in the signal of the C2 carbon atom from $\delta = 138.2$ ppm in the imidazolium salt to $\delta = 173.5$ ppm in the complex (carbene carbon atom) was observed. This behavior is in agreement with the already reported data (see ref.[13](#) and the references cited therein).



Scheme 1: Synthesis of the investigated NHC gold(I) complexes.

Replacing the chlorido ligand on some (phosphine)gold(I) complexes by tetra-*O*-acetyl-1-thio- β -D-glucopyranosido was reported to result in an enhancement in both cytotoxic effects and uptake.¹¹ Thus, we decided to substitute the chlorido ligand of **2** by both tetra-*O*-acetyl-1-thio- β -D-glucopyranosido and 1-thio- β -D-glucopyranosido (Scheme 1). Whereas tetraacetyl derivative **3** was obtained by deprotonation of the thiol by using 1 M NaOH in acetone,¹⁴ 1-thio- β -D-glucopyranosido complex **4** was synthesized by direct reaction of the commercially available sodium 1-thio- β -D-glucopyranoside with carbene(chlorido)gold complex **2**. Both compounds were fully characterized by ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR, as well as IR spectroscopy and high-resolution mass spectrometry, and their purity was checked by elemental analysis. A far-IR spectrum confirmed the formation of an S–Au bond in both cases through the appearance of a novel absorption band around 370 cm^{-1} and the absence of the band at 330 cm^{-1} corresponding to $\nu_{\text{Au-Cl}}$ stretching.¹⁵ The ^1H NMR spectra of **3** and **4** show the expected 1:1 ratio of the signals of the NHC with respect to the sugar moiety. The signal of the carbene carbon atom in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **3** resonates downfield from that in **2** ($\Delta\delta = -3.6$ ppm) and is not observed in the case of **4**. However, by ^1H – ^{13}C correlation NMR spectroscopy, we observed a correlation spot between both the *N*-methyl and the methylene bridge signals in the ^1H NMR spectrum and a signal at $\delta \approx 181$ ppm in the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of **4** (see the ^1H – ^{13}C HMBC spectrum in the Supporting Information, Figure S1). Noteworthy, elemental analysis of **4** shows that it is in the form of an adduct with 1 equiv. of NaCl, probably through the hydroxy groups of the glucose moiety, as already reported for different sugars.¹⁶ The photophysical characterization was performed for the four compounds (see details of the study in the Supporting Information). No significant change was observed between imidazolium salt **1** and NHC Au^I complexes **2**–**4**. The compounds display a maximum absorption around

325 nm, a maximum emission around 400 nm, and the molar absorption coefficient remains almost the same for the different compounds. The quantum yields are low, maybe due to a photoinduced electron transfer (PET) between the coumarin and the imidazolium/carbene.¹⁷

In Vitro Cell Viability Assays

The antiproliferative properties of imidazolium salt **1** and NHC Au^I complexes **2–4** (with cisplatin used as a comparison) were assessed by monitoring their ability to inhibit cell growth by using the classical MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in the human ovarian cancer A2780 cell line, in human mammary carcinoma MCF-7 cells, as well as in the human lung cancer A549 cell line (Table 1). In addition, to evaluate the selectivity of the compounds for cancerous cells relative to that for healthy cells, these coumarin derivatives were also tested in human embryonic kidney HEK-293 T cells. Overall, the compounds are markedly less effective in all the selected cell lines in comparison to cisplatin. Only in the case of the MCF-7 cells did compound **3** show better activity ($IC_{50} = 11.6 \pm 0.8 \mu\text{M}$) than cisplatin. Notably, **3**, bearing the tetra-*O*-acetyl-1-thio- β -D-glucopyranosido ligand, is also the most active of the series in the A2780 and MCF-7 cells, as well as the one relatively selective for cancer cells with respect to noncancerous ones. The inactivity of **4** in all tested cells rules out the idea that the 1-thio- β -D-glucopyranosido ligand may enhance the uptake of the compounds through GLUT-1 transporters.

Table 1. Effects of compounds **1–4** on cell viability in human ovarian carcinoma A2780 cells, in human lung cancer A549 cells, in human mammary carcinoma MCF-7 cells, and in embryonic kidney cells (HEK-293 T) after 72 h incubation.

Compound	IC_{50} [μM] ^[a]			
	A2780	A549	MCF-7	HEK-293T
1	46.9 ± 13.3	52.5 ± 17.9	71.2 ± 5.2	>100
2	45.5 ± 7.5	95.8 ± 5.3	39.7 ± 11.8	48.0 ± 8.4
3	11.6 ± 0.8	55.6 ± 12.6	12.9 ± 3.8	15.2 ± 1.7
4	>100	>100	64.5 ± 11.5	>100
Cisplatin	1.9 ± 0.6	8.0 ± 0.5	20.0 ± 3.1	11.0 ± 2.9

- [a] Values are the mean ± standard error of at least three determinations.

Because TrxR is a potential target for gold compounds, in vitro inhibition of rat TrxR by **1–4** was studied by using an established protocol. The results summarized in Figure 1 and Table 2 show that the NHC gold(I) complexes inhibit both TrxR1 and TrxR2, although less efficiently than the gold(I) compound auranofin. Notably, ligand **1** is ineffective as an inhibitor at least until 30 μM concentration (Table 2). Moreover, the new complexes are much less efficient with respect to both the closely related selenium enzyme Gpx and the Se-free GR having IC_{50} values > 1000 nM. Notably, minor Gpx inhibition is shown for different NHC gold(I) complexes previously reported.¹⁸

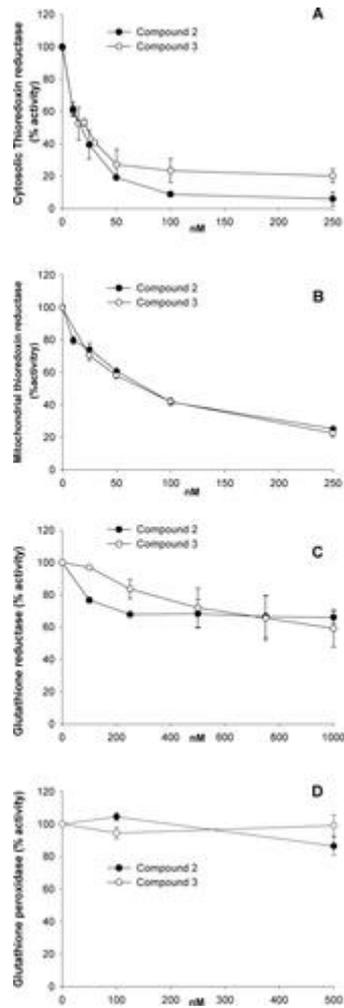


Figure 1 Effect of compounds **2** and **3** on (a) cytosolic and (b) mitochondrial TrxRs and the (c) GR and (d) Gpx enzymes. The compounds were tested in isolated and purified enzymes.

Table 2. IC₅₀ values for TrxRs, GR, and Gpx inhibition calculated for **1–4**.

Compound	IC ₅₀ [nM] ^[a]			
	TrxR1	TrxR2	GR	Gpx
1	>10000	>10000	>10000	>10000
2	16.38 ± 1.32	76.52 ± 3.2	>1000	>500
3	17.90 ± 1.8	78.66 ± 1.2	>1000	>500
4	6.49 ± 0.2	54.02 ± 1.3	>1000	>1000
Auranofin	1.51 ± 0.2	24.3 ± 1.2	>1000	>1000

- [a] Values are the mean ± standard error of at least three experiments.

Additional complementary information on the interaction of TrxR with compounds **1–3** and possible binding sites was obtained through the application of a specific biochemical assay relying on the thiol-tagging reagent BIAM. BIAM selectively alkylates TrxR in a pH-dependent manner; at pH = 6.0 only selenocysteines and low-pK_a cysteines are alkylated. In our experiments, TrxR1 was treated with metal complex, and afterwards, sample aliquots were treated with BIAM at pH = 6.0. Thus, the samples were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). BIAM-labeled proteins were detected with horseradish peroxidase conjugated streptavidin (see Figure S3 and the Experimental Section for further details). The immunoblotting indicated that the tested gold complexes are able to target, although to slightly varying extents, the selenocysteine residues present in the enzyme redox-active motif.

To assess whether TrxR inhibition by the compounds under study could contribute to the observed antiproliferative effects on cells, enzyme activity was also evaluated on protein extracts obtained from A2780 cells pretreated with 10 μM of **3** (close to the IC₅₀ for the antiproliferative effects) for 48 h. In this case, we observed an inhibition of thioredoxin reductase activity of about 30 % with respect to control samples (Figure S4). Conversely, glutathione reductase, tested in the same cell lysates, appears to be largely stimulated. This fact can be explained as a response to the stress that cells are subjected to after inhibition of the thioredoxin system. In fact, as a control, isolated GR is not inhibited by this compound.

Fluorescence Microscopy

Notably, among the various strategies to achieve the imaging of metal compounds in biological environments, fluorescence microscopy is certainly one of the most explored, and an increasing number of publications has appeared reporting on bifunctional metal compounds bearing fluorescent moieties for both therapeutic and imaging applications.¹⁹ Thus, we recently described an NHC gold(I) complex bearing a fluorescent anthracenyl ligand, for which the cytotoxic effects were investigated in vitro in different lines of normal and cancerous human cells.²⁰

Similarly, the uptake of **3** was evaluated in A2780 cells by using fluorescence confocal microscopy. Figure 2 shows typical fluorescence images of cells treated with **3** (20 μM) at 37 °C for 2 h. Unfortunately, the low fluorescence of the compounds allowed visualization only at

20 μM or higher. We avoided longer incubation times because of the relatively high tested concentrations of the compounds, which may have induced rapid cell death. In the obtained images, preserved cell morphology confirms viability after treatment, and the uptake of the compound is evident, as is its accumulation in the nuclei (colocalization with propidium iodide staining).

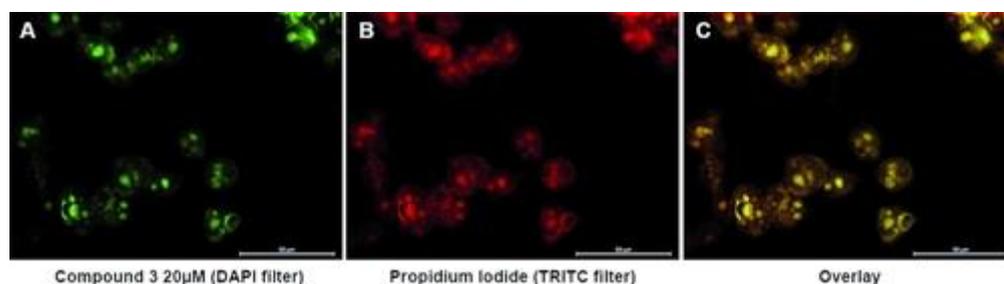


Figure 2: Visualization of the NHC gold(I) compound by using confocal microscopy. A2780 cells were incubated with 20 μM **3** at 37 °C for 2 h. (a) Fluorescence of the compound; (b) propidium iodide nucleic acid staining; (c) merge.

Conclusions

We synthesized and characterized a new family of NHC gold(I) complexes bearing a fluorescent coumarin-type carbene ligand by using a limited number of steps (two to three). Two of the reported compounds bear 1-thio- β -D-glucopyranosido groups as a second ligand. Notably, this study was initiated in the frame of ongoing studies in our laboratories aimed at developing new organometallic gold compounds as anticancer agents. In fact, as demonstrated by numerous studies, regulating the reactivity of gold compounds through the optimization of an appropriate organometallic scaffold may constitute a strategy to achieve selectivity for cancer tissues, a feature that is often lacking with other types of coordination gold complexes.

Thus, the new compounds were tested in different cancer cell lines and showed moderate antiproliferative properties, in particular derivative **3** bearing the tetraacetyl-1-thio- β -D-glucopyranosido ligand, for which the uptake was studied by fluorescence microscopy. Preliminary mechanistic studies demonstrated that the compounds are able to inhibit the cancer-relevant enzyme thioredoxin reductase, most likely by targeting the selenocysteine residue in the active site. Presently, further studies are ongoing in our laboratories to validate this protein target, as other interactions may occur between the gold(I) compounds and other relevant biomolecules. As an example, a recent study for the first time described the properties of a cationic bis(NHC) gold(I) complex containing a caffeine-based ligand as a DNA G-quadruplex stabilizing agent with exquisite quadruplex-over-duplex DNA selectivity.[21](#)

Supporting Information (see footnote on the first page of this article): Experimental section and additional figures.

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