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Gold(III) complexes with 2-substituted pyridines as experimental anticancer agents: Solution behavior, reactions with model proteins, antiproliferative properties

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ABSTRACT

Gold(III) compounds form a family of promising cytotoxic and potentially anticancer agents that are currently undergoing intense preclinical investigations. Four recently synthesized and characterized gold(III) derivatives of 2-substituted pyridines are evaluated here for their biological and pharmacological behavior. These include two cationic adducts with 2-pyridinyl-oxazolines, $[Au(pyox^R)Cl_2][PF_6]$, [$pyox^R$ = (S)-4-benzyl-2-(pyridin-2-yl)-4,5-dihydrooxazole, I; (S)-4-iso-propyl-2-(pyridin-2-yl)-4,5-dihydrooxazole, II] and two neutral complexes $[Au(N,N'OH)Cl_2]$, III, and [Au(N,N′,O)Cl], IV, containing the deprotonated ligand N-(1-hydroxy-3-iso-propyl-2-yl)pyridine-2-carboxamide, N,N′H,OH, resulting from ring opening of bound $pyox^R$ ligand of complex II by hydroxide ions. The solution behavior of these compounds was analyzed. These behave as classical prodrugs: activation of the metal center typically takes place through release of the labile chloride ligands while the rest of the molecule is not altered; alternatively, activation may occur through gold(III) reduction. All compounds react eagerly with the model protein cyt c leading to extensive protein metalation. ESI MS experiments revealed details of gold–cyt c interactions and allowed us to establish the nature of protein bound metal containing fragments. The different behavior displayed by I and II compared to III and IV is highlighted. Remarkable cytotoxic properties, against the reference human ovarian carcinoma cell lines A2780/S and A2780/R were disclosed for all tested compounds with IC_{50} values ranging from 1.43 to 6.18 μM in the sensitive cell line and from 1.59 to 10.86 μM in the resistant one. The common ability of these compounds to overcome cisplatin resistance is highlighted. The obtained results are thoroughly discussed in the frame of current knowledge on cytotoxic gold compounds.

1. Introduction

In the course of the last decade gold(III) compounds have greatly attracted researchers' attention for their outstanding cytotoxic actions [1]. Indeed, various classes of gold(III) compounds were prepared and characterized showing an appreciable stability under physiological-like conditions and manifesting at the same time important antiproliferative effects *in vitro*. We refer, in particular, to gold(III) dithiocarbamates [2], gold(III) porphyrinates [3], dinuclear gold(III) complexes [4], and to a variety of organogold(III) compounds [5]. Potent cytotoxic actions *in vitro* were documented for most of the investigated compounds, as well as the evident ability to overcome cisplatin resistance. In addition, for a few gold(III) compounds, preliminary but truly encouraging *in vivo* data were obtained [6].

Several lines of evidence suggest that gold(III) compounds produce their antiproliferative effects through innovative and non-conventional modes of action. For instance, the hypothesis that their biological effects are mediated by an antimitochondrial mechanism rather than by direct DNA damage, as it is the case for cisplatin and its analogs, has gained much credit during the last few years [7]. Other targets were proposed in recent times such as the proteasome [1,8], histone deacetylases [9,10], a few kinases [11,12], transcription factors containing zinc finger motifs, *etc*. It is now a quite accepted opinion that the remarkable cytotoxic effects documented for several gold compounds are mainly the consequence of metalation and inactivation of selected crucial proteins.

For the present study, we have considered four representative gold(III) compounds with 2-substituted pyridines, recently prepared and characterized in our laboratories. These include two cationic adducts with 2-pyridinyl-oxazolines, $[Au(pyox^R)Cl_2][PF_6]$, [$pyox^R$ = (*S*)-4-benzyl-2-(pyridin-2-yl)-4,5-dihydrooxazole, **I**; (*S*)-4-iso-propyl-2-(pyridin-2-yl)-4,5-dihydrooxazole, **II**] and the neutral complexes $[Au(N,N'OH)Cl_2]$, **III**, and [Au(N,N′,O)Cl], **IV**, containing,

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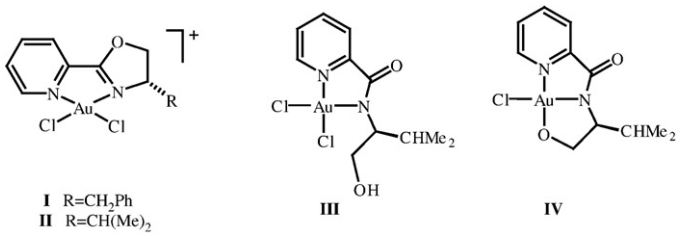

Chart 1. Schematic drawing of the gold(III) compounds.

respectively, the mono- and bis-deprotonated ligand *N*-(1-hydroxy-3-iso-propyl-2-yl)pyridine-2-carboxamide, N,N′H,OH, resulting from ring opening of bound pyox^{R} ligand of complex **II** by hydroxide ions [13]. We have examined here their solution behavior, their reactions with cyt c used as a "*model protein*", their antiproliferative effects *in vitro*. Notably, these novel gold(III) compounds revealed very encouraging chemical and biological properties, as it will be shown below, and warrant, in our opinion, a further and deeper pharmacological evaluation.

2. Experimental section

2.1. General

Complexes **I–IV** were prepared as previously reported [13]. UV/vis spectra were recorded on a Varian Cary 50 UV–vis spectrophotometer. Mass spectra were recorded in an LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source.

2.2. Solution chemistry

The solution chemistry of the above compounds was analyzed by absorption UV–visible spectroscopy. UV–vis absorption spectra of the gold compounds were recorded on a Varian Cary 50 spectrophotometer. Solutions of **I–IV** (10^{-4} M) in 10 mM phosphate buffer pH = 7.4 (1% DMSO).

2.3. ESI mass spectrometry

Horse heart cytochrome c was purchased from Sigma (C7752) and used as received. All samples were prepared in tetramethylammonium acetate buffer (TMAA) pH 7.4, with a protein concentration of 10^{-4} M, and a gold to protein molar ratio of 3:1. The reaction mixtures were incubated for 24 h at 37 °C, and the resulting products analyzed by ESI MS. After a 20-fold dilution with MilliQ water, ESI MS spectra were recorded by direct introduction on an LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA), equipped with a conventional ESI source. The working conditions were the following: spray voltage 3.1 kV, capillary voltage 45 V and capillary temperature 220 °C. The sheath and the auxiliary gases were set, respectively, at 17 (arbitrary units) and 1 (arbitrary units). For acquisition, Xcalibur 2.0 software (Thermo) was used and monoisotopic and average deconvoluted masses were obtained by using integrated Xtract tool.

2.4. Antiproliferative tests

Exponentially growing cells were seeded in 96-well microplates at a density of 5×10^3 cell/well and incubated with various compound concentrations. After 72 h of exposure to compounds, the cells were fixed with 100 μL of ice-cold 10% trichloroacetic acid for 60 min at 4 °C, rinsed 6 times with water and air-dried. Fixed cells were stained with 50 μL of sulforhodamine B (SRB) solution (0.4% SRB/0.1% acetic acid), rinsed with 0.1% acetic acid and air-dried. At the end of the staining period, SRB was dissolved in 150 μL of 10 mM Tris–HCl solution (pH 10.5) for 10 min on a gyratory shaker. Optical density was read in a microplate reader interfaced with the software Microplate Manager/PV version 4.0 (Bio-Rad Laboratories, Milan, Italy) at 540 nm.

3. Results and discussion

3.1. Structural chemistry

The chemical structures of the four compounds chosen for the present study are shown in Chart 1. Notably, all of them feature a square planar geometry at the gold(III) center bound to chelating N,N, N,N′ and N,N′,O ligands, respectively; tetracoordination is

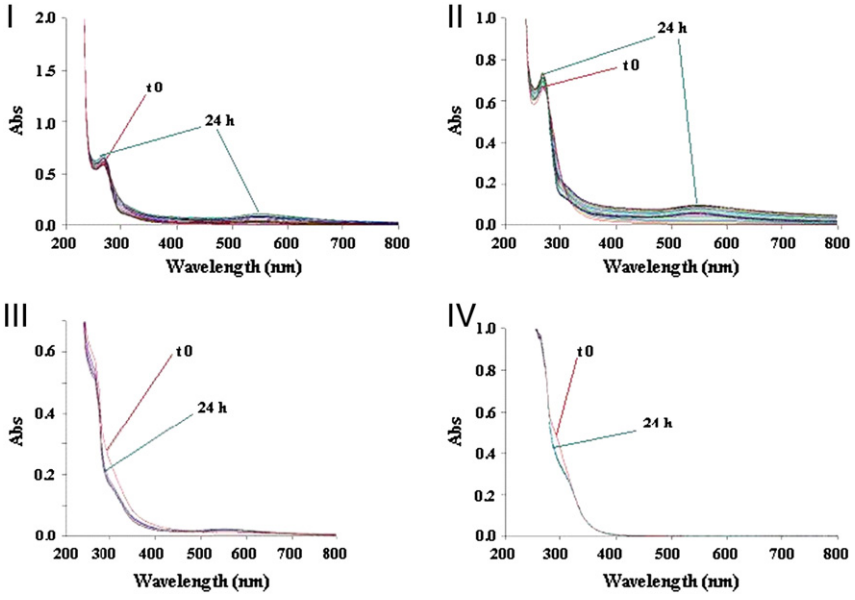

Fig. 1. Hydrolysis profiles of complexes **I–IV** dissolved in phosphate buffer 10 mM pH 7.4. Spectra were recorded at different times over 24 h at room temperature.