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Original article

A potential antitumor agent, (6-amino-1-methyl-5-nitrosouracilato-N3)-triphenylphosphine-gold(I): Structural studies and *in vivo* biological effects against experimental glioma

Nuria A. Illán-Cabeza [a], Antonio R. García-García [a], José M. Martínez-Martos [b], María J. Ramírez-Expósito [b], Tomás Peña-Ruiz [c], Miguel N. Moreno-Carretero [a,*]

[a] Department of Inorganic and Organic Chemistry, University of Jaén, Spain
[b] Department of Health Sciences, University of Jaén, Spain
[c] Department of Physical and Analytical Chemistry, University of Jaén, Spain

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ABSTRACT

The synthesis and molecular and supramolecular structures of the compound (6-amino-1-methyl-5-nitrosouracilato-N3)-triphenylphosphine-gold(I) with interesting abilities to inhibit tumor growth in an animal model of experimental glioma are reported. Thus, its antitumor properties, effects on both enzyme and non-enzyme antioxidant defense systems and the response of several biochemical biomarkers have been analyzed. After seven days of treatment, the gold compound decreased the tumor growth to *ca.* one-tenth and reduced oxidative stress biomarkers (thiobarbituric acid-reactive substances (TBARS) and protein oxidation levels) compared to animals treated with the vehicle. Also, gold compound maintained non-enzyme antioxidant defense systems as in non-tumor animals and increased enzyme antioxidant defenses, such as superoxide dismutase and glutathione peroxidase activities, and decreased catalase activity. Analysis of serum levels of electrolytes, nitrogenous compounds, glucose, lipids, total protein, albumin, transaminases and alkaline phosphatase indicated that gold compound treatment showed few adverse effects, while effectively inhibiting tumor growth through mechanisms that involved endogenous antioxidant defenses.

1. Introduction

Malignant gliomas, the most frequent brain tumors, are currently non-curable central nervous system neoplasias and unfortunately there has been little improvement in the efficacy of adjuvant therapies [1,2]. Brain tumorigenesis is associated with oxidative stress. This is reflected by an imbalance between free radicals production and antioxidant mechanism. In pathological conditions, free radicals are generated in excess from endogenous sources such as mitochondria, peroxisomes, inflammatory cell activation or neurotransmitters oxidation, and exogenous sources, including environmental agents, drugs, irradiation or chemicals. The resulting oxidative stress promotes various pathologic reactions which contribute to illness. In response to various inducers, large amounts of free radicals (superoxide anion, hydrogen peroxide and hydroxyl groups) trigger lipid peroxidation of the cellular membranes, oxidation of proteins and DNA and lead to changes in chromosome structure, genetic mutations and/or modulation of cell growth. In fact, it was shown that oxygen-derived free radicals play an important role in tumor development. Although the induction of cancer represents a multistage, multistep process, involving multiple molecular and cellular events, the transformation of a normal cell into a malignant one, initiation, promotion and progression stages have been described [3,4]. But brain tumor development involves not only oxidative aggression but also a reduced response of antioxidant defense. During prolonged oxidative stress, changes in brain non-enzyme (reduced glutathione, GSHr) and enzyme antioxidant activities

Abbreviations: GSHr, glutathione reduced; GSSG, glutathione disulfide; SOD, superoxide dismutase; GPx activity, glutathione peroxidase activity; TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; CAT, catalase; ASP, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

* Corresponding author. Dpto. Química Inorgánica y Orgánica, Universidad de Jaén, Campus Las Lagunillas B3, 23071 Jaén, Spain. Tel.: +34 953212738; fax: +34 953211876.
E-mail addresses: mmoreno@ujaen.es, m.moreno.carretero@gmail.com (M.N. Moreno-Carretero).

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(superoxide dismutase, SOD, catalase and glutathione peroxidase, GPx), appear. These enzyme and non-enzyme systems act to prevent or decrease brain damages caused by free radicals in excess. However, they are controlled by polymorphic genes which can be altered by free radicals, leading to dysfunctions.

A critical step in introducing clinical trials of treatments for gliomas after in vitro studies is to examine the efficacy and toxicity *in vivo* animal models. In studying malignant gliomas, rat C6 glioma models are widely used to evaluate the effects of novel therapies [5–8].

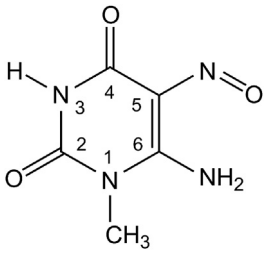
Gold and its complexes have been known to display unique biological and medicinal properties [9]. Specifically, gold(I) complexes exhibit significant biological properties that can be used for the development of novel therapeutic agents. Particularly important are the studies on gold(I)–phosphine complexes since the discovery of auranofin, because of their anticancer activity, especially in some cisplatin-resistant cell lines. The mechanisms of action of anticancer gold(I) and gold(III) complexes appear in general to be DNA independent and essentially cisplatin unrelated. Several proteins with important functional roles in cells are effective targets for cytotoxic gold compounds, such as thioredoxin reductase [10], cathepsins [11], tyrosine phosphatase [12], proteasomes [13], iodothyronine deiodinase [14] and, recently, zinc finger proteins such as PARP-1 [15]. The different mechanisms which contribute to the pharmacological profile of gold complexes are based on their different ligands, different kinetic properties, geometries and other features [9,16–18]. A possible therapy in the treatment of cancer could be through the potentiation of antioxidant defenses.

In previous reports the evaluation of the biological properties against different tumoral cell lines in metalated uracils have been carried out with interesting results [19–21]. Thus, in the present work, we report the structure of a new gold(I) compound, [Au(MANUH$_{-1}$)(PPh$_3$)], containing 6-amino-1-methyl-5-nitrosouracilato ligand (MANUH$_{-1}$) binding the metal in a quite unusual monodentate N3 mode, and its antitumor properties in an animal model glioma. Also, we describe its effects on both non-enzyme and enzyme antioxidant defense systems and on several biochemical serum biomarkers, to analyze putative adverse effects of the treatment in several physiological functions.

2. Chemistry of [Au(MANUH$_{-1}$)PPh$_3$]

2.1. Synthesis and spectral characterization

The synthesis of the precursor organic ligand 6-amino-1-methyl-5-nitrosouracil (MANU) was already described (Scheme 1) [21]. The complex [Au(MANUH$_{-1}$)PPh$_3$] was obtained by mixing [AuCl(PPh$_3$)] and MANU (1:4) in a methanolic alkaline medium. Precursor and gold complex were characterized by IR, MS and NMR spectra. The IR spectra display the bands due to the endocyclic carbonyl groups which appear at around 1729 and 1705 cm^{-1} in the free ligand, whereas in the complex they are shifted to lower wavenumber (about 50–90 cm^{-1}); these bands are primarily sensitive to the loss of the pyrimidine proton H3. In the 600–200 cm^{-1} range, the stretching vibration associated to M–P has been assigned in accordance with data found in literature [22]. EI-MS shows the peak of the monoprotonated specie [M$^+$] at *m*/*z* 629 and those of the corresponding loss of MANU and Au–MANU fragments.



Scheme 1. Structure of the 6-amino-1-methyl-5-nitrosouracil (MANU) showing the IUPAC's numbering system.

On comparing the ^{1}H NMR spectra of both metalated and free-metal ligand, the most remarkable feature is the disappearance of the signal corresponding to the H3 proton. ^{13}C NMR data show a general deshielding as consequence of the nitrogen N3 coordination, with important downfield shifts for the signals assignable to C2 and C4 carbon atoms due to their proximity to N3 atom (5–7.5 ppm). Double signals in CP–TOSS spectra are observed for C1 and C2 atoms due to an insufficient rotation which induces the splitting of the signals [23]. The ^{31}P{^{1}H} NMR spectrum of the complex displays a singlet at 31.38 ppm, which is downfield shifted *ca.* 1 ppm with respect to its position in the [AuCl(PPh$_3$)] spectrum; this signal is very close to those found for other triphenylphosphine complexes containing the N–Au–P fragment [24,25]. In order to check the stability of the title compound in DMSO solution and bearing in mind that the treatments of animals were performed along one week, the ^{1}H NMR spectrum (DMSO) was recorded at intervals of two days during ten days; the spectra indicate no significant decomposition.

2.2. XRD single-crystal study

The crystal structure of [Au(MANUH$_{-1}$)PPh$_3$]·¼H$_2$O obtained by X-ray diffraction analysis consists in asymmetric units containing two independent and virtually identical molecules; one of them is shown in Fig. 1. The Au(I) center is coordinated by a deprotonated endocyclic N3 atom of the MANU ligand and the phosphorus of a triphenylphosphine, displaying a typical quasi-linear geometry with P–Au–N angles of 169.3(2) and 173.2(2)°. The distances Au–P (2.215(2) and 2.218(2) Å) and Au–N (2.058(5) and 2.057(5) Å) are similar to those found in other P–Au–N compounds [26–28].

The uracil ligand is roughly planar and coordinates to the metal in a quite unusual monodentate N3 binding mode, this behavior is also observed in complexes with 1-methylthymine or 1-methylcytosine which often act as bidentate ligands through the deprotonated N3 atom but also the partially enolyzed O4 atom [29–31]. Despite an extensive electronic delocalization existing, the MANUH$_{-1}$ anion can be best described as a 6-amino-5-nitroso-2,4-dioxo

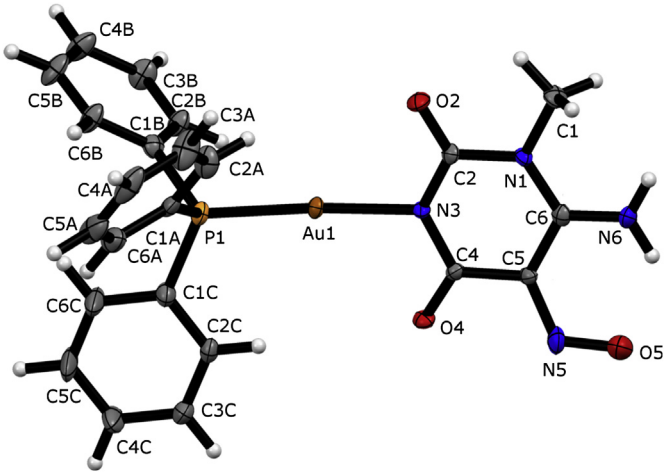


Fig. 1. ORTEP plot of one [Au(MANUH$_{-1}$)PPh$_3$] molecule (ellipsoids at 30% probability).