Inorganica Chimica Acta 393 (2012) 304–317

Contents lists available at SciVerse ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

t-Butylsarcosinedithiocarbamato gold(III)-based anticancer agents: Design, *in vitro* biological evaluation and interaction with model biomolecules

Giulia Boscutti [a], Lisa Feltrin [a], Debora Lorenzon [b], Sergio Sitran [a], Donatella Aldinucci [b], Luca Ronconi [a], Dolores Fregona [a,*]

[a] Department of Chemical Sciences, University of Padova, Via F. Marzolo 1, Padova 35131, Italy
[b] Division of Experimental Oncology 2, Centro di Riferimento Oncologico (CRO-IRCSS), Via F. Gallini 2, Aviano (PN) 33081, Italy

ARTICLE INFO

Article history:
Available online 23 July 2012

Metals in Medicine Special Issue

Keywords:
Gold
Dithiocarbamates
Anticancer activity
Nucleotides
Albumin
Glutathione

ABSTRACT

As a further extension of our research work focusing on the development of gold(III)-dithiocarbamato derivatives as potential anticancer agents, the new complexes [$Au^{III}X_2$(dtc-Sar-O(*t*-Bu))] (X = Cl (**1**)/Br (**2**)) are here reported. The compounds were characterized by means of FT-IR, ESI mass, and mono- and multidimensional NMR spectroscopy. In order to get further insights into the possible behavior under physiological conditions, their affinity toward selected model biomolecules was spectroscopically investigated in detail. In this regard, they seem to react very slowly with isolated dAMP (but not dGMP), forming a species identified as [Au^{III}(dtc-Sar-O(*t*-Bu))(dAMP-N^1,C^6NH)]$^+$. In presence of GSH they undergo sudden reduction to the gold(I) counterpart [Au^{III}_2(dtc-Sar-O(*t*-Bu))$_2$], whereas only secondary interactions seem to occur when reacted with BSA. According to *in vitro* cytotoxicity studies, both complexes turned out to be highly effective toward all the human tumor cell lines evaluated (HeLa, L540 and U937), reporting IC_{50} values lower than cisplatin. Apoptosis was proved a major cell death mechanism and, accordingly, DNA fragmentation was observed. Remarkably, cell cycle progression was negligibly affected, thus supporting the hypothesis of a different mechanism of action from clinically-established platinum-based drugs.

1. Introduction

The discovery of the anticancer properties of cisplatin (*cis*-dichloridodiammineplatinum(II), *cis*-[$Pt^{II}Cl_2(NH_3)_2$]) in the mid-1960s, opened up new prospects in the exploitation of metal-based chemotherapeutics to fight cancer. Since its introduction in the clinical practice in 1978, cisplatin is still among the most used drugs for the treatment of a wide spectrum of malignancies [1]. Anyway, this kind of therapy shows severe drawbacks in the patients, the most relevant being neuro- and nephrotoxicity, along with the evidence of either induced or intrinsic resistance to the treatment in some tumor types [2]. Thus, researchers have been focusing on the development and *in vitro* testing of both novel platinum- and other metals-based compounds with the aim of obtaining complexes with higher effectiveness, increased selectivity for tumor tissue, reduced toxicity, wider spectrum of activity, and able to overcome tumor resistance often arisen from cisplatin treatment [3].

Since gold compounds have been used for centuries in the treatment of rheumatoid arthritis [4], their well-known antiinflammatory and immunosuppressing properties made them promising candidates as potential drugs in oncology. Additionally, gold(III) and platinum(II) complexes share some important chemical features (*i.e.* they are isoelectronic and isostructural), thus allowing to hypothesize, at least in principle, a resemblance between their mechanisms of action [5]. The formation of platinum–DNA adducts through the covalent binding to (preferentially) neighboring nucleobases, is commonly acknowledged as the main mechanism involved in cisplatin's anticancer activity [6]. This interaction is believed to induce a distortion of the DNA double-strand that prevents main cellular processes, such as replication and transcription, eventually leading to apoptosis as final outcome [7].

In this context, during the last decade, some gold(III)-dithiocarbamato derivatives have been designed and tested by our research

Abbreviations: BrdU, bromodeoxyuridine; BSA, bovine serum albumin; dAMP, 2′-deoxyadenosine-5′-monophosphate; dGMP, 2′-deoxyguanosine-5′-monophosphate; DMSO, methyl sulfoxide; DSC, differential scanning calorimetry; dtc, dithiocarbamate; ELISA, enzyme-linked immunoadsorbent assay; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; GSH, glutathione; GSSG, oxidized glutathione (glutathione disulfide); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HMBC, heteronuclear multiple bond correlation; HSA, human serum albumin; PBS, phosphate-buffered saline; PI, propidium iodide; PS, phosphatidylserine; RPMI, Roswell Park Memorial Institute (culture medium); Sar, sarcosine (*N*-methylglycine); TG, thermogravimetry; TMS, tetramethylsilane.

* Corresponding author. Tel.: +39 (0)49 8275159; fax: +39 (0)2 700500560.
E-mail address: dolores.fregona@unipd.it (D. Fregona).

0020-1693/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ica.2012.06.048

group as potential anticancer agents, showing promising and outstanding properties [8,9]. In particular, the choice of dithiocarbamates as ligands was driven by their potential chemoprotective action toward renal toxicity, a major limiting factor in the use of platinum-based therapeutics in the clinic. Strong and irreversible binding of platinum to sulfur-containing biomolecules (including thiol groups of renal enzymes) is believed to alter the conformation of the biomolecules themselves and to lead to substantial changes of their biological functions up to inactivation, thus inducing gastro-, nephro-, and bone marrow toxicity [10–12]. Among the sulfur-containing compounds tested as chemoprotectants [13,14], the accurate use and dosing of sodium diethyldithiocarbamate was proved successful in reducing cisplatin nephrotoxicity without decreasing its antitumor properties [15]. On the other hand, the overall nephroprotective benefits were somewhat limited by the acute toxicity profile, especially at neuronal level, exhibited by the free (*i.e.* not coordinated) dithiocarbamate [16]. Consequently, the design of metal-dithiocarbamato derivatives has potential owing to the combination of the cytotoxicity of the metal center with the chemoprotective activity of the dithiocarbamato ligand, thus resulting in anticancer agents with high antiproliferative activity as well as reduced toxic side-effects. Moreover, along with the prevention of the intrinsic toxicity of the free dithiocarbamate, in the case of gold(III) complexes the chelating effect of the dithiocarbamato moiety is likely to stabilize the metal center in the +3 oxidation state, thus preventing reduction to gold(I) species or metallic gold (a well-known drawback of gold(III) derivatives often occurring under the normally reducing physiological conditions) [17].

Based on such considerations, we have previously reported on some gold(III)-dithiocarbamato derivatives of the type [$Au^{III}X_2$(dtc)] (X = Cl, Br; dtc = various dithiocarbamates), some of which showed excellent *in vitro* antiproliferative properties, even toward human tumor cell lines resistant to cisplatin [18]. Moreover, a couple of selected compounds were proved to exert promising *in vivo* anticancer activity on xenografts [19,20] together with negligible acute toxicity, no organ toxicity, and the almost lack of nephrotoxic side-effects *in vivo* [21].

Following these positive results, we have been exploring alternative routes to improve the therapeutic effectiveness of this class of compounds. In the actual research work, we mainly modified the steric hindrance of the dithiocarbamato ligand with the aim at tuning the lipophilic profile of the complexes and, as a consequence, their cell uptake and metabolic pathway. Accordingly, we report here on the synthesis and chemical characterization of the gold (III)-dithiocarbamato derivatives [$Au^{III}X_2$(dtc-Sar-O(*t*-Bu))] (X = Cl (**1**), Br (**2**)).

Owing to the above mentioned structural similarity with cisplatin, the possibility that these gold complexes could exert their antineoplastic activity through direct coordination to DNA was taken into account by studying their interaction with isolated model nucleotides. In fact, some previous studies have suggested that possible binding sites for gold(III) compounds might be the same as for platinum(II) drugs (in particular the N(7) atoms of purine residues) [22,23]. Therefore, the behavior of compounds **1** and **2** in presence of either 2′-deoxyguanosine-5′-monophosphate or 2′-deoxyadenosine-5′-monophosphate was investigated by NMR in order to evaluate the potential binding of the purine bases to the metal center and preliminarily hypothesize whether cellular DNA might represent a suitable biological target.

Among the intracellular biomolecules, glutathione (γ-Glu-Cys-Gly) is one of the most abundant non-protein thiol in cells and it is involved in various physiological functions in defense of cells, including detoxification and inactivation of drugs [12]. In this regard, we here report also on detailed NMR and UV–Vis studies aimed at elucidating the potential interaction of the gold(III)-dithiocarbamato complexes with glutathione.

The assessment of the stability and the behavior of metal complexes upon administration to the patients is crucial so as to understand what is the effective metabolite that reaches cells. Consequently, we evaluated by means of UV–Vis spectroscopy the stability of the object complexes in phosphate-buffered saline as a mimicking environment for a physiological-like medium. Once in the bloodstream, metal derivatives can bind to serum proteins, thus acting as active transporters. Among all, serum albumin is the most abundant plasma protein and was proved to be involved in the transport of a number of compounds, including metallodrugs [24]. The interaction of serum albumin with metal-based chemotherapeutics is of paramount importance since it can determine the overall drug distribution and excretion, together with differences in effectiveness, activity and toxicity [25]. Therefore, we have also investigated by CD and UV–Vis spectroscopy the interaction of the two gold(III) derivatives with the model plasma protein bovine serum albumin.

From the biological point of view, complexes **1** and **2** have been tested for their *in vitro* cytotoxic activity toward human squamous cervical adenocarcinoma (HeLa), Hodgkin's lymphoma (L540), and histiocytic lymphoma (U937) cells, together with their capability to induce apoptosis and affect cell cycle progression. Results are here discussed and compared to those obtained with the reference drug cisplatin under the same experimental conditions.

2. Experimental

2.1. Materials

t-Butylsarcosine hydrochloride, dAMP, dGMP, FBS, DMSO, DMSO-d_6, $CDCl_3$, carbon disulfide, PI, PBS tablets (1 tablet in 200 mL of water: 0.01 M phosphate pH 7.4, 0.138 M NaCl, 0.0027 M KCl), GSSG, BSA in PBS (0.01 M phosphate pH 7.4, 0.138 M NaCl, 0.0027 M KCl, 1% w/v BSA), trypan blue (Aldrich), potassium tetrachloro- and tetrabromoaurate(III) dihydrate (Alfa Aesar), GSH (Calbiochem), RPMI medium, penicillin, streptomycin, L-glutamine, (Biochrom KG), and cisplatin (Pharmacia and Upjohn) were of reagent grade or comparable purity and were used as supplied. All other reagents and solvents were used as purchased without any further purification.

2.2. Instrumentation

Melting points were determined using an Electrothermal IA9300 apparatus and were not corrected.

CHNS elemental analyses were carried out on either a Fisons EA1108 or a Thermo Scientific FLASH 2000 CHNS-O microanalyzer.

Electrospray mass spectra (ESI-MS) were obtained by means of an ESI-TOF Mariner 5220 mass spectrometer (PerSeptive Biosystems). The samples were prepared in a 1:1 mixture of water/acetonitrile containing 0.1% formic acid, to a final concentration of 0.1 mM. Samples were injected directly (16 μl min^{-1}) and the ions produced in an atmospheric pressure ionization (API) ESI ion source. The source temperature was 413 K, and the drying gas (N_2) flow rate was 300 L h^{-1}. A potential of 4.3 kV (capillary voltage) was applied to the probe tip, and the ions accelerated at 10–30 eV. Mass spectra were recorded over the scan range 100–4000 Da at a scan rate of 5 s and a resolution of 2 *m*/*z*. Data acquisition and processing were carried out using DATA EXPLORER TM version 4.0 (Applied Biosystems).

FT-IR spectra were recorded in Nujol on a Nicolet Nexus 870 spectrophotometer (1000 scans, resolution 2 cm^{-1}) for the range 50–600 cm^{-1}, and in solid KBr on either a Nicolet spectrophotometer (32 scans, resolution 2 cm^{-1}) for the range 400–4000 cm^{-1}.